The Role of Antibodies to Plasmodium falciparum Merozoite Antigens in the Resistance of Young Infants to Febrile Malaria and Their Place as a Biomarker in the Detection of Malaria Transmission Hotspots

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The role of antibodies to *Plasmodium falciparum* merozoite antigens in the resistance of young infants to febrile malaria and their place as a biomarker in the detection of malaria transmission hotspots

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Thesis submitted to the Open University (UK) for the degree of Doctor of Philosophy

Life and Biomolecular Sciences

Sponsoring Institution

Centre National de Recherche et de Formation sur le Paludisme

Ouagadougou, Burkina Faso

Collaborating Institution

Kenya Medical Research Institute-Wellcome Trust Research Programme

Kilifi, Kenya

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Candidate Contribution

The work was based on two collaborative projects, the first conducted in Banfora, Burkina Faso and Keur Soce, Senegal and the second conducted in Kilifi, Kenya. I was responsible for the design of the studies.

In the Banfora cohort study:

I have had primary responsibility for:

- Designing the study case report forms for data collection,
- Applying for ethical clearance to the Institutional Review Board,
- Recruiting the study fieldworkers,
- Training the fieldworkers and nurses in study procedures,
- Recruiting the study participants,
- Planning and supervising the field work,
- Supervising the passive follow up at the study dispensaries,
- Supervising the work of the community representatives,
- Performing the data management and quality assurance,
- Performing the enzyme-linked immunosorbent assay in KEMRI-WTRP laboratories,
- Preparing and implementing the statistical analysis plan.

In the Keur Soce cohort study:

I have had primary responsibility for:

- Preparing the first drafts of the study case report forms,
- Performing the enzyme-linked immunosorbent assay in KEMRI-WTRP laboratories,
• Performing the statistical analysis of the serology data.

**In the Kilifi MTI study:**

I have had primary responsibility for the design and implementation of statistical analysis.

In all three studies I have had primary responsibility for conducting the write-up and interpretation.
Publications


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Abstract

Over the last 15 years the malaria burden has globally declined, but not evenly across endemic areas. In areas with substantial decline, elimination became realistic. However, malaria elimination has to, in a cost-effective way, overcome problems such as increasing drug and insecticide resistance and the increasing heterogeneity in transmission as the transmission intensity declines. Vaccines are proven cost-effective tools in infectious disease control and substantial progress was made with the RTS, S vaccine. However, vaccine development is hampered by the lack of reliable immune correlates of protection.

We have analysed antibody responses in relation to the incidence of febrile malaria in young children, with the specific objective of investigating their contribution to the apparent resistance of young infants to febrile malaria. We have also analysed the dynamics of antibodies in relation to previously established protective thresholds.

We found that the antibody responses to 6 different falciparum antigens were not associated with protection against febrile malaria in young children and that their levels were consistently below the protective thresholds. Furthermore, we found that antibody titres were often actually associated with increasing risk of febrile malaria. A likely explanation is that the antibodies were markers of exposure and hence associated with higher risk.

We therefore analysed geo-spatial data on malaria risk to identify hotspots of clinical malaria and their association with hotspots of serological responses to malaria antigens.

We found that 1) antibody responses correlated well with asymptomatic parasitaemia detected by polymerase chain reaction, and 2) there was substantial overlapping between the hotspots detected using these markers.
Our data suggest that other mechanisms are responsible for the apparent resistance of infants to febrile malaria. Moreover, our data suggest that serology or polymerase chain reaction results may be used as markers for the detection of hotspots when the transmission declines to very low levels.
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Glossary of Terms and Abbreviations

ACT Artemisinin-based Combination Therapy

ADRB Antibody-Dependent Respiratory Burst

AMA1 Apical membrane Antigen 1

AU Arbitrary Unit

BSA Bovine Serum Albumin

CD Cluster of Differentiation

CDC Centers for Disease Control and Prevention

CI Confidence Interval

CNRFP Centre National de Recherche et de Formation sur le Paludisme

CSP Circumsporozoite Protein

CV Coefficient of Variation

DMFA Direct Membrane-Feeding Assay

EDTA Ethylenediaminetetraacetic acid

EI Exposure Index

EIR Entomological Inoculation Rate
ELISA Enzyme-Linked Immunosorbent Assay

EMA European Medicines Agency

EPI Expanded Programme on Immunization

FcRn Neonatal Fc receptor

G6PD Glucose-6-Phosphate Dehydrogenase

GLURP Glutamate-Rich Protein

GPI Glycosylphosphatidylinositol

GPS Global Positioning System

GST Glutathione S-Transferase

Hb Haemoglobin

HbA1 Haemoglobin Alpha 1

HbA2 Haemoglobin Alpha 2

HLA Human Leukocyte Antigen

HPLC High-Performance Liquid Chromatography

HR Hazard Ratio

IgG Immunoglobulin G

IPTp Intermittent Preventive Treatment of malaria in pregnancy
IQR Inter-Quartile Range

IRR Incidence Rate Ratios

ITN Insecticide-Treated Nets

KEMRI-CGMRC Kenya Medical Research Institute-Centre for Geographic Medicine Research Coast

LSA-1 Liver Stage Antigen 1

MBP Maltose-Binding Protein

MIG Malaria Immune Globulin

MSP1 Merozoite Surface Protein 1

MSP2 Merozoite Surface Protein 2

MSP3 Merozoite Surface Protein 3

MUAC Mid Upper Arm Circumference

NAI Naturally Acquired Immunity

NANP Four amino acid repeat region of the circumsporozoite protein

OD Optical Density

PBST Phosphate Buffered Saline solution with the detergent Tween

PCR Polymerase Chain Reaction
PF155/RESA *Plasmodium falciparum* Ring-infected Erythrocyte Surface Antigen

PfEMP1 *Plasmodium falciparum* Erythrocyte Membrane Protein 1

PK Pyruvate Kinase

RBC Red Blood Cell

RDT Rapid Diagnostic Test

ROS Reactive Oxygen Species

RR Relative Risk

SAO Southeast Asian Ovalocytosis

SPf66 Chimeric peptide vaccine combining fractions of three *Plasmodium falciparum* blood stage antigens and the circumsporozoite protein

VIF Variance Inflation Factor

WBC White Blood Cell

WHO World Health Organization
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1. CHAPTER 1: Literature review

1.1 The global problem of malaria

In 2015, around 3.2 billion (about half the world’s population) people were at risk of malaria worldwide (WHO 2015). An estimate of 214 (149-303) million malaria cases have been reported globally the same year, resulting in 438000 (236000-635000) deaths, of which 90% were recorded in the WHO African region (WHO 2015) where some countries have 100% of their population at risk of malaria (Gething, Patil et al. 2011). However, these figures may have been underestimated since many cases are likely to be unreported (Hay, Okiro et al. 2010, Murray, Rosenfeld et al. 2012). Malaria contributed to 7.4%, 7.3% and 10% of the global mortality of children under five years of age in 2010 (Liu, Johnson et al. 2012) 2013 (Liu, Oza et al. 2015) and 2015 (WHO 2015) respectively. In 2015, 70% of the 438000 malaria deaths occurred globally in children under-five (WHO 2015). The global pattern of the distribution of malaria burden remains unchanged with sub-Saharan Africa being disproportionately affected with 90% of malaria deaths (WHO 2015).

Malaria affects the well-being of communities beyond the direct effect of clinical disease through the economic burden it engenders. A study conducted in a holo-endemic area of Nigeria found an estimate of household expenditure of between 12.57 US$ and 23.20 US$ per case for outpatient visits and inpatient stays respectively (Onwujekwe, Uguru et al. 2013). In Ghana, it has been estimated that a household spends on average 13.9 US$ for the management of each case of malaria (Dalaba, Akweongo et al. 2014). In high-burden malaria
countries, the malaria related expenses at household level can become quickly difficult to meet for the poorest households, pushing them further into poverty. There is growing evidence of an association between malaria and poverty. At country level, it has been shown that countries with the highest malaria burden have 1.3% lower annual economic growth than other countries (Gallup and Sachs 2001). These complex associations are often described as the “vicious circle of poverty and malaria” (Tusting, Willey et al.).

The global malaria control effort has yielded encouraging results in the last fifteen years with the estimated malaria incidence and mortality rate falling respectively by 37% and 60% globally and the number of deaths averted estimated at 6.2 million lives (WHO 2015). Similar findings, 40% decline in malaria incidence, were reported by Bhatt and colleagues for Africa especially for the same period (Bhatt, Weiss et al. 2015).

These recent gains in malaria control, driven to a large degree by early treatment with artemisinin-based combination therapies, universal coverage of insecticide-treated nets and indoor residual spraying, are however fragile considering emerging drug and insecticide resistance (Ashley, Dhorda et al. 2014, Sovi, Djegbe et al. 2014), the underlying poverty in the most affected areas in sub-Saharan Africa (Gallup and Sachs 2001) and the potential adverse effects of climate and environmental change on vector distribution and vectorial capacity in the long run especially in areas of declining or unstable malaria transmission. On the latter factor, substantial work is being done to try predict malaria vectors redistribution under different climatic change scenarios (Ermert, Fink et al. 2012, Tonnang, Tchouassi et al. 2014). The consolidation of the current gains in malaria control is expected to benefit from a vaccine and a better understanding of the immune targets and mechanisms would guide and accelerate the development of a malaria vaccine with high and long lasting protective efficacy. The vaccine received a positive scientific opinion by the European
Medicines Agency (EMA) for use outside the European Union, although its protective efficacy is limited (Morrison 2015). Improved vaccination strategies may be guided by immuno-epidemiological data on appropriate antigens to target.

1.2 The human malaria parasites

Malaria is caused by a parasitic single-cell apicomplexan micro-organism of the genus Plasmodium that was first identified in 1880 within red blood cells using light microscopy by Alphonse Laveran in Algeria (Bruce-Chuavatt 1981). To date, human malaria has been linked to five species of the genus Plasmodium: P. falciparum, P. malariae, P. ovale (curtisi and wallikeri), P. vivax and P. knowlesi. The malaria global public health problem is essentially driven by the virulence and/or the spread of P. falciparum and P. vivax (Gething, Patil et al. 2011, Gething, Elyazar et al. 2012).

1.2.1 Plasmodium falciparum

The population at risk of P. falciparum transmission in 2010 was estimated at 2.57 billion people living in highly variable transmission intensity areas; of the 1.44 billion people living in stable P. falciparum transmission areas, Africa and Asia are home to respectively 52 % and 46 % of them (Gething, Patil et al. 2011). P. falciparum is the most virulent species in humans. It has biological features that predispose it to cause severe forms of malaria under certain immunological conditions, especially in children aged less than five years (Marsh, Forster et al. 1995) and pregnant women (Desai, ter Kuile et al.). The origin of P. falciparum is not clearly established, but there is some evidence of a close relationship with the chimpanzee malaria parasite P. reichenowi (Escalante, Freeland et al. 1998, Conway, Fanello et al. 2000).
1.2.2 Plasmodium vivax

In 2010, it has been estimated that 2.48 billion people were at risk of \textit{P. vivax} transmission worldwide. Central Asia was home to the majority of these people (82 \%). Africa accounted for only 3 \% of this at risk population (Gething, Elyazar et al. 2012). The very limited transmission of \textit{P. vivax} in African populations (especially West and Central Africa) has been linked to the lack of a red blood cell membrane protein, the Duffy antigen, expressed on the surface of erythrocytes and reticulocytes and acting as a receptor for their invasion by the parasite (Miller, Mason et al. 1976). However, infections have been reported in Duffy-negative individuals, suggesting the existence of alternative RBC invasion pathways (Ménard, Barnadas et al. 2010). \textit{P. vivax} was usually considered as a causal agent of mild malaria, but recent reports suggest it may also cause severe clinical disease (Andrade, Reis-Filho et al. 2010). \textit{P. vivax} is known for its ability to establish dormant forms in the liver that are a source of subsequent relapses few weeks to one year later (White 2011). The stimuli that trigger the relapses are unclear, but other infectious diseases including \textit{P. falciparum} malaria have been suggested as risk factors (Shanks and White 2013). Southeast Asian non-human primates (macaques) were previously thought to be the origin of \textit{P. vivax} (Escalante, Cornejo et al. 2005) but there is now evidence of an African origin (African apes) where the high prevalence of Duffy negative phenotype is hypothesized to have resulted from the selective pressure of \textit{P. vivax} (Liu, Li et al. 2014).
1.2.3 Plasmodium ovale

A number of *P. ovale* malaria cases have been reported in Southeast Asia, Western Pacific islands and the Middle East but it is mainly established in sub-Saharan Africa (Lysenko and Beljaev 1969, Collins and Jeffery 2005, Mueller, Zimmerman et al. 2007). *P. ovale* has been historically described as a cause of mild clinical malaria with usually low parasitaemia and low incidence (Faye, Spiegel et al. 2002, Roucher, Rogier et al. 2014). *P. ovale* shares with *P. vivax* the ability to establish dormant forms in the liver, from where it can cause relapses (Collins and Jeffery 2005). Arisue and colleagues have recently related *P. ovale* to rodent malaria parasites (*P. yoelii, P. berghei, P. chabaudi*) in a phylogenetic study (Arisue, Hashimoto et al. 2012). Based on genetic characterization, it has been recently proposed that *P. ovale* be now viewed as two different species, *P. ovale curtisi* and *P. ovale wallikeri* (Sutherland, Tanomsing et al. 2010).

1.2.4 Plasmodium malariae

*P. malariae* appears as the least studied human malaria parasite. Although less prevalent, its distribution is broad, following that of *P. falciparum*, with the highest prevalence reported in sub-Saharan Africa (Collins and Jeffery 2007). *P. malariae* is characterized by a longer pre-patent period (16-59 days), a longer development cycle in RBC (an extra 24 hours compared with the other human malaria parasites), lower parasitaemia levels and its ability to cause long-lasting (decades) asymptomatic infections despite absence of latent forms (Collins and Jeffery 2007). The illness caused by *P. malariae* is usually mild but it has been recognized in earlier studies as a possible cause of chronic glomerulopathy through the formation of immune complexes (Gilles and Hendrickse 1963, Ward and Kibukamusoke
1969). The phylogenetic origin of *P. malariae* is still unclear (Arisue, Hashimoto et al. 2012).

1.2.5 *Plasmodium knowlesi*

*P. knowlesi* is primarily a Southeast Asian simian malaria parasite; it has been recently recognized as the fifth human malaria parasite (Singh, Sung et al. 2004). It should actually currently be considered as a zoonosis since direct human to human transmission has yet to be evidenced. *P. knowlesi* infection has been clearly associated with severe and fatal illness (Cox-Singh, Hiu et al. 2010, Seilmaier, Hartmann et al. 2014). There is growing evidence on the extent of the morbidity attributable to *P. knowlesi* in Southeast Asia (William, Rahman et al. 2013, Yusof, Lau et al. 2014) but not enough to map its geographical distribution with reasonable certainty. However, Moyes and colleagues have established a map of the geographical distribution of its potential reservoir which is limited to Southeast Asia and Western Pacific Islands (Moyes, Henry et al. 2014). An important current hurdle to the study of the epidemiology of *P. knowlesi* is its frequent misdiagnosis with *P. malariae* using light microscopy, the current standard for malaria species identification (Barber, William et al. 2013, Jeremiah, Janagond et al. 2014).

1.2.6 A potential emerging malaria parasite: *Plasmodium cynomolgi*

Other simian malaria parasites are currently recognized as zoonotic parasites (transmissible to humans) (Ramasamy 2014) but their natural transmission to humans has yet to be established. Ta and colleagues have recently reported the first identified case of natural infection with *P. cynomolgi* in Malaysia (Ta, Hisam et al. 2014). Land use changes (deforestation, farming, mining) as a result of the growing need of human populations for
natural resources create ideal conditions for increased human-wildlife interaction, hence a substantial potential for the emergence of these zoonotic malaria parasites.

1.2.7 Mixed malarial infections

In malaria endemic areas, several species of *Plasmodia* are often circulating concomitantly. In tropical Africa, the species involved in the most common co-infection are *P. falciparum* and *P. malariae* while in sub-tropical areas *P. falciparum* and *P. vivax* make up the most common mixed infection (Gilles 2002). Bousema and colleagues have found evidence for an association of mixed *malariae-falciparum* infections with higher *P. falciparum* gametocyte production (Bousema, Drakeley et al. 2008) which is in contradiction with the findings of Gneme and colleagues (Gnémé, Guelbéogo et al. 2013). In a study in West Africa, Doderer-Lang and colleagues recently found exceptionally high sero-prevalence of mixed infections; 45.3% of the participants were seropositive to the antigens of *P. falciparum*, *P. malariae* and *P. ovale* at the same time (Doderer-Lang, Atchade et al. 2014). In a study in Myanmar in Southeast Asia, Jiang and colleagues found that, for an overall *P. knowlesi* prevalence of 21.9 %, mono-infections accounted for only 2.7 %. *P. knowlesi* was preferentially involved in co-infection with *P. falciparum* or *P. vivax* (Jiang, Chang et al. 2010). Mixed infections with *falciparum* and *malariae* are less frequently reported. In a study in Ivory Coast in West Africa, a prevalence between 6-11 % (depending on the season) for mixed *falciparum-malariae* infection was reported(Black, Hommel et al. 1994) (Dossou-Yovo, Ouattara et al. 1994) while a study in India reported a prevalence of 1 % (Sri, Praveen et al. 2015).

1.3 The human malaria vectors
The relationship between malaria and mosquitoes was formally established in 1895 in India by Sir Ronald Ross who identified the causative parasite in the midgut of mosquitoes in his study of avian malaria. It was later, in 1898, that Giovani Battista Grassi provided evidence that malaria is transmitted by mosquitoes (Cox 2010). Only female mosquitoes of the genus *Anopheles* are able to transmit malaria. The genus *Anopheles* is part of the Anophelini tribe, Culicinae sub-family, Culicidae family, Nematocera sub-order, Diptera order (Gilles 2002).

Around 40 *Anopheles* species have been identified as capable of transmitting human malaria parasites with differing vectorial capacity ([http://www.map.ox.ac.uk/explore/mosquito-malaria-vectors/](http://www.map.ox.ac.uk/explore/mosquito-malaria-vectors/)).

### 1.3.1 Larval habitats, reproduction and fitness to survival

*Anopheles* mosquitoes breed on various types of habitats which may be natural, man-made or animal-made, standing or running, fresh or brackish water, permanent, semi-permanent or transient water bodies, with or without floating or emerging vegetation. The preference for the type of breeding site varies between species. The choice of a breeding site depends on factors such as ambient temperature, sunlight exposure, humidity, water salinity and organic content (Gilles 2002).

*Anopheles gambiae ss*, predominant in sub-Saharan Africa and reported as the most efficient malaria vector, typically breeds in temporary, still, shallow, clean and sunlit fresh water. However, there is evidence of its ability to adapt to polluted water in urban areas when clean water is not readily available (Awolola, Oduola et al. 2007). The reproduction of *Anopheles* mosquitoes follows a cycle termed gonotrophic (period between two subsequent ovipositions). It is believed that a single mating is enough to lead to all the subsequent
gonotrophic cycles. The short duration of the gonotrophic cycle (48 hours) allows the female Anopheles to lay multiple batches of eggs, provided the blood meal sources necessary for egg maturation are readily available (Gillies 1953). The eggs develop successively into larvae, pupae and adult form; the duration of this cycle is highly variable, depending on the ambient temperature and the species, between 7 (at 31°C) and 20 (at 20°C) days (Gilles 2002).

Anopheles mosquitoes are susceptible to ecological and climatic challenges represented by a wide range of predators and the long, harsh dry season that occurs in some sub-Saharan geographical areas. Indeed, natural predators (Araneae, Diptera, Coleoptera, Amphibia) are responsible of a high larval mortality rate of Anopheles gambiae such that less than 10 % reach adult stage (Service 1973). Given the limited resistance of Anopheles larvae to desiccation and the relatively short life span of adult mosquitoes, it seems surprising that even after long and severe droughts, the mosquito population almost immediately reconstitutes after the return of rains. There are two main hypotheses to explain this mosquito fitness to survival: long distance migration and aestivation. Lehmann et al. have provided definitive evidence for Anopheles gambiae aestivation in a mark-release-recapture experiment in the Sahel. Indeed, they have recaptured a female Anopheles gambiae marked 7 months earlier at the end of the previous rainy season (Lehmann, Dao et al. 2010), confirming the aestivation hypothesis suggested by earlier laboratory experiments (Holstein 1954, Omer and Cloudsley-Thompson 1968). The hypothesis of long distance migration remains to be demonstrated.
1.3.2 *Anopheles* dispersal, feeding and resting habits

*Anopheles* mosquito dispersal can be described as active, when the mosquito moves by means of its own wings, and passive when mosquitoes are displaced by mechanical means such as wind or any kind of vehicle (truck, boat, airplane or train) (Gilles 2002). Active dispersal of mosquitoes is mainly studied by mark-release-recapture experiments where mosquitoes can be marked either by fluorescent substance or radioactivity. *Anopheles* mosquitoes seldom actively spread more than two kilometres from their breeding sites (Constantini, Li et al. 1996, Midega, Mbogo et al. 2007, Liu, Liu et al. 2012, Thomas, Cross et al. 2013). Passive dispersal of infected mosquitoes by aeroplane, is responsible of “airport malaria” (malaria cases occurring in the vicinity of international airports) and may serve as a route for reintroduction of malaria in areas where it has been previously eliminated (Tatem, Rogers et al. 2006). Climatic factors such as wind direction, that is a major force for passive mosquito dispersal, may be of public health importance as it can help identify major larval sites in relation to the location of malaria hotspots (Midega, Smith et al. 2012).

Male *Anopheles* mosquitoes feed exclusively on natural sugar sources (flower nectar, fruit juice) whereas females feed mainly on warm-blooded animals. Based on host preferences, female *Anopheles* mosquitoes are classified as anthropophylic (preference for feeding on humans) or zoophylic (preference for feeding on animals). Based on their preferred place of feeding, they are classified as endophagic (indoor-biting preference) or exophagic (outdoor-biting preference) mosquitoes. Based on their preferred place for resting, they are classified as endophylic (preference for indoor-resting) or exophylic (preference for outdoor-resting) mosquitoes. These behavioural features have critical importance for vector control strategies.
*Anopheles gambiae*, described as the most efficient malaria vector, is typically anthropophylic, endophagic, endophilic and nocturnal but actually adapts its behaviour to the location and accessibility of the blood meal source (Faye, Konate et al. 1997, Mwangangi, Mbogo et al. 2003, Lefèvre, Gouagna et al. 2009). There is empirical evidence of a high variability in human attractiveness to *Anopheles* mosquitoes. Earlier studies suggested that the distribution of bites is uneven with regards to age with older children and adults bearing the bulk of bites compared with children below two years of age (Muirhead-Thomson 1951, Carnevale, Frezil et al. 1978). A recent study suggested that body odour, heat and moisture are the major explanatory factors (Mukabana, Takken et al. 2002). A wide range of other biological and physical factors have been studied, but no conclusive explanation of the variability of human attractiveness to mosquitoes has been established yet.

There is evidence that once the blood source is chosen, the choice of biting site on the human body is not random. A recent study reported that the *Anopheles gambiae* complex preferentially bites closer to ground level, whatever the position of the body is. When sitting, feet, ankles and legs are the most affected by bites. When lying down, the lower edge of the body is the most affected (Braack, Hunt et al. 2015).

The recent scale up of vector control measures in the last decade has elicited *Anopheles gambiae* survival responses. Universal coverage of ITNs and IRS campaigns have modified the feeding and resting behaviour of the malaria vectors and triggered the emergence of resistant strains. Behavioural shifts from nocturnal to diurnal biting, endophagic to exophagic and anthropophylic to zoophylic phenotype have been reported (Ndiath, Mazenot et al. 2014).
1.4 The vertebrate host

The vertebrate host is considered as intermediary since it does not support the sexual reproduction. The host preference for the female *Anopheles* seeking blood meal is species-specific. *Anopheles* mosquitoes are either anthropophilic or zoophilic but there is no clear-cut limit between both phenotypes. Depending on the conditions, host availability and accessibility, the anthropophilic *Anopheles* mosquitoes may occasionally feed on animals and the zoophilic on humans. The major human malaria vectors, *Anopheles gambiae ss* and *Anopheles funestus ss*, have been reported to feed on a wide range of domestic animals kept in the vicinity of households (cattle, sheep, goats, pigs, donkeys, horses, dogs, cats, poultry) (Sousa, Pinto et al. 2001, Lefèvre, Gouagna et al. 2009). This observed preference (*Anopheles arabiensis*) or alternative (*Anopheles gambiae, Anopheles funestus*) feeding on domestic animals has led to a concept termed zooprohylaxis. Indeed, pyrethroids and Ivermectin have been extensively studied in vector control livestock-based interventions with variable results; besides evidence for reduction of mosquito lifespan, egg-laying capacity, malaria incidence and prevalence, a lot of potential drawbacks were reported (Rowland, Durrani et al. 2001, Fritz, Siegent et al. 2009). This approach has been recently applied to the human vertebrate host in the treatment of uncomplicated malaria using a combination of Ivermectin and ACTs. Direct membrane feeding assays (DMFA) following administration of this combination showed evidence of association with mosquito lifespan reduction (Ouedraogo, Bastiaens et al. 2015).

1.5 The malaria parasite life cycle

The different species of the genus *Plasmodium* have in common a complex life cycle made of an asexual multiplication (schizogony) in a vertebrate (intermediary) host and a sexual
reproduction (sporogony) in an invertebrate (definitive) host. The life cycle of *Plasmodium spp* is illustrated in figure 1.1.

1.5.1 The sporogonic phase

The sporogonic cycle starts when the female *Anopheles* mosquito takes a blood meal from an infected human host. The circulating sexual stages of the parasite (male and female gametocytes) are then ingested by the mosquito and the digestion process starts in the midgut lumen. It is believed that the sudden drop in temperature, rise in pH and concentration of xanthurenic acid, a byproduct of tryptophan catabolism in the mosquito, trigger the activation of gametocytes, which then egress from the parasitophorous vacuole membrane and the RBC plasma membrane (Billker, Shaw et al. 1997, Garcia, Wirtz et al. 1998) thanks to pore-forming proteins (Wirth, Glushakova et al. 2014). The male gametocyte undergoes nuclear division that yields four to eight motile microgametes in a process termed exflagellation. The fertilization of the macrogamete (female gamete) requires only one microgamete. Following adherence of the microgamete to the macrogamete, their plasma membranes and their nuclei merge resulting in a rounded diploid body called the zygote. The zygote then undergoes meiosis to achieve tetraploidy and differentiates into a motile invasive elongated form called the ookinete equipped with an apical complex. The ookinete then moves to and traverses the peritrophic matrix and the midgut epithelium before it establishes on the outer surface of the midgut wall, between this epithelium and its basal lamina. It then develops into an oocyst that undergoes several nuclear divisions to form the haploid sporozoites. When the oocysts are mature, they burst and release the sporozoites in the haemolymph stream that bathes the outer surface of the basal lamina. The sporozoites then migrate up to the salivary glands where they traverse the basement membrane and epithelium to accumulate inside and remain there until the next blood meal. It has been hypothesized
that sporozoites localize in salivary glands through chemotactism to high molecular mass proteins or carbohydrate-binding proteins (Akaki and Dvorak 2005) and shown that sporozoite entry in the glands is a receptor mediated process (Mueller, Kohlhepp et al. 2010). Parasite population dynamics studies suggest that the sporogonic phase is an inefficient process. Indeed, it has been shown that a 40-fold and 69-fold decrease occur respectively in the transition from macrogametocytes to ookinete and from ookinete to oocyst (Vaughan, Noden et al. 1992) but this contrasts with in vivo experiments which found that very low gametocytemia (less than 1 gametocyte/µL blood) is sufficient to establish infection in Anopheles mosquitoes (Churcher, Bousema et al. 2013). From the 2000-5000 sporozoites released in the haemocel by each mature oocyst (Rosenberg and Rungsiwongse 1991), 80-90% never get to the salivary glands (Hillyer, Barreau et al. 2007). There is compelling evidence that the sporogonic phase kinetics is highly influenced by ambient temperature with suitable development temperatures ranging from 16 to30°C. Higher temperatures were shown to be detrimental to early stages of sporogony while the transitions ookinete-oocyst-sporozoite appear less affected by temperatures above 30°C (Vanderberg and Yoeli 1966, Okech, Gouagna et al. 2004). The events occurring from the end of the blood meal uptake to the crossing of the midgut by the ookinete constitute the “early sporogony” and last approximately 48 hours. That early sporogony is followed by the “mid-sporogony” covers the development of the oocyst and lasts approximately one week. The release of the sporozoites by the mature oocyst and their migration to the mosquito salivary glands constitute the last phase called “late sporogony”. Overall, the sporogony lasts ten to sixteen days (Zollner, Ponsa et al. 2006).
1.5.2 The schizogonic cycle

The schizogonic cycle occurs in the human host and may be divided into two phases: the pre-erythrocytic and erythrocytic phases.

1.5.2.1 The pre-erythrocytic phase

The pre-erythrocytic phase starts when a blood meal is taken by an infected female *Anopheles* mosquito from a human host. The saliva, injected to prevent pain and blood coagulation during the blood meal (Ribeiro 1995, Ha, Oh et al. 2014), conveys and deposits the sporozoites in the skin. Incidentally, few sporozoites may be injected directly in the probed blood vessel. It has been estimated that a median number of 15 sporozoites (range 0-978) were injected during an infected mosquito blood meal (Rosenberg 2008). Once deposited in the dermis, three scenarios may happen. Some sporozoites migrate through skin cells until they reach a blood vessel and traverse its endothelium to enter the blood stream (Kebaier, Voza et al. 2009). Alternatively, it has been estimated that around 20% of sporozoites end up in skin lymphatic vessels and are drained to skin-draining lymph nodes where they are stopped, phagocytized and processed by antigen presenting cells (Chakravarty, Cockburn et al. 2007, Yamauchi, Coppi et al. 2007, Radtke, Kastenmüller et al. 2015). Finally, other sporozoites may not be able to leave the skin where it has been shown in mice models that they can complete the pre-erythrocytic life cycle to develop into infective merozoites (Gueirard, Tavares et al. 2010). Once in the blood stream, the sporozoites quickly home to the liver. They migrate through the fenestrated sinusoidal endothelium, Kupffer cells, space of Disse and several hepatocytes before they finally settle within one of them (Mota, Pradel et al. 2001, Tavares, Formaglio et al. 2013). Kupffer cells are liver resident macrophages but do not seem to prevent sporozoite invasion of
hepatocytes. There is evidence that sporozoites are capable of Kupffer cells immunosuppression through prevention of reactive oxygen species (ROS) release, downregulation of pro-inflammatory cytokines and upregulation of anti-inflammatory cytokines (Usynin, Klotz et al. 2007, Klotz and Frevert 2008). In the home hepatocyte, the sporozoite forms a parasitophorus vacuole, expels its invasion machinery and develops into a liver trophozoite, the feeding form of the parasite. The trophozoite grows and undergoes several rounds of nucleus and organelles replication and becomes a schizont. In mature liver schizonts, each nucleus is packaged with the necessary organelles by repeated parasite plasma membrane invaginations to form daughter merozoites (Lindner, Miller et al. 2012). The sporozoites undergo a single schizogonic cycle in the hepatocytes.

Following enzymatic degradation of the parasitophorous vacuole membrane, the merozoites are packaged with the hepatocyte membrane into vesicles called merozomes (few to thousands merozoites) that are expelled into the liver sinusoides to initiate the erythrocytic phase (Graewe, Rankin et al. 2011).

1.5.2.2 The erythrocytic phase

The merosomes circulate in the blood stream, evading the immune system wrapped by the hepatocyte plasma membrane, up to the lung microvasculature where they rupture and release the non-motile merozoites into the blood stream (Lindner, Miller et al. 2012). The half-life of free merozoites was estimated to approximately 5 minutes at 37°C and they can remain invasive up to 10 minutes following contact with the erythrocytes (Boyle, Wilson et al. 2010). The process of the merozoites attachment to erythrocytes through their surface proteins is rapid, subsequent to which they reorient so that their apex come into contact with the erythrocyte membrane realizing a tight junction. The merozoites then enter the
erythrocytes through a membrane invagination process, leaving their protein coat outside (Figure 1.2). This process is completed quickly within 30 to 60 s (Gilson and Crabb 2009). At the end of the process, the parasite loses its internal organelles and settles within a parasitophorous vacuole. It is now called a young trophozoite. The young trophozoite feeds, enlarges and becomes ameboid (mature trophozoite) before it starts several rounds of nuclear division. At this stage it is called an early schizont. When the cytoplasm divides and packs around the daughter nuclei, it is called a mature schizont. During the intra-erythrocytic development of the parasite, alteration of the infected erythrocyte cytoskeleton leads to a reduced deformability and the formation of small protrusions termed knobs spread across the infected erythrocyte surface (Mills, Diez-Silva et al. 2007, Shi, Liu et al. 2013). The duration of each cycle is 48 hours for *P. falciparum* at the end of which the mature schizonts burst and release the merozoites in the blood stream and the cycle is repeated (Millholland, Chandramohanadas et al. 2011).

A number of hypotheses on the stimuli for trophozoites differentiation into sexual stages during the erythrocytic phase have been advanced, but none of them has been conclusive to date (Baker 2010). Gametocytes have been reported to appear in the peripheral blood between one and three weeks after the start of the erythrocytic phase and circulate in the blood stream for up to 60 days (Shute and Maryon 1951, Bousema and Drakeley 2011). Gametocytogenesis has been characterized into five distinct morphological phases (I-V) during which the early rounded gametocyte progressively turns into crescent-shaped body (Hawking, Wilson et al. 1971). Early gametocytes (I) are difficult to differentiate from the young trophozoites from which they derive and only mature stages (V) are detectable in peripheral circulation. Indeed, there is evidence that immature gametocytes sequester in the bone marrow to achieve maturation (Farfour, Charlotte et al. 2012). There is evidence that
the sexual determination of schizonts occurs prior to nuclear division and that each sexually-committed schizont yields either male or female gametocytes. The sex ratio of gametocytes is variable with a tendency to be biased towards females (Smith, Lourenco et al. 2000). A better understanding of the mechanisms of gametocytogenesis is critical as it may lead to the development of new and additional tools for malaria transmission control.

Figure 1.1. The life cycle of malaria parasite. Evidence of dormant forms exists only for *P. vivax* and *P. ovale*. Adapted from (Winzeler 2008).

Figure 1.2. Erythrocyte invasion process by *Plasmodium falciparum* merozoite. Adapted from (Kats, Cooke et al. 2008)
1.6 Spatial epidemiology of malaria

Epidemiology is the study of the distribution and determinants of diseases in human populations (Woodward 2014). In order to conduct an epidemiological study one must select a specific marker/definition to identify cases. In any epidemiological study, the population described as being at a certain prevalence will contain sub-populations with heterogeneities in prevalence. It would therefore be instructive to analyse the spatial and temporal distribution of the health event of interest. In the case of malaria, parasitological, serological and/or molecular markers have been used.

1.6.1 Global and regional determinants of the global distribution of malaria

Malaria is a disease of tropical and sub-tropical areas, predominant in sub-Saharan Africa, South-East Asia and South America and climatic factors determine the distribution of malaria at a global level. Indeed, the above-mentioned most affected regions are characterized by warm temperatures, substantial rainfall and humidity, though seasonal variations exist in some areas. Paaijmans and colleagues have shown that the gonotrophic and sporogonic cycles are very sensitive to daily temperature variability (Paaijmans, Blanford et al. 2010). The optimal temperature for sporogony ranges between 25-30°C with extremes at 16-35°C beyond which a considerable slowing down is observed (Gilles 2002). The association between rainfall and malaria transmission is more apparent in areas of seasonal rains and in dry areas when they experience unusually high rainfall (Grover-Kopec, Kawano et al. 2005). In the first situation, although the proposed methods for defining seasonality of malaria transmission may not include rainfall (Roca-Feltrer, Schellenberg et al. 2009, Cairns, Walker et al. 2015), there is evidence of a strong correlation between rainfall and febrile malaria incidence with a few weeks’ time lag accounting for the vector
and parasite development (Krefis, Schwarz et al. 2011). Altitude and land cover have also been associated with malaria distribution. Githeco and colleagues have shown that altitude was inversely correlated with anopheline mosquito densities and malaria prevalence on the one hand, and positively correlated with parasite density in young children on the other hand (Githeko, Ayisi et al. 2006). This could be explained by the strong inverse correlation between altitude and temperature with an average 0.5°C drop for every 100 m increase (Gilles 2002). Although the association is weak at the extremes (desert areas and forests), a strong correlation exists between land cover and rainfall (Ding, Zhang et al. 2007).

Despite the substantial evidence for association between climatic factors (temperature, rainfall) and the malaria vector biology, the effect of climate change on malaria transmission is still unclear. The general concern is that climate change, especially global warming, may worsen the global malaria burden, although there is limited evidence in support to this hypothesis. Indeed, there is a striking contrast between the global warming over the last century (Hansen, Sato et al. 2006) and the global decline in the global malaria geographical distribution and endemicity (Lafferty 2009, Gething, Smith et al. 2010). The relationship between global warming and malaria epidemiology is complex and conflicting results seen in different studies. While Hay and colleagues conclude that factors other than climate change have driven malaria resurgence in the East African highlands, Siraj and colleagues predict that malaria would spread vertically to the usually cooler highlands as a consequence of warmer temperatures, more suitable for malaria parasite development within the mosquito vector (Hay, Cox et al. 2002, Siraj, Santos-Vega et al. 2014). Droughts often have a detrimental effect on mosquito vector populations and therefore malaria transmission (Mouchet, Faye et al. 1996) but they have also been associated with epidemics, increased morbidity and mortality in the subsequent year. For instance, one of the worst malaria
epidemics in history took place in Russia after drought-breaking rains following a two-year drought (Bruce-Chwatt 1959). While some studies suggest that global warming would exacerbate droughts (Dai 2013), other studies suggest that it is not associated with global drought (Sheffield, Wood et al. 2012) or it is associated with increased rainfall in arid regions, especially the Sahel (Dong and Sutton 2015). Increased rainfall in association with increased temperature is predicted to have variable effects on mosquito densities and vectorial capacity (Yamana and Eltahir 2013). Areas with current intense and stable transmission are less likely to experience a significant increase in malaria incidence compared with highlands and areas at the fringes of endemic zones where malaria transmission has been unstable (Martens, Niessen et al. 1995).

Malaria is also considered a disease of poverty on a global scale. Although the distribution of malaria is primarily determined by ecological factors suitable for the development of the vector, its distribution overlaps the world poorest countries.

Figure 1.3. *P. falciparum* endemicity map. Adapted from (Hay, Guerra et al. 2009).
1.6.2 Microepidemiological determinants of malaria

The heterogeneity of the distribution of malaria is relatively easy to observe at global and regional scales, as shown in figure 1.3, but also exists at finer scales. As malaria elimination becomes a realistic objective in an increasing number of areas, the need of a better characterization of fine-scale space-time patterns of malaria distribution and determinants grows. There is evidence that clustering of malaria infections can be detected down to the level of the smallest spatial unit (homestead for instance) (Bejon, Williams et al. 2014).

A wide range of factors have been associated with increased risk of malaria at the community and household level including temperature, altitude, wind direction, agricultural practices, dams construction and irrigation projects, heritable genetic traits, housing, household income, natural disasters and mass population displacement (Protopopoff, Van Bortel et al. 2009).

Income, literacy, housing and nutrition are somewhat related and low education level, poor housing and poor nutrition are all proxies for low-income. Tusting and colleagues have shown that within the same area, the poorest children have twice the odds of malaria infection than the least poor ones (Tusting, Willey et al. 2013). Although it is not a causal relationship, it suggests that wealth has a protective effect against malaria. There is a growing body of evidence that poor housing is associated with an increased risk of malaria (Sonko, Jaiteh et al. 2014, Snyman, Mwangwa et al. 2015, Tusting, Ippolito et al. 2015). Poor housing is mostly defined as mud-walled and thatched-roofed houses (Snyman, Mwangwa et al. 2015); they are predominant in rural areas and characterized by open eaves that allow traffic of malaria mosquito vectors between the indoor and outdoor space (Njie, Dilger et al. 2009).
In slum habitats, non-brick walled houses have been associated with increased risk of malaria (Sur, von Seidlein et al. 2006).

Forcibly displaced populations, whether it is an internal movement or a refugee movement and whatever the reason is (natural disaster, inter-community violence, and war), are often established in overcrowded temporary settlements with poor housing, poor sanitation, and limited access to standard healthcare, health education and control measures. These local conditions put them at higher risk of malaria, although heterogeneity of exposure to malaria is possible within this vulnerable group. Within a refugee camp, Bayoh and colleagues have observed a spatial clustering of malaria infections near clusters of larval sites (Nabie Bayoh, Akhwale et al. 2011). Outbreaks of malaria are more likely to occur if the displacement is from low to higher endemicity area.

Natural disasters, especially flooding, can also increase the burden of malaria in resident populations by creating local environmental conditions that enhance the development of the local malaria mosquito vectors (Saenz, Bissell et al. 1995).

Bejon and colleagues have shown that malaria infections can cluster at the homestead level (Bejon, Williams et al. 2014) and Fernandez-Grandon and colleagues found a significantly higher correlation of attractiveness of body odour to mosquitoes in identical twins compared with non-identical twins (Fernández-Grandon, Gezan et al. 2015). Genetic factors might then be contributing to fine-scale clustering of malaria infection through increased malaria exposure of the entire household.

Water bodies, that serve as mosquito breeding sites, are critical elements of malaria epidemiology and their proximity to homesteads has been associated with increased risk of
malaria (Zhou, Zhang et al. 2012). Similarly, wind direction has been shown to determine the location of hotspots of homesteads with increased risk of malaria (Midega, Smith et al. 2012). Although there is evidence that some malaria mosquito vectors can adapt to unusual breeding sites (Awolola, Oduola et al. 2007), not all water bodies are suitable breeding sites for malaria mosquito vectors.

1.6.3 Malaria transmission markers, metrics, classification and seasonality

In the study of the epidemiology of malaria, the choice of the exposure/transmission marker matters a lot as they have different sensitivity and specificity at differing transmission levels. Several human-based or vector-based, direct or indirect markers have been used, including clinical, parasitological, serological, biomolecular, haematological and entomological markers, each with its advantages and drawbacks (Tusting, Bousema et al. 2014). Malariometric indices are then derived from these markers and serve mainly to classify malaria endemicity level and measure spontaneous or intervention-driven changes in malaria transmission intensity over time and space. These markers have also been used to study the space-time distribution of malaria in areas of differing endemicities (Ernst, Adoka et al. 2006, Bejon, Williams et al. 2010, Bousema, Drakeley et al. 2010).

The traditional gold standard vector-based metric for malaria transmission is the entomological inoculation rate (EIR) i.e. the number of infectious bites per individual per year. The EIR is itself derived from two other metrics: the human biting rate (number of bites/person/year) and the sporozoite rate (proportion of sporozoite-infected mosquitoes). Although considered as the gold standard, the EIR suffers from the lack of standardization of its methods, logistical and ethical constraints (Tusting, Bousema et al. 2014). In practice, malaria transmission intensity (endemicity) was initially graded using the prevalence of a
clinical manifestation, the spleen enlargement improperly termed “spleen rate”, in children aged 2-9 years as follows: hypoendemic, mesoendemic, hyperendemic and holoendemic for a prevalence of <10%, 11-50%, 51-75% and >75% respectively. It has the advantage of being non-invasive but, lacks specificity (Hay, Smith et al. 2008). The prevalence of asexual parasitaemia detected by light microscopy in children aged 2-9 years (improperly termed “parasite rate”) later supplanted the “spleen rate” as the metric for malaria endemicity classification with the only difference being that only children aged less than one year were considered in the holoendemic level definition (WHO 1950). Parasite rate has since then been widely used (Smith, Guerra et al. 2007, Hay, Guerra et al. 2009). The correspondence between the different classifications is shown in table 1.1.

Table 1.1. Classification of malaria endemicity. The spleen rate and parasite rate are measured in children aged 2-9 years (Adapted from (Reyburn and Drakeley 2005)).

<table>
<thead>
<tr>
<th>Spleen Rate *</th>
<th>Parasite Rate*</th>
<th>EIR</th>
<th>Endemicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10</td>
<td>&lt; 10</td>
<td>&lt; 1</td>
<td>Hypoendemic</td>
</tr>
<tr>
<td>10-50</td>
<td>10-50</td>
<td>1-10</td>
<td>Mesoendemic</td>
</tr>
<tr>
<td>51-75</td>
<td>51-75</td>
<td>11-100</td>
<td>Hyperendemic</td>
</tr>
<tr>
<td>&gt; 75</td>
<td>&gt; 75</td>
<td>&gt; 100</td>
<td>Holoendemic</td>
</tr>
</tbody>
</table>

A different more recent classification is challenging the one based on parasite rate and defines two main levels of transmission intensity: stable and unstable malaria. “Stable malaria transmission” denotes areas where the pattern of transmission is maintained over the years. The transmission may be seasonal or not, malaria inoculation rate is variable but regular, clinical immunity is acquired earlier and malaria epidemics are unlikely. “Unstable malaria transmission” denotes areas where the pattern of malaria transmission in space and time is highly variable. The malaria challenge is irregular and low to moderate, clinical
immunity is acquired later and quickly lost, epidemics and severe malaria are more likely to occur (Carter and Mendis 2002).

Serology plays a growing use in malaria epidemiology, although its use is not new (Otieno, Lelijveld et al. 1971), especially in low transmission conditions (Bruce-Chwatt, Draper et al. 1973, Bruce-Chwatt, Draper et al. 1975). When malaria transmission declines to low levels as it is currently the case in a number of settings worldwide (WHO 2015), entomological metrics become much less reliable because of the scarcity and heterogeneity in vector distribution, and parasitological metrics such as the prevalence of asymptomatic parasitaemia by microscopy are either of low sensitivity or may not be cost-effective for large scale and long term surveillance (molecular methods). Serology reflects past cumulative exposure and may be insensitive to short-term variations in transmission. Antibody responses therefore appear more appropriate to study historical changes in transmission, especially where baseline data is missing. Malaria seroconversion rate (annual rate at which individuals change from seropositive to seronegative), derived from age-specific seroprevalence, has been proposed to estimate variations in malaria transmission (Drakeley, Corran et al. 2005) although it has limitations in estimating recent changes, especially intervention-induced ones. This limitation could be offset by the concomitant direct measure of actual transmission marker (parasitaemia) using molecular methods (PCR) that have higher sensitivity compared with microscopy (Bejon, Andrews et al. 2006).

The basic reproductive number ($R_0$), defined as the number of secondary cases that can stem from an index infected individual introduced in a completely susceptible population (Dietz 1993), would also make an ideal transmission metric. However, $R_0$ is not directly measurable and requires statistical modelling based on the human and entomological parameters described above (Smith, McKenzie et al. 2007).
Other less widely used host-based metrics include febrile malaria incidence, slide positivity rate or malaria positive fraction, proportion of fevers with *P. falciparum* malaria, seroconversion rate, force of infection, multiplicity of infection (Bejon, Williams et al. 2014, Tusting, Bousema et al. 2014).

Malaria transmission is often described as seasonal, but how seasonality is defined is generally unclear. It is believed that there is some degree of seasonality in most endemic settings as peaks of a given metric is often reported (Roca-Feltrer, Schellenberg et al. 2009). In a study conducted in a high transmission setting in Tanzania, Smith and colleagues did not find any seasonal pattern in fevers, parasite prevalence or density in children and adults; however, they reported a marked seasonal variation in the EIR (Smith, Charlwood et al. 1993). Roca-Feltrer and colleagues have proposed a method that discriminates well two categories: “marked seasonality” when 75% or more febrile episodes occur in six months or less of the year and “no marked seasonality” if the 75% episodes occur in more than six months (Roca-Feltrer, Schellenberg et al. 2009).

In a global context of declining malaria transmission, the study of the fine scale distribution of malaria is of increasing importance as targeting control interventions to restricted areas with the highest risk is expected to be more effective than unfocused control, benefiting individuals in the foci and the surrounding community. The choice of the marker to identify and map these transmission foci / hotspots should be studied since hotspots need to be identified accurately for targeted control to be effective.
1.6.4 The use of serology for malaria risk mapping

The use of serological markers to map malaria transmission is relatively recent. Traditional markers of transmission, including parasite prevalence and entomological inoculation rate have been used to map the risk of malaria often at a global scale (Kleinschmidt, Omumbo et al. 2001, Gething, Patil et al. 2011, Gething, Elyazar et al. 2012). Other methods using remote sensing (satellite-based measure of vegetation index, land surface and air temperature, altitude…) have also been used for large scale mapping of malaria transmission (Omumbo, Hay et al. 2002, Dambach, Sié et al. 2009). The interest in serological markers has grown in a context where a number of countries have progressed to pre-elimination stage unveiling the issue of controlling malaria in residual transmission foci whose identification is challenging. Drakeley and colleagues have compared seroprevalence with others malaria transmission markers and found that seroprevalence reflected better spatial heterogeneity of transmission than the alternative markers (parasite rate, haemoglobin concentration) (Drakeley, Corran et al. 2005). Teun and colleagues found that age-specific sero-conversion rates (annual rate at which individuals change from seropositive to sero-negative) well correlated with malaria incidence and were better predictors of clinical hotspots than entomological markers or seroprevalence (Bousema, Drakeley et al. 2010). In their study, Bejon and colleagues found that antibody titres predicted clinical and parasitological hotspots better than seroprevalence or sero-conversion rates (Bejon, Williams et al. 2010). Serological markers have then been used in a number of studies to map the risk of malaria (Noor, Mohamed et al. 2011, Kobayashi, Chishimba et al. 2012, Ashton, Kefyalew et al. 2015, Rosas-Aguirre, Speybroeck et al. 2015, Lynch, Cook et al. 2016) using different methods.
1.7 Immunity to malaria

Bruce Chwatt, as cited in (Doolan, Dobaño et al. 2009), defined immunity to malaria as “the state of resistance to the infection brought about by all those processes which are involved in destroying the plasmodia or by limiting their multiplication. Natural (innate) immunity to malaria is an inherent property of the host, a refractory state or an immediate inhibitory response to the introduction of the parasite, not dependent on any previous infection with it. Acquired immunity may be either active or passive. Active (acquired) immunity is an enhancement of the defence mechanism of the host as a result of a previous encounter with the pathogen (or parts thereof). Passive (acquired) immunity is conferred by the prenatal or postnatal transfer of protective substances from mother to child or by the injection of such substances.

1.7.1 Innate immunity to malaria

1.7.1.1 The organization and function of the innate immune system

The innate immune system can be divided into cells (phagocytes and lymphocytes) and humoral factors (complement and cytokines). The cells involved in the innate immune system are ubiquitous and are found either patrolling in peripheral blood, or stationed in lymphoid organs as well as peripheral non-lymphoid tissues (Ferlazzo and Münz 2004). Monocytes which can differentiate into macrophages and dendritic cells are phagocytes which express pattern recognition receptors (TLR2, TLR4, TLR9 for malaria parasite) to recognize pathogen-associated molecules (e.g. haemozoin or GPI for malaria). In addition to recognition, uptake and destruction (lysis) of parasites, a major role of these phagocytes is to present the pathogen antigens to the other effectors of the innate and adaptive immune systems. Professional antigen-presenting cells include dendritic cells, macrophages and...
neutrophils. Pathogen antigens bound to TLR9 are further bound to MHC class II molecule before they are presented on the surface of the APC to the T and B cells, activating the adaptive response. The third important function of these APC is to enhance the recruitment of cells involved in the innate response. The tissue-resident macrophages in the skin, the lymph nodes, the liver and the spleen are called Langerhans cells, histiocytes, Kupffer cells and red pulp macrophages respectively.

There is evidence for an association between interferon gamma (IFN-γ) production and resistance to malaria and stimulation of nitric oxide production (directly involved in parasite killing) has been advanced as the effector mechanism. Though IFN-γ production is not specific to innate immunity cells (King and Lamb 2015), its production in the early stages of the infection has been attributed to natural killer cells (lymphocytes of the innate immune system) (Artavanis-Tsakonas and Riley 2002). NK cells are also able to sense directly, stick to and destroy infected erythrocytes by direct cytotoxicity through the release of perforin, granzymes A, B and M.

The complement system is a family of over 30 soluble or membrane-bound proteins synthetized primarily in the liver (hepatocytes) and secondarily by various extra-hepatic tissues / cells (macrophages, monocytes, neutrophils, lymphocytes, platelets, epithelial & endothelial cells, adipocytes, fibroblastes, glial cells, glomerular mesangial, epithelial and endothelial cells) (Marsh, Zhou et al. 2001). They circulate in the blood stream in precursor forms that are activated through three possible pathways (classical, alternative, lectin) by molecules present at the surface of pathogen surfaces or antigen-antibody complexes (Sarma and Ward 2011). The complement system is involved in opsonic phagocytosis and direct killing of pathogens (through membrane attack complexes) (Merle, Noe et al. 2015). However, a number of studies suggest that excessive activation of the complement system...
may be associated with severe malaria (Silver, Higgins et al. 2010, Biryukov and Stoute 2014, Berg, Otterdal et al. 2015).

1.7.1.2 The major inherited traits that protect against malaria

Most of the inherited protective biological traits are actually genetic characteristics that occur at high frequencies in high-burden malaria endemic areas. This forms the basis of the “malaria hypothesis” which stipulates that these genetic anomalies result from the strong natural evolutionary selection pressure for genes (even deleterious in some circumstances) that confer fitness to malaria mortality (Tzoneva, Bulanov et al. 1980, Hill, Flint et al. 1987, Piel, Patil et al. 2010).

Sickle cell haemoglobin

Sickle haemoglobin is widespread across continents, but the burden is highest in equatorial Africa that is home to around 85% of cases of sickle cell disease (Modell and Darlison 2008). Piel and colleagues have shown that areas with high frequencies of sickle cell trait approximately overlap areas with high burden of *P. falciparum* malaria, especially in sub-Saharan Africa, in keeping with the “Malaria hypothesis” (Piel, Patil et al. 2010). Sickle haemoglobin is the result of a single point mutation of the gene coding for the beta-chain of haemoglobin where the glutamate at position 6 is replaced by a valine (Ingram 1958). It has been shown that the mutation occurred independently in four areas in Africa (Senegal, Benin, Central African Republic, Cameroun) and one area in Asia (Saudi Arabia/India) (Pagnier, Mears et al. 1984, Kulozik, Wainscoat et al. 1986, Lapoumeroulie, Dunda et al. 1992) but another point of view assumes one African origin and one Asian origin (Piel, Patil et al. 2010). Heterozygous individuals are said to be sickle cell trait carriers and homozygous
individuals are said to have sickle cell disease. Sickle cell trait is associated with reduced risk of severe and mild *P. falciparum* malaria (Allison 1954, Williams, Mwangi et al. 2005, Taylor, Parobek et al. 2012) but this protection comes at the cost of an early high mortality rate for homozygous individuals (Makani, Cox et al. 2011). The existence of a protective effect against asymptomatic infection is still controversial (Williams, Mwangi et al. 2005, Billo, Johnson et al. 2012). The mechanism of the protective effect of sickle cell trait is not clearly understood and a number of biochemical and immunological hypotheses based on theory, *in vitro* or mouse models have been proposed (Gong, Parikh et al. 2013). Increased sickling (Luzzatto, Nwachuku-Jarrett et al. 1970), higher levels of antibodies to PfEMP1 (Marsh, Otoo et al. 1989, Cabrera, Cot et al. 2005, Verra, Simpore et al. 2007) and enhanced phagocytosis of infected red blood cells by monocytes (Ayi, Turrini et al. 2004) have been reported. Tolerance to infection mediated by an increased expression of heme oxygenase-1 (Ferreira, Marguti et al. 2011), impaired rosette formation (possibly related to increased sickling and/or reduced expression of surface adhesion proteins) (Carlson, Nash et al. 1994) and impaired cytoadherence of infected erythrocytes (possibly related to altered expression of PfEMP1) (Cholera, Brittain et al. 2008) have been hypothesized as mechanisms of protection against severe malaria.

**Haemoglobin C**

Haemoglobin C is a red blood cell polymorphism that is geographically predominant in West Africa, where it coexists at high frequencies with sickle haemoglobin (Piel, Howes et al. 2013). In haemoglobin C, the glutamate at position 6 of the beta-chain is replaced by a lysine1 (Itano and Neel 1950). The mutation is believed to have initially occurred in West Africa (Boehm, Dowling et al. 1985) although an isolated different haplotype has been reported in Thailand (Sanchaisuriya, Fucharoen et al. 2001). Compared to sickle cell disease,
the homozygous form (CC) is less pathogenic and chronic haemolytic anaemia occurs to a lesser extent than in sickle cell disease when it is combined with haemoglobin S (SC) (Ballas, Lewis et al. 1982). Heterozygous (AC) and homozygous (CC) forms of haemoglobin C have been associated with reduced risk of severe *P. falciparum* malaria (Agarwal, Guindo et al. 2000, Modiano, Luoni et al. 2001, Mockenhaupt, Ehrhardt et al. 2004) but a protective effect against asymptomatic and uncomplicated malaria has not been found (Taylor, Parobek et al. 2012). An hypothesized mechanism is an impairment of cytoadherence and sequestration through reduced PfEMP1 expression and abnormal knobs display (Fairhurst, Bess et al. 2012).

**Haemoglobin E**

Haemoglobin E is predominant in South-East Asia with reported prevalence exceeding 50% at the borders of Thailand, Laos and Cambodia, in what has been termed the “haemoglobin E triangle” (Fucharoen and Winichagoon 2011). It results from a single point mutation of the gene coding for the beta-chain of haemoglobin where the glutamate at position 26 is replaced by a lysine. There is evidence supporting independent origins of this mutation in Asia and Europe (Antonarakis, Orkin et al. 1982, Kazazian, Waber et al. 1984). The phenotypes AE, EE, SE are associated with mild disease. Severe manifestations are observed in Individuals carrying the association Hb E-beta-thalassemia (Vichinsky 2007). Compared to haemoglobin S and C, fewer clinical studies have been conducted on haemoglobin E and no convincing evidence of a protective effect of haemoglobin E has been established yet. A retrospective study in Thailand found that haemoglobin E trait may protect against severe malaria, but recent studies yielded opposite conclusions (Naka, Ohashi et al. 2008, Taylor, Parobek et al. 2012).
**Thalassemias**

Thalassemias are inherited haemoglobin chain production disorders which result in reduced production of the alpha globin chain (alpha-thalassemia) or the beta globin chain (beta-thalassemia) of haemoglobin (Harteveld and Higgs 2010). Thalassemias are spread worldwide. They have been described in Africa, the Mediterranean region, Asia, north and west America and are now considered a major public health problem (Vichinsky 2005, Galanello and Origa 2010).

Alpha-thalassemia results from the deletion of a variable number of the genes (HBA1, HBA2) coding for the alpha globin chain. The severity of manifestations of alpha-thalassemia is variable, increasing with the number of deleted alpha globin genes (Galanello and Cao 2011). α+ thalassemia is the condition in which one linked pair of the alpha globin genes is deleted and α0 thalassemia the condition in which both the linked pairs are deleted. The latter condition is lethal. Evidence of the association of α+ thalassemia with reduced risk of severe malaria has been firmly established (Williams, Wambua et al. 2005).

Contrary to alpha-thalassemia, deletions of the beta globin gene are relatively rare. However, more than 200 mutations of the beta globin gene have been described (Galanello and Origa 2010). It has been suggested that beta-thalassemia trait is associated with resistance to malaria infection (Willcox, Bjorkman et al. 1983) but this association has been much less studied compared to the protective effect of alpha-thalassemia and remains to be confirmed.

Thalassemias are often inherited in combination with other haemoglobin variants that confer protection to clinical malaria, but the resulting associations are not necessary have additive
or multiplicative protective effects. Indeed, evidence of possible negative epistasis has been reported between alpha-thalassemia and haemoglobin S (Williams, Mwangi et al. 2005).

**The red blood cell enzyme disorders**

Glucose-6-phosphate-deshydrogenase (G6PD) is a cytosolic enzyme of the pentose phosphate pathway which produces nicotinamide adenine dinucleotide phosphate (NADPH), an important molecule in the mechanism of protection of RBCs against oxidative damage. Mutations of the X-linked gene coding for G6PD, most of which are single point substitutions, translate into deficits in production of the enzyme, resulting in the condition termed G6PD deficiency. The G6PD gene is highly polymorphic with several variants, of which only few encountered variants have been associated with severe clinical symptoms (Minucci, Moradkhani et al. 2012, Monteiro, Franca et al. 2014). In general, individuals harbouring the trait remain asymptomatic until exposed to dietary, pharmacologic, infectious or metabolic factors triggering haemolysis with possible life-threatening complications (Eziefula, Pett et al. 2014, Monteiro, Franca et al. 2014). G6PD is the most common enzymopathy in humans, widespread worldwide with a global prevalence estimate of 8% and the highest prevalences reported in sub-Saharan Africa (Nkhoma, Poole et al. 2009, Howes, Piel et al. 2012). G6PD A- common in Africa and America, and G6PD Mediterranean common in the Mediterranean area, Middle East and Asia, are the most prevalent allelic variants, but both alleles coexist in several populations (Howes, Piel et al. 2012, Howes, Dewi et al. 2013, Monteiro, Val et al. 2014). There is compelling evidence of the protective effects of G6PD deficiency against severe malaria (Malaria Genomic Epidemiology Network 2014), but reports have been conflicting as regards to the heterozygous females carrying the trait. Guindo et al. provided some evidence that it protects hemizygous males from *P. falciparum* severe malaria, but not heterozygous females
(Guindo, Fairhurst et al. 2007) while Manjourano et al. later provided evidence of protection against severe malaria in heterozygous females harbouring the trait in a larger study (Manjurano, Sepulveda et al. 2015). Leslie et reported protection against \textit{P. vivax} infection in hemizygous males and heterozygous or homozygous females (Leslie, Briceño et al. 2010). An increased susceptibility to phagocytosis at an early stage of parasite development in infected-RBCs may be the mechanism of protection (Cappadoro, Giribaldi et al. 1998).

\textit{Pyruvate kinase deficiency}

Pyruvate kinase is a critical enzyme of glycolysis. Its deficiency is due to mutations of the pyruvate kinase gene. Several studies reporting its prevalence in African, European, Asian, American populations support its worldwide distribution (Baronciani, Magalhaes et al. 1995, Beutler and Gelbart 2000, Machado, Manco et al. 2012, Warang, Kedar et al. 2013). PK deficiency is emerging as a protective factor that fits to the “malaria hypothesis”. Indeed, a study based on mouse models has reported a protective effect against murine malaria conferred by PK deficiency (Mi-N-Oo, Fortin et al. 2003). A human \textit{in vitro} study later provided support for this hypothesis by providing evidence of resistance of PK deficient-erythrocytes to \textit{P. falciparum} infection. The actual protective mechanism is currently unknown (Durand and Coetzer 2008).

\textit{Southeast Asian ovalocytosis}

Southeast Asian ovalocytosis (SAO) is a RBC membrane disorder resulting from a deletion on the gene coding for the band 3 protein involved in trans-membrane anion exchange (Jarolim, Palek et al. 1991). It is believed to have originated from Southeast Asia around 10000 years ago (Paquette, Harahap et al.). SAO seems to be confined to Southeast Asia and
the Southwest Pacific where it remains at relatively low frequencies. Its presence in the Malgashi population has been interpreted as a vestige of the original occupation of the island by people of Indonesian origin (Rabe, Jambou et al. 2002). Heterozygous SAO has been reported to cause only mild disease, but homozygous forms are presumed to be lethal and recently, a case of homozygous SAO was reported with severe clinical manifestations (Liu, Jarolim et al. 1994, Picard, Proust et al. 2014). SAO has been compellingly associated with protection against severe *P. falciparum* malaria (Foo, Rekhraj et al. 1992, Allen, O'Donnell et al. 1999, Rosanas-Urgell, Lin et al. 2012). In vitro studies have shown that RBC invasion by merozoites is impaired (Kidson, Lamont et al. 1981) but whether this is the protective mechanism is still unclear.

### 1.7.1.3 Other putative protective biological parameters

Human leucocyte antigens (HLA class I, II and III) are antigens expressed on the surface of all the body cells. They are involved in the initiation of immune responses by activating cytotoxic T lymphocytes (HLA class I) or Helper T lymphocytes (HLA class II) through antigen presentation. Alleles in HLA class I and class II that are prevalent in West African populations have been associated with protection against severe malaria (Hill, Allsopp et al. 1991, Hill, Allsopp et al. 1992) and the global distribution patterns of HLA alleles, in relation to the distribution of populations at risk of malaria, is highly suggestive of a positive selective pressure of malaria (Garamszegi 2014).

The predominance of blood group O observed in areas of high malaria burden has made it a candidate protective factor positively selected by *P. falciparum* malaria pressure. Though different studies have yielded conflicting results about the association of blood group O and the risk of uncomplicated malaria (Uneke 2007), there is now conclusive evidence that blood
group O confers resistance to severe malaria through reduced rosetting (Rowe, Handel et al. 2007). In pregnancy, blood group O has been associated with reduced risk of placental malaria in primiparous, but not in multiparous (Bedu-Addo, Gai et al. 2014) while other previous studies found no association (Boel, Rijken et al. 2012). Foetal haemoglobin (HbF) in young infants has been suggested as a putative protective mechanism, though convincing evidence from field observational studies is scarce (Mmbando, Mgaya et al. 2015). It has been compellingly shown that *P. falciparum* invade and grow normally in HbF-containing cells and suggested that foetal haemoglobin mediates protection by impairing cytoadherence of infected cord blood erythrocytes through reduction of expression and alteration of display of PfEMP1 at the surface these cells (Amaratunga, Lopera-Mesa et al. 2011, Sauerzopf, Honkpehedji et al. 2014).

1.7.2 Naturally acquired immunity to malaria

As compared with other infectious diseases, immunity to malaria is a very complex phenomenon and there is no unique definition as levels of resistance may vary in respect of the symptomatology of the disease (asymptomatic infection, mild and severe malaria) (Langhorne, Ndungu et al. 2008). Natural immunity to malaria has been classified into two main types: innate resistance to malaria and acquired immunity to malaria (Gilles 2002).

1.7.2.1 Population-level dynamics of naturally acquired immunity to malaria

Naturally acquired immunity (NAI) to malaria can be defined as the resistance to malaria that develops in response to repeated parasite challenge. The main interest in studying immunity is to gather the knowledge that would guide the design of a vaccine that would protect better than natural immunity.
Naturally acquired immunity to malaria is age-dependent and builds gradually as it is parasite species and strain-specific and theoretically requires repeated exposure to the different circulating strains of a parasite in a given setting. There is epidemiological evidence that immunity to severe malaria develops first quickly after relatively few infective bites (Gupta, Snow et al. 1999) although a recent study suggested that it could be being acquired more gradually than previously thought (Griffin, Hollingsworth et al. 2015). The acquisition of this immunity to severe malaria is followed by immunity to mild malaria and then anti-parasite immunity (Langhorne, Ndungu et al. 2008) as illustrated in figure 1.4. The final “equilibrium state” where clinical manifestations are absent in presence of a chronic low parasitaemia is called “premunition” (Perignon and Druihe 1994). To date, sterile immunity (resistance to infection) has been achieved in humans only under experimental conditions using irradiated whole sporozoites (Hoffman, Goh et al. 2002) and no observed case of sterile NAI has been reported.

Figure 1.4. Population indices of immunity to malaria. Adapted from (Marsh and Kinyanjui 2006).
The age pattern of the acquisition of natural immunity is also strongly influenced by the transmission intensity. In high transmission settings, the risk of life-threatening malaria (mainly malaria with impaired consciousness, severe respiratory distress and/or severe anaemia (Marsh, Forster et al. 1995)) increases from around four months of age up to four years before it declines sharply after five years of age (Gilles 2002). In areas of low transmission intensity, the risk of life-threatening malaria also declines with age. However, immunity to severe malaria establishes much later as even adults become at risk and though the predominant clinical form differs (impaired consciousness in adults vs severe anaemia in children) (Luxemburger, Ricci et al. 1997). This underlines a potential consequence of effective malaria control interventions that would bring transmission to such low levels that immunity would take longer to establish, increasing the upper limit of the age range of mild and life-threatening malaria up to adults (Ghani, Sutherland et al. 2009).

An important characteristic of NAI is memory. Anti-malaria antibodies have a short half-lives (a few days to a few months) that has been attributed to short-lived (a few days) plasma cell production (Kinyanjui, Conway et al. 2007). However, antibody levels can be maintained by long-lived (few months) plasma cell production (Slifka, Antia et al. 1998) or re-stimulation of memory B cells by chronic infections (Gatto, Martin et al. 2007). There is now evidence that even without re-exposure to infective bites, malaria memory B cells can be maintained up to sixteen years (Ndungu, Lundblom et al. 2013) arguing against the idea of a loss of memory after long periods of non-exposure. Akpogheneta and colleagues found that, in the absence of persistent infection, a rapid decline of antibodies occurs in the youngest children aged less than years (attributed to the predominance of short-lived plasma cells) compared with older children aged more than 5 years (attributed to the predominance of long-lived plasma cells) (Akpogheneta, Duah et al. 2008). However, it has also been
assumed that both short-lived and long-lived components coexist and a bi-phasic decline is fitted to the serology data revealing an initial rapid decay followed by slower and longer decay (White, Griffin et al. 2014, White, Verity et al. 2015). Average catabolic half-life of human antibody subclasses (1, 2 and 4) has been estimated at 21 days (Morell, Terry et al. 1970). The half-life of anti-malaria antibodies have been estimated using different models and children populations of differing age groups with variable results, but most estimates lie between to 4-31 days, although older children tend to have longer antibody half-lives (Kinyanjui, Conway et al. 2007, White, Griffin et al. 2014). In a vaccine trial where a biphasic decay has been assumed, the half-lives of antibodies have been estimated to 45 days and 591 days for the short-lived and long-lived components respectively (White, Verity et al. 2015).

1.7.2.2 Mechanisms and measures of naturally acquired immunity to malaria

T cells and plasma cells (antibody producing cells differentiated from B cells) constitute the effector cells of the adaptive components of the natural immunity to malaria (CA, P et al. 2001).

Circulating antibodies bind to proteins expressed on the surface of the exposed circulating invasive parasites (sporozoites and merozoites) or to proteins expressed on the surface of infected cells (hepatocytes and erythrocytes) and mediate protection through many ways.

They can then inhibit the host cell entry by the parasite by binding to the parasite surface proteins involved in the invasion process and therefore prevent them from binding to their ligands displayed on the surface of the host cells: this mechanism is called neutralization. It has been recently shown that antibodies interact with an innate humoral factor, complement,
to prevent invasion. Indeed, Boyle and colleagues have demonstrated that most antibodies were non-inhibitory without complement fixation on merozoites and that complement fixation was strongly positively associated with age and clinical protection; this mechanism is called antibody-mediated complement dependent invasion-inhibitory activity (Boyle, Reiling et al. 2015).

By sticking to the surface antigens of infected erythrocytes or merozoites, they mark them for phagocytosis by antigens presenting cells (macrophages and monocytes): this mechanism is called opsonic phagocytosis (Chua, Brown et al. 2013, Osier, Feng et al. 2014). Other studies suggested that opsonization of infected erythrocytes and merozoites promote phagocytosis by neutrophils (Celada, Cruchaud et al. 1983, Pleass, Ogun et al. 2003).

Since both cyto-adherence (binding of infected erythrocytes to endothelial cells) and rosetting (binding of infected erythrocytes to other infected or uninfected erythrocytes) are mediated by a protein expressed on the surface of P. falciparum infected erythrocytes (PfEMP1), antibodies can also prevent or limit the occurrence of these phenomena.

The interaction of opsonized merozoites and monocytes receptors to antibodies (Fc receptors) trigger the release of soluble factors that can inhibit parasite growth in infected erythrocytes: this mechanism is called antibody-dependent cellular inhibition (ADCI) (Khusmith and Druilhe 1983). It has been also shown that opsonized merozoites can promote the release of oxygen radicals by peripheral blood monocytes and neutrophils (Kharazmi, Jepsen et al. 1987), and that oxygen radical generation was inversely correlated with parasite clearance time (Greve, Lehman et al. 1999).
The findings of Pombo and colleagues in a challenge study, where malaria-naïve individuals were challenged with subclinical infections and treated, are suggestive of the existence of an antibody-independent protective role of T cells in (Pombo, Lawrence et al. 2002). However, the effector mechanisms in T cell responses to malaria are less clear than those of antibodies.

Some experimental studies in mouse models suggested that antibodies, T-cells (CD8+ and CD4+) have independent anti-parasitic effects against pre-erythrocytic stages and thus may all contribute to pre-erythrocytic immunity (Rodrigues, Nussenzweig et al. 1993) while other studies (mice, Rhesus monkeys) suggested that pre-erythrocytic immunity is mediated primarily by CD8+ T-cell activity (Schofield, Villaquiran et al. 1987, Weiss, Sedegah et al. 1988, Weiss and Jiang 2012). Evidence for associations between antibodies to pre-erythrocytic stages and protection against malaria have been found in human observational studies (John, Moormann et al. 2005, John, Tande et al. 2008). Interventional (CSP-based vaccine) and experimental studies (radiation-attenuated sporozoites vaccine) in humans have shown that CD8+ responses (Ewer, O’Hara et al. 2013) and CD4+ T-cell responses (Reece, Pinder et al. 2004) play a major role in pre-erythrocytic immunity. T cell-mediated immunity to malaria has been attributed to interferon gamma production by CD4+, CD8+ T cells (Sun, Schwenk et al. 2003) as well as the promotion of inducible nitric oxide synthase production by peripheral blood mononuclear cell (Pombo, Lawrence et al. 2002). Sterile protective immunity induced by immunization with radiation-attenuated sporozoites has yet to be achieved outside experimental conditions (Hoffman, Goh et al. 2002).

Protective immunity has been practically difficult to measure and define, and a number of metrics have been proposed. These metrics are based on antibody responses only (seropositivity, protective thresholds) or antibody-dependent cellular responses (growth inhibition assay, antibody-dependent cellular inhibition, antibody-dependent respiratory
burst, opsonic phagocytosis) but there is increasing advocacy to use functional assays (Osier, Fegan et al. 2008, Joos, Marrama et al. 2010, Duncan, Hill et al. 2012, Murungi, Kamuyu et al. 2013, Osier, Mackinnon et al. 2014, Tiendrebeogo, Adu et al. 2015). To date, standardized and validated correlates of protection against malaria that meet agreement for generalized use have yet to be established.

1.7.2.3 Immune responses to malaria: marker of immunity or marker of exposure?

Acquired (adaptive) immune responses develop in response to exposure of antigen-presenting cells to the parasite antigens. In endemic settings, all residents are theoretically exposed to malaria, but this exposure is not constant over space and time. Malaria transmission is often seasonal and the heterogeneity of exposure to malaria has been observed at global, continental, regional up to the finest geographical scale (homestead). Since immune responses and exposure to infection are both associated with the risk of malaria, in any study of the association between immune responses and risk of malaria, confounding by heterogeneity of exposure need to be accounted for to avoid biased results (Bousema, Kreuels et al. 2011).

Various approaches have been suggested. Randomization is the usual approach to control confounding factors in clinical trials, but even trial estimates of vaccine efficacy may be biased by heterogeneity of exposure (White, Griffin et al. 2010). A simpler approach is to define zones of differing exposure intensity for a given area and adjust accordingly. These zones may be defined based on altitude (Drakeley, Carneiro et al. 2005), distance to mosquito breeding sites (Clarke, Bogh et al. 2002), wind direction (Midega, Smith et al. 2012), distance to forest fringe (Kreuels, Kobbe et al. 2008). Administrative divisions are used with the limit that they are arbitrary, especially when the zones are contiguous. A
different approach consists of the use of baseline serological markers with the limit that antibody titres may not reflect recent / current exposure to malaria (Osier, Fegan et al. 2008). Bejon and colleagues have proposed an approach in which uninfected (assumed unexposed) individuals are removed from the analysis, leaving only asymptotically infected individuals and individuals with febrile malaria in the analysis (Bejon, Warimwe et al. 2009). The findings of Greenhouse and colleagues suggest that restricting the analysis to the periods when individuals are parasitaemic reveals the protective effect of antibodies (Greenhouse, Ho et al. 2011). More recently, Olotu and colleagues have developed a quantitative spatial approach with the advantage of an estimation of exposure at the individual level (Olotu, Fegan et al. 2012). There is currently no consensus or validated method for adjusting for heterogeneity of exposure to malaria.

1.7.2.4 Plasmodium falciparum merozoite antigens associated with immunity

The genome of Plasmodium falciparum (clone 3D7) made of 14 chromosomes harbouring about 5300 genes encoding an equivalent number of proteins. At least 1.3% of the genes are known to be involved in interaction with host cells and 3.9% in immune evasion. Other proteins are involved in “housekeeping activities” (transport activities, DNA replication and repair…etc.) but the exact function of most proteins remains unknown (Gardner, Hall et al. 2002). Any protein displayed on the surface of the parasite, especially free invasive forms (sporozoites and merozoites), is a potential target for the immune system. We describe below some of the most studied Plasmodium falciparum antigens that have been or are being tested as candidate vaccines.
The apical membrane antigen (AMA1)

*Plasmodium falciparum* AMA1 is an 83 kDA micronemal type I transmembrane protein (Peterson, Marshall et al. 1989) encoded by a single-locus gene (PF3D7_1133400) located on chromosome 11 [http://www.genedb.org](http://www.genedb.org). This protein is present in the invasive forms of malaria parasites (sporozoite and merozoite), synthetized in late schizont stage during asexual multiplication and has homologues in all *Plasmodium* species (Triglia, Healer et al. 2000). The 83 kDA precursor protein is then processed into a 66 kDA protein in the micronemes before it translocates to the parasite surface just prior to the host cell invasion process where there it undergoes further processing (Howell, Withers-Martinez et al. 2001). AMA1 has been reported as involved in the parasite reorientation following initial attachment, the formation of the parasite-host cell tight-junction and invasion in cooperation with another parasite protein called rhoptry neck protein 2 (RON2) (Mitchell, Thomas et al. 2004, Srinivasan, Beatty et al. 2011, Yap, Azevedo et al. 2014). In contrast, other studies suggest that AMA1 may not be essential for host cell invasion (Bargieri, Andenmatten et al. 2013). Invasion assays have shown that antibodies to AMA1 can inhibit erythrocyte invasion and impaired proteolytic processing or binding to AMA1 to prevent interaction with its partner proteins have been suggested as possible effector mechanisms (Dutta, Haynes et al. 2005, Collins, Withers-Martinez et al. 2009). In humans, antibodies to AMA1 have been associated with protection from clinical malaria in some sero-epidemiological studies (Polley, Mwangi et al. 2004, Osier, Fegan et al. 2008, Greenhouse, Ho et al. 2011). While immunization in non-human primates (Stowers, Kennedy et al. 2002, Dutta, Sullivan et al. 2009) and phase 1 clinical trials (Dicko, Diemert et al. 2007, Dicko, Sagara et al. 2008) showed encouraging results, the results of phase 2 clinical trials of monovalent vaccine candidates were rather disappointing (Sagara, Dicko et al. 2009, Thera, Doumbo et al. 2011).
**The merozoite surface protein (MSP3)**

*Plasmodium falciparum* MSP3 is an abundant 40 kDa soluble protein (no transmembrane domain or GPI anchor) in the form of oligomeric (dimers and tetramers) aggregates (Imam, Singh et al. 2014) encoded by a single-locus gene (PF3D7_1035400) on *Plasmodium falciparum* chromosome 10 ([http://www.genedb.org](http://www.genedb.org)). It appears as a 5 to 15 μm long filamentous structure attached to the merozoite surface (Imam, Singh et al. 2014) possibly through protein-protein interaction (Trucco, Fernandez-Reyes et al. 2001). Though its exact functional role is poorly understood, MSP3 is believed to play a role in host cell invasion and parasite protection. Indeed, MSP3 is shed with other surface proteins at the tight junction during the host cell invasion and this suggests that MSP3 plays a role in the initial contact and invasion events (Boyle, Langer et al. 2014). MSP3 has also heme-binding properties and this is suggestive of a possible role in the protection of the parasite from potential heme-related damages (Imam, Singh et al. 2014). MSP3 was identified as an antigen capable of eliciting protective immune response using the antibody-dependent cellular inhibition (ADCI) assay in which the parasite growth inhibition is mediated by monocytes in cooperation with antibodies (Oeuvray, Bouharoun-Tayoun et al. 1994). This protective response elicited by MSP3 has been evidenced in a number of field studies (Soe, Theisen et al. 2004, Nebie, Tiono et al. 2008, Osier, Fegan et al. 2008, Greenhouse, Ho et al. 2011) and Fowkes and colleagues have reported the lowest pooled relative risk (RR=0.46) for antibodies to MSP3 among other anti-merozoite antibodies (Fowkes, Richards et al. 2010). Immunization in non-human primates yielded evidence of protective effect (Hisaeda, Saul et al. 2002) and after satisfactory phase 1 clinical trials (Lusingu, Gesase et al. 2009, Sirima, Tiono et al. 2009) the testing of MSP3 progressed to a phase 2 trial in Mali.
Unfortunately to date the results are not published.

**The merozoite surface protein 2 (MSP2)**

*Plasmodium falciparum* MSP2 is an approximately 30 kDA GPI-anchored merozoite surface protein (Gerold, Schofield et al. 1996) encoded by a single-locus gene (PF3D7_0206800) on the *Plasmodium falciparum* chromosome 2 ([http://www.genedb.org](http://www.genedb.org)). MSP2 has no ordered three-dimensional structure (intrinsically unstructured protein); it forms amyloid-like fibrils in physiological conditions and is thus believed to be a component of the fibrillar coat on the surface of the merozoite (Adda, Murphy et al. 2009). The exact role of MSP2 is unclear. Failure of genetic deletion of MSP2 suggest that the protein plays a critical role in parasite biology (Sanders, Kats et al. 2006). MSP2 is not shed during host cell invasion by the merozoite; it is internalized and immediately degraded (within 10 minutes) upon completion of the invasion, suggesting a function during the invasion process (Boyle, Langer et al. 2014). As for other *Plasmodium falciparum* antigens, antigens to MSP2 have been associated with protection against malaria in some (Metzger, Okenu et al. 2003, Polley, Conway et al. 2006, Osier, Fegan et al. 2008, Reddy, Anders et al. 2012), but not all studies. MSP2 was initially tested in humans as a multicomponent vaccine (Combination B: MSP1, MSP2-3D7 and RESA) (Genton, Al-Yaman et al. 2000, Genton, Betuela et al. 2002, Genton, Al-Yaman et al. 2003). The allele-specific component of the humoral response to MSP2 induced by this vaccine (Flück, Smith et al. 2004) was not clearly seen in sero-epidemiological studies (Osier, Murungi et al. 2010). However, a subsequent clinical trial of a vaccine made of recombinant forms of the two allele families (3D7 and FC27) yielded good immunogenicity but poor tolerance, raising safety questions and the trial was terminated early (McCarthy, Marjason et al. 2011).
The merozoite surface protein 1 (MSP1)

*Plasmodium falciparum* MSP1 is a GPI-anchored merozoite surface protein encoded by a single-locus gene (PF3D7_0930300) on *Plasmodium falciparum* chromosome 9 ([http://www.genedb.org](http://www.genedb.org)). It is the most abundant and the first merozoite surface protein to be identified. After its synthesis during schizogony (liver and blood phases), the 195 kDa precursor MSP1 traffics to the parasite plasma membrane and its processing starts in late schizogony just before merozoites are released in the blood stream (Das, Hertrich et al. 2015). This initial processing yields four fragments of varying molecular weight (MSP1-83, MSP1-30, MSP1-38 and MSP1-42 named after their respective molecular weights 83, 30, 38 and 42 kDa) that form a non-covalent complex at the parasite surface (McBride and Heidrich 1987). MSP1-42 is further cleaved into a 19 and 33 kDa fragments just prior to the host cell invasion and MSP1-33 is shed from the surface in the form of a protein complex with other proteins and the fragments from the primary cleavage; MSP1-19, the only fragment that remains on the surface of the merozoite during the invasion process is carried into the parasitized erythrocyte (Blackman, Heidrich et al. 1990, Blackman and Holder 1992). The lethality of the genetic disruption of MSP1 suggests that it plays a critical function in parasite biology (O'Donnell, Saul et al. 2000). MSP1-19 is believed to play a critical role in initial parasite attachment to erythrocyte and in the invasion process through interaction with the RBC most abundant surface receptor: band 3 (Goel, Li et al. 2003). Though some field studies reported no association with protection (Dodoo, Theander et al. 1999), many others found that antibody responses to MSP1 were protective against malaria supporting its testing as a candidate malaria vaccine (Riley, Allen et al. 1992, Al-Yaman, Genton et al. 1996, Cavanagh, Dodoo et al. 2004, Dodoo, Aikins et al. 2008, Osier, Fegan et al. 2008). MSP1 (block 3 and 4) was first tested as a mixture of 3 antigens (Combination
B) (Genton, Betuela et al. 2002). MSP1-42 has reached phase 2 clinical trial, but the results did not warrant further testing (Ogutu, Apollo et al. 2009). MSP1-19 has been tested in association with other antigens, AMA1 in PfCP-2.9 (Hu, Chen et al. 2008), EBA175 in JAIVAC-1 (Chitnis, Mukherjee et al. 2015).

_The glutamate-rich protein (GLURP)_

*Plasmodium falciparum* GLURP is a 220 kDA GPI-anchored protein encoded by a single copy gene (PF3D7_1035300) located on *Plasmodium falciparum* chromosome 10 ([http://www.genedb.org](http://www.genedb.org)). GLURP is expressed in all the stages of the parasite in the human host as evidenced by the reactivity of anti-GLURP antibodies with asexual liver and blood stages of the parasite (Borre, Dziegiel et al. 1991). GLURP is shed from the merozoite surface during the invasion and anti-GLURP antibodies do not directly inhibit host cell invasion (Theisen, Soe et al. 1998). Though its role is unknown, the presence of GLURP in the different stages of the parasite in the human host is suggestive of a critical function in the parasite biology (Borre, Dziegiel et al. 1991). It has been shown that antibodies targeted at GLURP inhibit parasite growth through a cooperation with monocytes (antibody-dependent monocyte-mediated growth inhibition) (Theisen, Soe et al. 1998). In field studies, antibodies to GLURP have been associated with protection against clinical malaria in some studies (Oeuvray, Theisen et al. 2000, Soe, Theisen et al. 2004, Lusingu, Vestergaard et al. 2005, Dodoo, Aikins et al. 2008, Nebie, Tiono et al. 2008) but not all. After a phase 1 clinical trial, the testing of GLURP continued as a hybrid protein GMZ2 in which it is fused to MSP3. After satisfactory phase 1 studies (Esen, Kremsner et al. 2009, Mordmüller, Szywon et al. 2010, Bélard, Issifou et al. 2011) GMZ2 testing has progressed to phase 2 clinical trial and elicited low vaccine efficacy (Sirima, Mordmüller et al. 2016).
Genetic polymorphism and implication for field and clinical studies

Genetic polymorphism has been defined as a variation in the DNA sequence that occur with a frequency ≥ 1% in a population (Brookes 1999, Karki, Pandya et al. 2015). There is extensive evidence for allelic polymorphism (antigenic diversity) in *Plasmodium falciparum* genes in many different populations (Hoffmann, Da Silveira et al. 2001, Zhong, Afrane et al. 2007, Osier, Weedall et al. 2010, Mwingira, Nkwengulila et al. 2011). This is believed to be a parasite strategy for immune evasion (Healer, Murphy et al. 2004) and represents a major hurdle in vaccine development, interpretation of the results of sero-epidemiological studies and vaccine efficacy trials, as adaptive immunity may be strain-specific and acquired progressively with repeated encounters with various strains of the parasite (Doolan, Dobaño et al. 2009, Griffin, Hollingsworth et al. 2015). Indeed, allele-specific immunity was evidenced in some sero-epidemiological studies (Osier, Polley et al. 2007, Polley, Tetteh et al. 2007) and vaccine trials (Genton, Betuela et al. 2002, Ouattara, Takala-Harrison et al. 2013).

Heterologous expression of *Plasmodium falciparum* proteins

Copies of *Plasmodium falciparum* native proteins (recombinant proteins) can be produced in desired quantity for structural and functional studies using heterologous expression systems (bacterial or yeast systems). For instance, diverse bacteria (*Escherichia coli* (Theisen, Vuust et al. 1995), *Lactococcus lactis* (Theisen, Soe et al. 2004), *Mycobacterium bovis* (Nurul and Norazmi 2011)) and yeast (*Saccharomyces cerevisiae* (Kaslow, Hui et al. 1994), *Pichia pastoris* (Kocken, Withers-Martinez et al. 2002)) have been used. Alternative expression systems, less frequently used than bacteria and yeast, include baculovirus-infected insect cells, other parasites (amoeba *Dictyostelium discoideum*, *Toxoplasma*...
*gondii)*, mammalian cells, frog cells, tobacco plant cells and cell-free expression systems (reviewed in (Birkholtz, Blatch et al. 2008)). Basically, in these expression systems, a clone (copy) of the gene (piece of DNA) of interest is transfected into the DNA of the host cell using a vector (plasmid, virus); the desired protein is then expressed by the resulting recombinant DNA along with the host cell protein (Nurul and Norazmi 2011). A major shortcoming in protein expression is the risk of incorrect protein folding (3-dimensional structure) that is critical to its function and that may bias the results of immunoassays. Recombinant proteins are often expressed fused to a carrier protein (tag) to enhance solubility and achieve native protein folding among other objectives. Maltose binding protein (MBP) and glutathione-S-transferase (GST) are among the most commonly used tag for *Plasmodium falciparum* recombinant proteins (Esposito and Chatterjee 2006, Bell, Engleka et al. 2013).

### 1.7.2.5 The choice of the study design

As described above, some protective factors are inherited and last lifelong but other factors are fluctuating. Many previous field studies have measured naturally acquired immunity and susceptibility to malaria infection and / or febrile malaria using cross-sectional studies, but it has been pointed out that the risk of misclassification is high with this design (Marsh and Kinyanjui 2006) given that anti-malaria antibodies are short-lived and that the body temperature and parasitaemia show very short-term temporal variations (Delley, Bouvier et al. 2000). Longitudinal studies with repeated measurements have then been recommended (White, Griffin et al. 2013) as they are more likely to capture the true picture of the immune responsiveness and susceptibility to malaria. However, for logistical reasons, when the aim is large-scale and / or long-term surveillance, cross-sectional measures of the same parameters may be more suitable (Drakeley, Corran et al. 2005). For the study of protective
immunity to malaria, we have chosen an area of stable malaria transmission in western Burkina Faso as suggested by White and colleagues (White, Griffin et al. 2013) and for the study of malaria transmission hotspots, we have chosen a low transmission area in coastal Kenya as targeted interventions are expected to have more impact in pre-elimination conditions (Bousema, Griffin et al. 2012). We have also chosen a low transmission area in Senegal (Keur Soce) to study the comparative effect of low transmission condition on the dynamics of anti-malaria antibodies in young children.

In most studies of immunity to malaria, children represent a logical focus because of the high burden of the disease in this age group, but infants paradoxically exhibit a pattern of resistance to malaria similar to that of adults (Kitua, Smith et al. 1996, Snow, Nahlen et al. 1998), though their immune system is generally described as immature or actively suppressed (Gervassi and Horton 2014). The reasons for this observation are still unclear and a better understanding of this phenomenon may help in the development and / or improvement of malaria control tools and strategies, especially malaria vaccines. Targeting control interventions to spatial units of higher transmission intensity is also expected to be efficient in controlling malaria, but there is no consensus on how to reliably identify these spatial units called hotspots.
1.8 Study objectives

**General objective:**

The present work aimed at investigating the association between anti-malarial antibodies and the risk of febrile malaria in children.

**Specific objectives:**

- To investigate the role of antibodies in the resistance of young infants against febrile malaria
- To examine the dynamics and kinetics of antibodies to merozoite surface proteins in the first two years of life and compare the antibody levels measured in young children to previously established protective thresholds
- To examine the place of serological markers among other biomarkers in the detection of hotspots of malaria transmission in a context of declining malaria transmission.
2. CHAPTER 2: Are antibodies to some *P. falciparum* merozoite antigens protective against febrile malaria in children in their first two years of life

2.1 Introduction

Naturally acquired immunity to malaria is a complex phenomenon and despite advances achieved in a wealth of studies investigating the mechanisms of protection, defining immunity to malaria is still a challenge and there is no current universal consensus on the definition of immunity to malaria (Moormann and Stewart 2014). Evidence for an association between protection and immune responses in observational studies and malaria vaccine trials still mainly rely on epidemiological evidence with various approaches to address the issue of confounding by heterogeneity of exposure (Bejon, Warimwe et al. 2009, Ali, Bakli et al. 2012, Olotu, Fegan et al. 2012, Diop, Richard et al. 2014, Helb, Tetteh et al. 2015).

Children under five bear the bulk of malaria burden (WHO 2015) and there is strong evidence of a substantial asymptomatic malaria prevalence in young infants (<6 months) (Serign, Lamine et al. 2015). However, severe malaria is exceptional, clinical malaria is rare and predominantly low density parasitaemias have been reported in the early months of life (McGuinness, Koram et al. 1998, Afolabi, Salako et al. 2001). Most studies suggest that the resistance to malaria lasts until around four months of age (Achidi, Salimonu et al. 1996, McGuinness, Koram et al. 1998, Wagner, Koram et al. 1998, Kitua, Urassa et al. 1999, Klein Klouwenberg, Oyakhirome et al. 2005).
A positive association between mosquito bites and age (Muirhead-Thomson 1951, Carnevale, Frezil et al. 1978), surface area or weight (Carnevale, Frezil et al. 1978, Port, Boreham et al. 1980), an exclusive breast feeding realising low para-amino-benzoic acid diet (Kassim, Ako-Anai et al. 2000, Kicska, Ting et al. 2003), foetal haemoglobin (Pasvol, Weatherall et al., Amaratunga, Lopera-Mesa et al. 2011) and transplacentally transferred gamma-globulins have been advanced as possible protecting factors against malaria in early life. However, the most robust evidence of protection against *P. falciparum* febrile malaria has been reported for circulating gamma-globulins. Indeed, the capacity of antibodies to control malaria (fever and parasitaemia) has been demonstrated in human gamma-globulin passive transfer therapeutic experiments (Cohen, McGregor et al. 1961, Edozien, Gilles et al. 1962, McGregor, Carrington et al. 1963, Sabchareon, Burnouf et al. 1991). Subsequent observational studies in adults, older children and young infants, that mainly investigated the role of specific immune responses to blood-stage antigens were less consistent in their findings but have served nonetheless as a basis of malaria vaccine candidate selection (Fowkes, Richards et al. 2010).


Antibodies circulating in early infancy mainly come from transplacental transfer and are mostly IgG (Pitcher-Wilmott, Hindocha et al. 1980). Thereafter they are endogenously
produced by the infant (IgM and IgG). In addition, there is evidence that very early in life, some foetuses are able to mount an immune response to prenatal exposure through transplacental transfer of soluble malaria antigens (Metenou, Suguitan et al. 2007, May, Grube et al. 2009) although this may be associated with immuno-suppression (Brustoski, Möller et al. 2006). Whether antibodies play a role in early life resistance to febrile malaria is still unclear.

Most sero-epidemiological studies investigating the implication of antibodies in the protection of infants against malaria did not find evidence for the hypothesised protective effect of specific antibodies to the individual antigens tested (CSP, LSA-1, crude schizont extract, MSP1, MSP2, Pf155/RESA and the vaccine candidate SPf66) (Achidi, Salimonu et al. 1996, Wagner, Koram et al. 1998, Kitua, Urassa et al. 1999, Riley, Wagner et al. 2000, Zhou, Xiao et al. 2002). However, two studies conducted independently in Liberia and Kenya (Høgh, Marbiah et al. 1995, Branch, Udhayakumar et al. 1998) yielded evidence for a protective effect of antibodies to MSP1-19. In the present study, we have followed recommendations for the design of studies of associations between markers of immunity and resistance to malaria (high transmission location, longitudinal prospective monitoring, active detection, repeated measures and analysis of immune responses as continuous variables) (White, Griffin et al. 2013) and we have investigated responses to two antigens that were not previously tested in young children.
2.2 Study objectives

We conducted the present longitudinal prospective infant cohort study in the south-western region of Burkina Faso in West Africa with the following objectives:

**Primary objective:**

To investigate the association between antibodies to some *P. falciparum* merozoite antigens and the risk of febrile malaria in the first two years of life. We initially planned to test a wide range of *P. falciparum* antigens including pre-erythrocytic (CSP or LSA-1) and merozoite antigens (EBA-175, MSP1-19, MSP2, MSP3, AMA1, GLURP) but in the end, we had access to MSP3 and two fragments of GLURP (R0 and R2), kindly provided by Statens Serum Institute, Copenhagen, Denmark.

**Secondary objectives:**

- To investigate the role of selected potential risk factors for febrile malaria in young children
- To investigate possible risk factors affecting antibody responses
- To determine the dynamics of antibody titres in the first two years of life
- To examine the spatial distribution of febrile malaria episodes in the study area

2.3 Methods

2.3.1 Ethical consideration

This study was approved by the Institutional Review Board of Centre National de Recherche et de Formation sur le Paludisme (CNRFP) in Burkina Faso. The study was conducted...
according to the principles of the Declaration of Helsinki. Individual written informed consent was obtained from the parents of each child before any study procedure was performed. For parents who could not read and write, the information was read to them and discussed in their own language in the presence of an independent witness who completed the consent form at the end of the discussion on their behalf before they sign it with their thumb print. The IRB approved this consent procedure.

2.3.2 Study site

The study was conducted in the Banfora department (Figure 2.1) where malaria transmission is stable in south-western Burkina Faso at around 500 km from the capital city Ouagadougou. The climate is of tropical Savanah type with a single pronounced dry season (October-May) and a single wet season (June-September). Banfora is located at an average altitude of 300 m from sea level with a total annual rainfall at 900-1200 mm.

Most of inhabitants rely on subsistence farming (maize, millet, yam, rice) but the substantial rainfall and permanent rivers of the Comoe hydrographic basin, in which Banfora lies, allow additional commercial crop farming (cotton, sugar cane, cashew nuts).

The study area encompasses four health catchment areas covered by four dispensaries reporting to Banfora district hospital. Two of these dispensaries (Flantama and Korona) are located within Banfora town with water and power supply and mainly cement brick houses with iron sheet roofing. The other two dispensaries are located in Banfora sub-urban villages (Nafona and Bounouna) with predominant adobe-walled houses with thatched roofing.
Malaria transmission is seasonal with an incidence in children under five estimated at 1.18 episodes/child/year using active case detection (Tiono, Kangoye et al. 2014) with *P. falciparum* being responsible for more than 90% malaria cases.

2.3.3 Study population

A cohort of 140 infants aged between four and six weeks was recruited into the study. The primary endpoint for the sample size calculation was the incidence of febrile malaria. Based on an assumption of an incidence of one or more episodes of febrile malaria of 20% during
one year of follow up, it was calculated that a sample size of 140 infants (including 10% lost to follow up) would allow this incidence to be determined within a 95% confidence interval of 13-27%. The parents were informed of the study aims and procedures during the early post-natal visits at the study dispensaries, prior to the children reaching one month of age. The primary endpoint of the larger multi-country study was not the study of the maternal antibodies, but the incidence of febrile malaria and the children were not recruited at birth but a few weeks later. Recruitment was carried out simultaneously at the four health catchment areas of the study site from November 2010 to February 2011.

The inclusion criteria were 1) age between four and six weeks, 2) signed informed consent given by the caregivers and 3) availability and willingness to remain within the study area for the follow up period. Children were excluded from the study if they had a documented malaria infection or a febrile malaria episode, haemoglobin level < 8 g/dL, a confirmed or suspected immuno-deficiency syndrome, prematurity (< 37 weeks gestation) (Lawn, Gravett et al. 2010), a congenital anomaly or any other clinical condition for which the risks related to participation in the study outweigh the benefits.

Infants who had either a documented previous episode of malaria or a positive blood smear at the baseline visit were then excluded from the study; nevertheless, some infants might have had malaria infections that were unobserved. After the recruitment of the study participants, the geodetic coordinates (longitude, latitude) of their homesteads were recorded using handheld Global Positioning System (GPS) devices (eTrex Summit® HC). The approximate centre of each family compound was the reference point to record these coordinates. The altitude of the homesteads involved in the study ranged from 259 to 344 m with a mean of 306.7 m above sea. The distance to the nearest study dispensary ranged from
0.15 to 4.34 km with a geometric mean at 0.8 km (95 % CI, 0.71-0.91). The biggest diameter of the study area is approximately 8 km.

2.3.4 Surveillance of malaria morbidity and healthcare

To detect malaria infections, the children were followed up actively by weekly home visits and passively by dispensary monitoring for two years.

The weekly home visits were performed by fieldworkers whose main tasks were to check the children’s health status, perform rapid malaria tests, perform thick and thin blood smears on the same slide in case of fever (i.e. a reported history of fever in the past 24 hours and/or axillary temperature ≥ 37.5°C). Febrile children with positive rapid malaria test were administered a first dose of paediatric fixed-dose combination of artesunate-amodiaquine by the study nurse and the subsequent doses were administered by the caregivers. Febrile children with positive RDT and a concomitant health condition unrelated to malaria or febrile children with negative rapid malaria test or children with afebrile health conditions were referred to the study dispensaries further clinical investigation. Those children who could not be properly managed at the study dispensary were referred to the clinical trial facility located within the Banfora regional referral hospital and then to the paediatric unit of that hospital if necessary for specialized healthcare. The time window for the weekly home visits was ± 2 days. In addition, to monitor the first occurrence of asymptomatic parasitaemia, blood smears were systematically collected on a monthly basis until the detection of the first malaria infection regardless of the axillary temperature.

In the passive follow-up, the caregivers were encouraged to bring their children to the nearby study dispensary or the clinical research unit at any time should the child appear unwell.
Thick and thin blood smears were performed on the same slide in case of fever (i.e. a reported history of fever in the past 24 hours and/or axillary temperature ≥ 37.5°C). The children who became ill over the course of the study received health care free of charge either at the dispensaries, the clinical research unit or the paediatric unit of the regional referral hospital when necessary.

Capillary blood samples (500 µL each) were collected in EDTA-coated Eppendorf tubes by finger or heel prick at 1-6, 9, 12, 15, 18, 21 and 24 months of follow up for malaria serology. Previous studies reported that maternal antibodies wane within the first six months (Achidi, Perlmann et al. 1995, Riley, Wagner et al. 2000) and sampling at one month interval during this period was a trade-off between risks and discomfort related to bleeding in the children and collecting enough data for a parameter that rapidly wanes. Continuing bleeding at this frequency for the remaining 18 months would have increased the risk of dropout and a quarterly bleeding was chosen instead. The plasma was separated by refrigerated centrifugation of the whole blood samples at 3000 rpm for 5 minutes, aliquoted into labelled cryotubes and kept at -20 °C on the site until transfer to the central storage facility where they were kept at -80 °C until testing.

2.3.5 Parasitological examination

The collected blood smears were air dried and GIEMSA-stained as described elsewhere (Tiono, Ouedraogo et al. 2014). The parasite density was estimated by two independent microscopists using 100x oil immersion objective. Asexual parasites were counted against white blood cells (at least 200 WBC and up to 1000 WBC if less than ten parasites were counted against the first 200 WBC). Before a slide is reported negative for malaria parasites, at least 200 thick film fields are screened and ascertained parasite-free. The density of sexual
forms was estimated by counting gametocytes against 1000 WBC. The estimated parasitaemia was extrapolated from the slide parasite density assuming a constant WBC count of 8000/µL of whole blood. The parasitaemia estimated by the two microscopists were then compared for consistency. When their results were concordant (parasitaemia ratio <1.5 or >0.67), the arithmetic mean was recorded as the final result. Otherwise a third microscopist was involved and the final result was the arithmetic mean of the two most concordant parasite densities.

2.3.6 Haemoglobin typing

High Pressure Liquid Chromatography (HPLC) was used to quantify the fractions of foetal haemoglobin and haemoglobin variants in the children. The assays were performed by an independent laboratory at the Faculty of Medicine of Ouagadougou University in Burkina Faso.

2.3.7 Antibody quantification

Enzyme-linked immunosorbent assay (ELISA) was used to quantify total IgG to *P. falciparum* MPS3 and GLURP as described elsewhere (Nebie, Tiono et al. 2008). Microtiter microplates were coated with *P. falciparum* antigens MSP3-LSP (Druilhe, Spertini et al. 2005) at 1 µg/ml, GLURP R₀ or R₂ (Theisen, Vuust et al. 1995) at 0.1 µg/ml and incubated overnight at room temperature. The plates were then washed four times with PBS-0.05% Tween 20 (PBST) and the binding sites of the wells blocked with 1% bovine serum albumin in PBST. After one hour of blocking, the plates were washed four times with PBST and reacted with plasma diluted at 1:200 in 0.5% BSA-PBST solution for one hour at room temperature. After washing the plates four times with PBST, a goat anti-human antibody
preparation diluted at 1:3000 in 0.5% BSA-PBST, was added to the wells and left for incubation for one hour at room temperature. The plates were then washed four times with PBST before a substrate (p-nitro-phenyl-phosphate (Sigma)) diluted at 1 mg/ml in 0.5% BSA-PBST was added to the wells. After 30 minutes, the reaction was stopped by the addition of sulphuric acid H$_2$SO$_4$ and the absorbance was read at 450 nm by a microplate reader. The assays were performed in duplicates. Assays were repeated for duplicates for which the coefficient of variation ([standard deviation/mean]*100) was > 20. In order to adjust for day-to-day variation, for each antigen tested, a positive control serum was added in duplicate on each plate. The absorbances of study serum samples were then adjusted for day-to-day variation by multiplying absorbance by an adjustment factor that is the ratio of the average absorbance of positive controls on each plate by that of a reference plate. The positive control was a pool of adult Burkinabe sera and the negative controls were Danish serum samples obtained through Statens Serum Institute (Copenhagen, Denmark).

2.3.8 Statistical analysis

Definition of febrile malaria

Fever was defined as an axillary temperature ≥ 37.5°C and / or a reported history of fever in the past 24 hours. Malaria infection was defined as any positive parasitaemia regardless of the axillary temperature. Two definitions were set for febrile malaria; definition 1 included all febrile episodes with any level of asexual *P. falciparum* parasitaemia, and definition 2 included only febrile episodes associated with asexual *P. falciparum* parasite density ≥10000/µL. This latter definition was derived after examining the distributions of the log-transformed parasite densities in children with and without fever in cross-sectional surveys.
Febrile malaria episodes occurring within a 21 days or less interval in the same individual were considered a single episode.

Figure 2.2. Parasite density threshold for febrile malaria. The box-and-whisker plot represents the median and the inter-quartile range of the parasite density in a log₁₀ scale in the febrile and afebrile children groups. Given the limited data available, I opted to pragmatically define a pyrogenic threshold as 10⁴ parasites, corresponding to the 75th centile of the afebrile cases and 25th centile of the febrile cases. More formal methods are available for determining sensitivity and specificity based on malaria attributable fractions from logistic regression modelling (Smith, Schellenberg et al. 1994) but I judged these to be unwarranted based on the limited data available.

**Calculation of individual malaria exposure index**

We have adapted a previously published method for calculating individual exposure indexes (EI) to our cohort with time-to-event data (Olotu, Fegan et al. 2012). The assumptions behind this method are that 1) the risk of malaria is not evenly distributed in the study area, 2) for a given index individual, the faster the time to the first malaria infection in his neighbourhood, the higher his risk of malaria infection is and 3) the estimated exposure index is constant over time. The individual malaria exposure index (EI) was computed as the median time to the first malaria infection in the surrounding neighbours of each index child.
within a circle of a given radius around him at the middle of the circle. We have computed median time to the first malaria infection using the neighbourhood included in circles of predefined radii (0.5 to 2 km in steps of 0.25 km). EIs were transformed to negative values so that the most exposed has the highest exposure index. We then tested each of the exposure variables generated in a univariate Cox regression model and found that exposure indexes calculated using a 1.5 km radius circle best predicted the risk of malaria (lowest log likelihood ratio) in our dataset (see Figure 2.3). The study area lies in a 4 Km radius circle and, on average, 21 homesteads were included in the neighbourhood (1.5 Km radius circle) of each index child.

Figure 2.3. Selection of the radius that yielded exposure indexes that best predicted the risk of malaria. The lowest log likelihood in the univariate Cox regression analysis was the selection criteria for the most appropriate radius.

**Detection of hotspots of high exposure intensity and febrile malaria cases**

We have examined the fine scale spatial clustering of febrile malaria cases and high individual malaria exposure indexes using the scan statistic method by Kulldorff (Kulldorff 1997) implemented in the SatScan software. Discrete Poisson and Normal probability models were used to detect hotspots of febrile malaria cases and hotspots of high malaria
exposure indexes respectively. The detection of malaria hotspots is an exploratory objective in this chapter and we set the scanning window shape to “circular” and the maximum cluster size to 50% of the population at risk (the default cluster size in SatScan). The p values are adjusted for multiple comparisons using the Bonferroni correction in SatScan.

**Analysis of risk of febrile malaria**

The antibody titres expressed in arbitrary units were log$_{10}$-transformed to approximate a normal distribution. We fitted a multiple fractional polynomial regression of antibody titres on age to estimate the nonlinear relationship between anti-malaria antibody titre and age. Indeed, naturally acquired immunity is often assumed to be nonlinear in its dynamics and multiple fractional polynomials represents a robust, flexible alternative to other approaches (logistic regression, splines) to model non-linear relationships, although the complexity of the regression equation makes the interpretation of coefficients almost impossible. We fitted a linear regression model to estimate the relationship between a set of potential predictors and anti-malaria antibody titres. In this linear model, antibody titre was included as a time-changing outcome, i.e. the antibody titre measured at the beginning of each time interval within which malaria infections were recorded, and age as fractional polynomials. Within-person lack of independence between repeated measurements was accounted for by the Huber-White Sandwich estimator.

We fitted a Cox regression model to estimate the relationship between the time to first febrile malaria episode and a set of covariates of interest. Cox regression was chosen among other possible models for time to event analysis of follow-up data (Bradburn, Clark et al. 2003)
because of its simplicity, flexibility and our literature review indicated that it is the most
commonly used regression analysis in studies similar to ours (Wagner, Koram et al. 1998,
Kitua, Urassa et al. 1999, Riley, Wagner et al. 2000). Resistance to malaria can also be
estimated based on the frequency of episodes over a time period and the count data analysis
makes it possible to use all the events that were recorded over the entire observation period
for each child, and not only the first occurrence. We also fitted a negative binomial
regression model to estimate the association between multiple malaria episodes and a set of
potential explanatory covariates. Indeed, it is often assumed that the distribution of malaria
episodes follows a Poisson distribution, but negative binomial regression fits the data better
when there is evidence for overdispersion (variance > mean) (Mwangi, Fegan et al. 2008,
RTSS_Clinical_Trial_Partnership 2014, RTS 2015). Antibodies were fitted in two ways; a)
applying the baseline antibody titre throughout the period of monitoring and b) applying
time-varying antibody titre, i.e. the antibody titre measured at the most recent time point,
which therefore changed throughout the period of monitoring. The log likelihood ratio test
was used to test the significance of categorical variables with multiple levels. The
assumption of proportional hazards for Cox regression was tested based on the Kaplan Meier
method and the Schoenfeld residuals. We used the Huber-White Sandwich estimator to
adjust for clustering by individual in negative binomial regression models. The significance
level for hypothesis testing was set to 0.05.

We used the bootstrap method to calculate the 95% CI for medians and Spearman correlation
test to examine the correlations between distance and incidence of febrile malaria episodes,
parasitaemia and age, antigen-specific antibodies and antibody titres at consecutive time
points for each antigen.

The data were analysed using Stata 13 (StataCorp, College Station, Texas).
2.4 Results

2.4.1 Study population characteristics

In total, 216 mothers of newborns were invited to attend study screening visits during early post-natal visits at the four dispensaries of the study area. Of these, 148 (68.5%) attended the study screening visit with their infants. A total of 140 infants were recruited during a three-month period from mid-November 2010 to mid-February 2011. The baseline characteristics of the infants and their mothers are summarised in Table 2.1.

2.4.2 Follow-up of participants and malaria morbidity

Twenty-three children (16.4%) were lost to follow-up before completing 24 months with a median [Inter Quartile Range-IQR] follow up time of 9.8 [2, 14.75] months. Among them, six migrated out of the study area, 10 withdrew their consent, four died and three dropped out of the study and were no longer reachable.

One or more episodes of febrile malaria (fever + asexual parasitaemia>0) were experienced by 79.6% of all children during follow-up with a median time to first febrile malaria episode of 9.8 months (95% CI: 8.3, 11.3). Sixty-three children (45.98%) had at least one malaria infection in their first year of life and 46 (76.66%) of the remaining 60 children in their second year. The monthly distribution of the febrile malaria cases is shown in figure 2.4.

As shown in Figure 2.4, the transmission of malaria has a pronounced seasonality with 93% of febrile malaria cases recorded between June and November. It starts approximately one month after the beginning of the rains and peaks at the end of the rains from where it starts declining up to the lowest levels from January to May.
Table 2.1. Study population baseline characteristics. MUAC: mid upper arm circumference. Hb: haemoglobin. EPI: expanded programme on immunization. ITN: insecticide-treated bednets. IPTp: intermittent preventive treatment in pregnancy. 1, 2, 3: number of observations with missing data.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>67 (47.9%)</td>
</tr>
<tr>
<td>Age infants (days), median [min, max]</td>
<td>33 [27, 42]</td>
</tr>
<tr>
<td>Weight (kg), median [min, max]</td>
<td>4.1 [2.8, 5.9]</td>
</tr>
<tr>
<td>Length (cm), median [min, max]</td>
<td>54 [48, 61]</td>
</tr>
<tr>
<td>MUAC (cm), median [min, max]</td>
<td>12 [8.5, 16]</td>
</tr>
<tr>
<td>Hb conc. (g/dL), median [min, max]</td>
<td>12.6 [8.7, 17.6]</td>
</tr>
<tr>
<td>Foetal Hb (%), median [min, max]</td>
<td>59.6 [20.1, 89.6]</td>
</tr>
<tr>
<td>Hb phenotype, n (%)</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>113 (80.71)</td>
</tr>
<tr>
<td>AC</td>
<td>18 (13.14)</td>
</tr>
<tr>
<td>AS</td>
<td>1 (0.73)</td>
</tr>
<tr>
<td>CC</td>
<td>5 (3.65)</td>
</tr>
<tr>
<td>Delivery way, n (%)</td>
<td></td>
</tr>
<tr>
<td>Natural</td>
<td>133 (95)</td>
</tr>
<tr>
<td>Cesarian section</td>
<td>5 (3.6)</td>
</tr>
<tr>
<td>Neonatal resuscitation, n (%)</td>
<td>12 (8.6)</td>
</tr>
<tr>
<td>Neonatal infection, n (%)</td>
<td>2 (1.43)</td>
</tr>
<tr>
<td>EPI (up to date at 1 month), n (%)</td>
<td>124 (88.6)</td>
</tr>
<tr>
<td>Age groups mothers (years), n (%)</td>
<td></td>
</tr>
<tr>
<td>≤19</td>
<td>10 (7.1)</td>
</tr>
<tr>
<td>20-29</td>
<td>89 (63.6)</td>
</tr>
<tr>
<td>≥30</td>
<td>37 (26.4)</td>
</tr>
<tr>
<td>ITN use during pregnancy, n (%)</td>
<td>123 (87.9)</td>
</tr>
<tr>
<td>IPTp courses, n (%)</td>
<td></td>
</tr>
<tr>
<td>No treatment</td>
<td>10 (7.14)</td>
</tr>
<tr>
<td>1 dose</td>
<td>28 (20)</td>
</tr>
<tr>
<td>2 doses</td>
<td>100 (71.43)</td>
</tr>
<tr>
<td>3 doses</td>
<td>2 (1.43)</td>
</tr>
<tr>
<td>Gravidity status, n (%)</td>
<td></td>
</tr>
<tr>
<td>Primigravidae</td>
<td>32 (22.86)</td>
</tr>
<tr>
<td>Multigravidae</td>
<td>106 (75.71)</td>
</tr>
<tr>
<td>Education level of mothers, n (%)</td>
<td></td>
</tr>
<tr>
<td>No formal education</td>
<td>78 (55.71)</td>
</tr>
<tr>
<td>Primary school</td>
<td>39 (27.86)</td>
</tr>
<tr>
<td>Secondary school or above</td>
<td>22 (15.71)</td>
</tr>
<tr>
<td>Distribution of study population, n (%)</td>
<td></td>
</tr>
<tr>
<td>Bounouna</td>
<td>34 (22.97)</td>
</tr>
<tr>
<td>Nafona</td>
<td>41 (27.7)</td>
</tr>
<tr>
<td>Korona</td>
<td>16 (10.81)</td>
</tr>
<tr>
<td>Flantama</td>
<td>49 (33.11)</td>
</tr>
</tbody>
</table>
Figure 2.4. Temporal distribution of febrile malaria (any parasitaemia with fever) cases over the monitoring period.

The distribution of the number of febrile malaria cases per child is summarized in Figure 2.5. This distribution takes into account the entire monitoring period.

Figure 2.5. Distribution of the number of febrile malaria episodes in the study population.
The geometric mean shortest straight-line distance to the healthcare facilities involved in the passive case detection is 0.8 km (95% CI, 0.71-0.91) which seem reasonable compared to the national standards (5 km). Looking at the correlation between shortest straight-distance to study dispensary (including the clinical trial facility) and the number of febrile malaria episodes recorded, we found a weak correlation (rho=0.22, p=0.01). Restricting the analysis to febrile episodes recorded in passive case detection reduced the strength and statistical significance of the correlation (rho=0.14, p=0.112).

In all, 296 febrile malaria episodes were recorded over 249 child-years, with the number of cases peaking in October each year, giving an incidence rate of 1.2 episodes / child / year (95%CI, 1.06-1.33). In the first six months of life, five infections (two asymptomatic and three febrile) were detected, of which three occurred in the rains, in children aged above five months. *P. falciparum* was present in all the positive slides. Only two mixed infections involving *P. falciparum* and *P. malariae* were detected in two children. The two year cumulative gametocyte prevalence was 13.6%. Asexual *P. falciparum* parasitaemia was weakly correlated to age (rho=0.216, p<0.001). The geometric mean (95% CI) *P. falciparum* asexual parasitaemia was 14646.53/µL (12320.07, 17412.31).

2.4.3 Fine scale spatial heterogeneity of malaria transmission

We found that febrile malaria cases and children with the highest exposure clustered in the northern peripheral area (Nafona) of the Banfora Town (Figure 2.6). We then examined the correlation between the frequency of febrile malaria episodes and individual malaria exposure indexes, we found a statistically significant and moderate correlation between both
the indicators (rho=0.43, p<0.001). However, around the two dispensaries located to the west of the study area, we noticed a cluster with high exposure to malaria with paradoxically low malaria incidence. Using the Kulldorff's spatial scan statistic, we formally tested the hypothesis of a spatial clustering of individuals with high exposure indexes, high incidence of febrile malaria and aimed at visualizing the extent of overlapping between the hotspots of febrile malaria cases and the hotspots of high exposure indexes. We found considerable overlapping between a single hotspot of high malaria exposure with three overlapping hotspots of febrile malaria (Figure 2.7).

Figure 2.6. Spatial distribution of febrile malaria episodes in the study area. Each dot represents a child. The colour shading increases with the frequency of febrile malaria episodes or intensity of exposure to malaria experienced by the child. Blue triangles represent study dispensaries. The red triangle represents the regional referral hospital.
In the present infant cohort study, we have detected hotspots of febrile malaria cases and this could serve as a basis for targeted malaria control interventions. However febrile malaria might not be an ideal marker in older children or adults, especially in a high and stable transmission context since high exposure would lead to a rapid development of clinical immunity and instability of the hotspots what would complicate the planning of control interventions (Bejon, Williams et al. 2010, Bousema, Griffin et al. 2012). Other transmission markers have been used in different studies, but data on the relationship between these markers, that might guide the choice of the marker, is scarce.
2.4.4 Anti-malaria antibody kinetics

Anti-GLURP R0 and anti-GLURP R2 antibodies were more closely correlated to each other (r=0.52, p<0.001) than either anti-GLURP sub-unit antibody was correlated with anti-MSP3 antibody titres (r=0.35, p<0.001 and r=0.4, p<0.001 respectively).

Antibody titres at one time point were weakly to moderately correlated with antibody titres at the next time point (rho ranging from -0.28 to 0.55; 0.31 to 0.58 and 0.15 to 0.60, respectively for anti-MSP3, anti-GLURP R0 and anti-GLURP R2 antibody titres) with the strongest correlations observed from baseline to month 3, and months 9 to 12 (Table 2.2).

Table 2.2. Variability in antibody titres. The correlations are examined between every two consecutive time points for antibody measurement. Antibody titres are in log₂ scale.

<table>
<thead>
<tr>
<th>Time points (months)</th>
<th>Anti-MSP3* r</th>
<th>Anti-MSP3* p</th>
<th>Anti-GLURP R0* r</th>
<th>Anti-GLURP R0* p</th>
<th>Anti-GLURP R2* r</th>
<th>Anti-GLURP R2* p</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0→M3</td>
<td>0.5460</td>
<td>&lt;0.0001</td>
<td>0.5514</td>
<td>&lt;0.0001</td>
<td>0.6022</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>M3→M6</td>
<td>0.1805</td>
<td>0.0852</td>
<td>0.3410</td>
<td>0.0009</td>
<td>0.3480</td>
<td>0.0007</td>
</tr>
<tr>
<td>M6→M9</td>
<td>0.4015</td>
<td>0.0001</td>
<td>0.4074</td>
<td>0.0001</td>
<td>0.2448</td>
<td>0.0187</td>
</tr>
<tr>
<td>M9→M12</td>
<td>0.4265</td>
<td>&lt;0.0001</td>
<td>0.5885</td>
<td>&lt;0.0001</td>
<td>0.4444</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>M12→M18</td>
<td>0.3054</td>
<td>0.0031</td>
<td>0.3657</td>
<td>0.0003</td>
<td>0.2819</td>
<td>0.0065</td>
</tr>
<tr>
<td>M18→M24</td>
<td>-0.2800</td>
<td>0.0069</td>
<td>0.3148</td>
<td>0.0022</td>
<td>0.1489</td>
<td>0.1565</td>
</tr>
</tbody>
</table>

The time-course of individual antibody kinetics is shown in figure 2.8. In follow-up studies, both analytical and biological variation contribute to the overall variation of repeated measurements (Monach 2012). The present study samples antibody titres were measured in the same laboratory following a standard operating procedure. Samples were not processed the same day, but the results were adjusted for plate to plate (duplicate testing) and day to day variation to limit analytical bias. Samples were also collected, processed and stored following a standard procedure. Though we cannot precisely quantify the contribution of analytical and pre-analytical bias resulting from laboratory variation in antibody titres, we hypothesize that biological variation explains most of the observed dynamics, since the
decline observed in the first six months of follow-up is in keeping with what is expected for the maternal antibodies from a biological perspective.

Figure 2.8. Individual trajectories of antibody responses to MSP3, GLURP R0 and GLURP R2.

Figure 2.9. Lines of best fit for antibody dynamics in the first two years of life.

In Figure 2.9, we show the individual antibody titres and the best-fit line using multiple fractional polynomials of age. There is an overall decline of total IgG titres to the three
antigens from one to four months of age, presumably indicating the waning of maternally-derived anti-malaria antibodies. Thereafter, both the anti-GLURP R2 and anti-GLURP R0 total IgG titres rise slightly with increasing age, while anti-MSP3 total IgG titres remain constant.

2.4.5 Predictive factors for the changing antibody titres.

In this analysis, there was one observation per individual per observation period and observations were clustered by individual in the analysis to adjust for repeated measures.

**Univariate analysis**

In the univariate analysis, the only common predictor that was statistically significantly and consistently associated with antibody titres was age. There was a tendency for residence in the urban zone and higher education level to be associated with lower antibody levels. Exposure index, number of previous episodes, haemoglobin type, number of IPTp courses had variable effects on the levels of antibodies to the different merozoite antigens. The univariate analysis is summarized in table 2.3.
Table 2.3. Predictive model for changing anti-malaria antibody titres using linear regression. Univariate analysis. *Powers of multiple fractional polynomials of age. **Only one participant had haemoglobin phenotype AS.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>IgG anti-MSP3</th>
<th>IgG anti-GLURP R0</th>
<th>IgG anti-GLURP R2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coef.  95% CI</td>
<td>Coef.  95% CI</td>
<td>Coef.  95% CI</td>
</tr>
<tr>
<td>Age power (-2/-5/5)*</td>
<td>0.02 [0.02, 0.03]</td>
<td>0.24 [0.10, 0.39]</td>
<td>0.001 0.51 [0.36, 0.66] &lt;0.001</td>
</tr>
<tr>
<td>Age power (NA/0/5)*</td>
<td>- - - -0.10 [-0.21, 0.02]</td>
<td>0.093 -0.19 [-0.24, -0.14] &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male 0 - - 0 - 0 -</td>
<td>Female -0.18 [-0.42, 0.07] 0.154 0.14 [-0.11, 0.40] 0.273 -0.22 [-0.54, 0.10] 0.179</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Weight (baseline) -0.04 [-0.25, 0.17] 0.723 -0.06 [-0.26, 0.15] 0.589 -0.20 [-0.46, 0.07] 0.139</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Length (baseline) 0.02 [-0.02, 0.06] 0.310 -0.002 [-0.05, 0.05] 0.944 -0.04 [-0.11, 0.02] 0.182</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MUAC (baseline) 0.05 [-0.07, 0.18] 0.425 0.002 [-0.11, 0.11] 0.976 -0.04 [-0.17, 0.09] 0.563</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Foetal Hb rate (baseline) 0.006 [-0.006, 0.02] 0.317 0.001 [-0.01, 0.01] 0.893 0.01 [-0.003, 0.02] 0.144</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin type</td>
<td>AA 0 - - 0 - -</td>
<td>AS** NA - - NA - - NA - -</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AC -0.22 [-0.44, -0.17] 0.276 0.05 [-0.38, 0.48] 0.832 0.04 [-0.49, 0.56] 0.885</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC 0.04 [-0.65, 0.73] 0.911 0.01 [-0.55, 0.58] 0.963 0.36 [0.003, 0.71] 0.048</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month of birth</td>
<td>October 0 - - 0 - -</td>
<td>November 0.30 [-0.07, 0.66] 0.108 0.17 [-0.25, 0.59] 0.421 0.07 [-0.40, 0.55] 0.757</td>
<td></td>
</tr>
<tr>
<td></td>
<td>December 0.07 [-0.30, 0.44] 0.724 0.10 [-0.32, 0.52] 0.636 -0.30 [-0.80, 0.20] 0.235</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>January -0.02 [-0.41, 0.38] 0.927 0.30 [-0.19, 0.80] 0.222 0.14 [-0.40, 0.68] 0.603</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPI status (Baseline)</td>
<td>Up to date 0 - - 0 - -</td>
<td>Not up to date 0.22 [-0.20, 0.63] 0.307 0.29 [-0.08, 0.66] 0.121 -0.01 [-0.45, 0.43] 0.961</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age mother (baseline) 0.02 [-0.001, 0.04] 0.057 0.01 [-0.005, 0.03] 0.138 0.02 [-0.01, 0.04] 0.166</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gravidity status</td>
<td>Primigravidae 0 - - 0 - -</td>
<td>Multigravidae 0.18 [-0.11, 0.47] 0.213 0.05 [-0.20, 0.29] 0.704 0.01 [-0.34, 0.37] 0.945</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ITN use (pregnancy) Yes 0 - - 0 - -</td>
<td>No -0.10 [-0.47, 0.27] 0.599 0.15 [-0.26, 0.55] 0.471 0.21 [-0.26, 0.68] 0.372</td>
<td></td>
</tr>
<tr>
<td>IPTp courses</td>
<td>0 0 - - 0 - -</td>
<td>1 0.56 [0.17, 0.94] 0.005 -0.22 [-0.93, 0.50] 0.549 -0.73 [-1.49, 0.03] 0.060</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 0.35 [0.07, 0.64] 0.017 -0.24 [-0.89, 0.40] 0.462 -0.75 [-1.47, 0.03] 0.040</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education level (mother)</td>
<td>3 0.20 [-0.06, 0.45] 0.127 -0.12 [-0.81, 0.57] 0.737 -1.88 [-3.06, -0.70] 0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zone of residence</td>
<td>None 0 - - 0 - -</td>
<td>Primary 0.18 [-0.07, 0.43] 0.150 -0.07 [-0.38, 0.25] 0.648 -0.21 [-0.57, 0.16] 0.260</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Secondary or above -0.36 [-0.76, 0.08] 0.116 -0.31 [-0.63, 0.01] 0.057 -0.50 [-0.87, -0.12] 0.010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Season</td>
<td>Rural 0 - - 0 - -</td>
<td>Urban -0.25 [-0.53, 0.02] 0.074 -0.44 [-0.72, -0.15] 0.003 -0.70 [-1.05, -0.36] &lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mixed 0.21 [-0.10, 0.53] 0.175 -0.43 [-0.77, -0.10] 0.010 -0.35 [0.04, 0.30] 0.072</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Number previous infections 0.05 [-0.06, 0.17] 0.373 0.28 [0.17, 0.38] &lt;0.001 0.28 [0.14, 0.41] &lt;0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Multivariable analysis

The Wald test was used to select variables. Some variables, nonsignificant in our univariate analysis, were retained in the final model based on compelling evidence for their association with immune responses in the published literature. Age and variables directly related to exposure such as ITN use were then kept in the final model. In the final multivariable model, age, season and the number of malaria episodes recorded immediately before the blood sample collection were the statistically significant predictors of the titres of antibodies to MSP3, GLURP R0 and GLURP R2. The results of multivariable analysis are shown in table 2.4.

Table 2.4. Multivariable predictive model for changing anti-malaria antibody titres. * Age is transformed in multiple fractional polynomials with the corresponding powers for antibodies to MSP3, GLURP R0 and GLURP R2 indicated in brackets. † Number of malaria infections recorded before the following time point for antibody measurement.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>IgG anti-MSP3</th>
<th>95% CI</th>
<th>p</th>
<th>IgG anti-GLURP R0</th>
<th>95% CI</th>
<th>p</th>
<th>IgG anti-GLURP R2</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age power (-2/-5/-5) *</td>
<td>0.02</td>
<td>[0.02, 0.03]</td>
<td>&lt;0.001</td>
<td>2</td>
<td>[1.49, 2.50]</td>
<td>&lt;0.001</td>
<td>-2.04</td>
<td>[-2.98, -1.10]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age power (NA/0/-5) *</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.19</td>
<td>[0.79, 1.58]</td>
<td>&lt;0.001</td>
<td>-0.92</td>
<td>[-1.21, -0.62]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ITN use (pregnancy)</td>
<td>Yes</td>
<td>0</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>-0.11</td>
<td>[-0.49, 0.26]</td>
<td>0.548</td>
<td>0.16</td>
<td>[-0.22, 0.53]</td>
<td>0.413</td>
<td>0.02</td>
<td>[-0.44, 0.48]</td>
</tr>
<tr>
<td>Season</td>
<td>Dry season</td>
<td>0</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Rains</td>
<td>0.35</td>
<td>[0.13, 0.57]</td>
<td>0.002</td>
<td>0.33</td>
<td>[0.19, 0.47]</td>
<td>&lt;0.001</td>
<td>0.28</td>
<td>[0.07, 0.50]</td>
</tr>
<tr>
<td>Malaria exposure index</td>
<td>0.02</td>
<td>[0.003, 0.04]</td>
<td>0.098</td>
<td>-0.01</td>
<td>[0.03, 0.04]</td>
<td>0.202</td>
<td>0.01</td>
<td>[-0.01, 0.04]</td>
<td>0.393</td>
</tr>
<tr>
<td>Number previous infections †</td>
<td>0.12</td>
<td>[-0.002, 0.24]</td>
<td>0.053</td>
<td>0.28</td>
<td>[0.17, 0.39]</td>
<td>&lt;0.001</td>
<td>0.31</td>
<td>[0.17, 0.45]</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
2.4.6 Antibody titres and risk of febrile malaria

Kaplan Meier estimates of survival to febrile malaria

By the end of the monitoring period, 74.4% of the children had experienced at least one episode of febrile malaria (fever + asexual parasitaemia $>10000/\mu L$). In Figure 2.10 we can also discern the seasonality and stability of the malaria transmission in the study area.

Figure 2.10. Kaplan Meier survival estimates of the children over the first two years of life.

The results of the test for the proportionality of hazards are summarized in Figure 2.11 and Figure 2.12. Overall, there was no significant variation in the proportionality of hazards over time, but there was borderline variation in varying hazards for the exposure index ($p=0.082$, in the direction of decreasing hazard over time) and for foetal haemoglobin ($p=0.098$, in the direction of increasing hazard over time).
Figure 2.11. Kaplan Meier estimates of survival to febrile malaria with respect to febrile malaria risk factors categorized into tertiles. The log-rank test compares the survival distribution between the three groups for each factor.

Figure 2.12. Test of proportional hazards assumption: Schoenfeld residuals plots.
Univariate analysis

Only month of birth and residence in the urban zone of the study site were associated with reduced risk of febrile malaria. Indeed, children born in January were at lower risk of malaria compared to those born in the last quarter of the year. Anti-MSP3 antibody titres were significantly associated with an increased risk of febrile malaria and no evidence of association between anti-GLURP antibody titres and risk of febrile malaria was found. The extrinsic predictors that were associated with higher risk of febrile malaria were the wet season and the individual malaria exposure. The results of the univariate analysis are shown in table 2.5.

Table 2.5. Univariate models of risk of malaria.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Cox regression</th>
<th>Negative binomial regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age</td>
<td>1.12</td>
<td>[1.10, 1.13]</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Female</td>
<td>0.86</td>
<td>[0.57, 1.30]</td>
</tr>
<tr>
<td>Weight (baseline)</td>
<td>1.06</td>
<td>[0.75, 1.50]</td>
</tr>
<tr>
<td>Length (baseline)</td>
<td>1.03</td>
<td>[0.95, 1.13]</td>
</tr>
<tr>
<td>MUAC (baseline)</td>
<td>1.13</td>
<td>[0.95, 1.34]</td>
</tr>
<tr>
<td>Foetal Hb rate (baseline)</td>
<td>0.98</td>
<td>[0.97, 0.97]</td>
</tr>
<tr>
<td>Haemoglobin type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>AS*</td>
<td>NA</td>
<td>-</td>
</tr>
<tr>
<td>AC</td>
<td>1.30</td>
<td>[0.71, 2.39]</td>
</tr>
<tr>
<td>CC</td>
<td>0.50</td>
<td>[0.12, 2.03]</td>
</tr>
<tr>
<td>Anti-MSP3 (changing)</td>
<td>1.41</td>
<td>[1.16, 1.70]</td>
</tr>
<tr>
<td>Anti-GLURP R0 (changing)</td>
<td>1.11</td>
<td>[0.90, 1.37]</td>
</tr>
<tr>
<td>Anti-GLURP R2 (changing)</td>
<td>1.03</td>
<td>[0.89, 1.20]</td>
</tr>
<tr>
<td>Anti-MSP3 (baseline)</td>
<td>0.96</td>
<td>[0.85, 1.07]</td>
</tr>
<tr>
<td>Anti-GLURP R0 (baseline)</td>
<td>1.01</td>
<td>[0.87, 1.16]</td>
</tr>
<tr>
<td>Anti-GLURP R2 (baseline)</td>
<td>1.04</td>
<td>[0.92, 1.18]</td>
</tr>
<tr>
<td>Month of birth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>October</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>November</td>
<td>1.41</td>
<td>[0.64, 3.08]</td>
</tr>
<tr>
<td>December</td>
<td>1.33</td>
<td>[0.61, 2.88]</td>
</tr>
<tr>
<td>January</td>
<td>2.49</td>
<td>[1.10, 5.64]</td>
</tr>
<tr>
<td>EPI status (baseline)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Up to date</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Not up to date</td>
<td>1.24</td>
<td>[0.69, 2.23]</td>
</tr>
</tbody>
</table>
Multivariate analysis

The results of negative binomial regression were consistent with the Cox regression analysis (Table 2.6). Changing anti-MSP3 antibody titres were significantly associated with increased risk of febrile malaria episodes in both models. Season and individual malaria exposure index were significantly associated with increased risk of febrile malaria episodes.

The baseline foetal haemoglobin fraction showed a protective effect in the multivariable model (HR=0.97, p=0.003); this was dependent on adjusting for exposure index in the multivariable model and we noted a non-significant correlation between foetal haemoglobin and exposure index (r=0.17, p=0.064). Belonging to the haemoglobin CC type group was
also significantly associated with decreased malaria risk (IRR=0.44, p=0.046) after adjusting for the other covariates.

Table 2.6. Multivariable models of risk of malaria using changing anti-malaria antibody titres. a MUAC: mid upper arm circumference. b Hb: haemoglobin. c NA not applicable; only one child had haemoglobin AS type. d HR: hazard ratio. e IRR: incidence rate ratio.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Cox regression</th>
<th>Negative binomial regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR d</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age</td>
<td>NA</td>
<td>-</td>
</tr>
<tr>
<td>MUAC (baseline) a</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Foetal Hb rate (baseline) b</td>
<td>0.97</td>
<td>[0.96, 0.99]</td>
</tr>
<tr>
<td>Haemoglobin type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>AS c</td>
<td>NA c</td>
<td>-</td>
</tr>
<tr>
<td>AC</td>
<td>1.29</td>
<td>[0.12, 2.19]</td>
</tr>
<tr>
<td>CC</td>
<td>0.52</td>
<td>[0.12, 2.19]</td>
</tr>
<tr>
<td>Anti-MSP3 (changing)</td>
<td>1.34</td>
<td>[1.08, 1.66]</td>
</tr>
<tr>
<td>Anti-GLURP R0 (changing)</td>
<td>1.15</td>
<td>[0.91, 1.44]</td>
</tr>
<tr>
<td>Anti-GLURP R2 (changing)</td>
<td>0.98</td>
<td>[0.83, 1.16]</td>
</tr>
<tr>
<td>ITN use (pregnancy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>No</td>
<td>0.86</td>
<td>[0.41, 1.79]</td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry season</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Rains</td>
<td>10.85</td>
<td>[2.80, 42.15]</td>
</tr>
<tr>
<td>Malaria exposure index</td>
<td>1.08</td>
<td>[1.04, 1.13]</td>
</tr>
</tbody>
</table>

2.5 Discussion

In this study, we observed a very low incidence of febrile malaria in the first six months of life with a median time to first infection of 9.8 months in a context of stable but markedly seasonal malaria transmission. The two-year cumulative febrile malaria incidence was 79.6% with an incidence rate of 1.2 episodes / child / year (95%CI, 1.06-1.33). Anti-malaria antibodies and foetal haemoglobin were investigated in relation to resistance to malaria in a cohort of 140 infants. Antibody titres to GLURP and MSP3 were found to decline in the first
four months of life, presumably due to the loss of maternal antibodies. Endogenous production was responsible for the subsequent increase in the case of GLURP and stabilization of the loss of antibodies for MSP3. Contrary to our expectations, there was no association between antibody titres to GLURP (R0 and R2) and the risk of febrile malaria in the first two years of life; antibody titres to MSP3 even appeared as a marker of exposure since it was statistically significantly positively associated with the risk of febrile malaria and inversely associated with time to first febrile malaria episode. Confounding between malaria risk and antibody titres by variation in exposure has previously been reported (Bejon, Cook et al. 2011, Greenhouse, Ho et al. 2011). Besides that, we observed a spatial clustering of highly exposed children and febrile malaria cases in the Northern part of the study area. We found that the incidence of febrile malaria in young infants was low in our study. However, the timing of the study was not ideal to assess the role of maternally-derived antibodies per se in this apparent resistance. The median time to first malaria infection in our study (9.8 months) was much higher than previously reported in a mesoendemic area in Benin (4.88 and 6.11 months in children born from infected and uninfected placentas respectively) (Le Port, Watier et al. 2011). This is likely due to the timing of the recruitment that took place during the dry season and there were 4 to 5 months of dry season remaining before the malaria season began. The observation of a low febrile malaria incidence in a cluster of high exposure may be explained by a more rapid acquisition of immunity to febrile malaria in children residing in this cluster due to a more intense exposure (Filipe, Riley et al. 2007). The spatial clustering of febrile malaria cases in the northern part of the study area is likely due to the presence of more suitable environmental factors for malaria vectors. Indeed, this zone is marshy and mainly rural in contrast to the southern part that overlaps with Banfora town. Long distances to dispensaries constitute a factor for poor accessibility.
to healthcare in impoverished areas (Müller, Smith et al. 1998) and we wanted to investigate if residing far from dispensaries affected the healthcare seeking behaviour. The weak positive correlation found between distance to health facility and incidence of febrile malaria, further reduced by restricting the analysis to passive case detection, do not suggest a bias in sensitivity for cases based on distance to dispensary.

The lack of association between antibodies to GLURP (R0 and R2) and protection against clinical malaria in our study is partially concordant with the findings of a previous study in older children in Burkina Faso. Nebie and colleagues investigated total IgG to GLURP (R0 and R2) among others antigens in children aged 6 months to 10 years and found a protective effect for antibodies to GLURP R0 but not to GLURP R2 when the antibody titres were analysed individually. When antibody titres to the four antigens we have tested (NANP, GLURP R0 and R2, MSP3) were included in the multivariable model, antibodies to GLURP (R0 and R2) were no longer associated with protection against malaria (Nebie, Tiono et al. 2008). In Ghana, Dodoo and colleagues reported a protective effect of total IgG and IgG subclasses to GLURP (R0 and R2) in the univariate analysis. However, neither total IgG nor IgG subclasses to GLURP (R0 and R2) were significantly protective when all the serological covariates were included in the final multivariable model (Dodoo, Aikins et al. 2008, Dodoo, Atuguba et al. 2011). In a study conducted in Tanzania by Lusingu and colleagues, total IgG to GLURP R0 was not associated with protection against febrile malaria; among IgG subclasses only IgG1 was associated with protection (Lusingu, Vestergaard et al. 2005).

In contrast, other studies have demonstrated a protective association for antibodies to GLURP (R0 and R2) in older children and adults (Dodoo, Theisen et al. 2000, Oeuvray, Theisen et al. 2000, Meraldi, Nebie et al. 2004, Courtin, Oesterholt et al. 2009). In a study in Myanmar that investigated antibodies to MSP1, MSP3, GLURP (R0, R1, R2), only
antibodies to GLURP R0 showed a protective effect when all the antibodies were included in the multivariable analysis (Soe, Theisen et al. 2004).

Antibodies to MSP3 have been associated with protection in previous sero-epidemiological studies (Meraldi, Nebie et al. 2004, Soe, Theisen et al. 2004, Roussilhon, Oeuvray et al. 2007, Osier, Fegan et al. 2008) and a vaccine trial (Sirima, Cousens et al. 2011) although the assessment of efficacy was not the primary objective in the latter. However, other studies did not find a protective effect of antibodies to MSP3 (Dodoo, Aikins et al. 2008, Courtin, Oesterholt et al. 2009, Dodoo, Atuguba et al. 2011) but none of these studies concluded on antibodies to MSP3 appearing as a marker of exposure.

Although antibodies to MSP3 and GLURP have not been previously studied in newborn cohorts, antibodies to other *P. falciparum* antigens have been investigated. In sero-epidemiological newborn cohort studies, antibodies to crude *P. falciparum* schizont extract and MSP2 were found to be associated with higher risk of malaria infection in infants (Wagner, Koram et al. 1998, Riley, Wagner et al. 2000). Evidence for an association with protection against clinical malaria has been reported only for antibodies to MSP1-19 (Høgh, Marbiah et al. 1995, Branch, Udhayakumar et al. 1998). Antibodies to MSP3 and GLURP (R0 and R2) were not associated with protection in our study, and we suggest that confounding due to exposure led to an apparent association with increased risk for antibodies to MSP3.

Interestingly, foetal haemoglobin was significantly inversely associated with febrile malaria risk, although the effect size was relatively small. The effect was only statistically significant on multivariable analysis, and appeared to depend on adjusting for exposure index. Furthermore the effect seems to be evident after 6 months of age, when we would
expect foetal haemoglobin to have been lost from the circulation. We speculate that an interaction between malaria exposure and foetal haemoglobin may be responsible for a delayed protective effect, perhaps due to an early but controlled infection in the presence of high levels of foetal haemoglobin leading to more rapid acquisition of immunity (Pombo, Lawrence et al. 2002). Children who carried the haemoglobin CC type appeared to have a significantly lower risk of malaria as compared to haemoglobin AA type children, as it has been previously reported (Modiano, Luoni et al. 2001, Bougouma, Tiono et al. 2012).

The limitations of our study include the fact that the high malaria transmission season began 5-7 months after recruitment. Therefore the majority of the maternal antibodies were likely gone by the time that febrile malaria episodes began, and children were exposed during a period of lower antibody titres. We did not use an external control to quantify malaria antibodies as performed elsewhere (Murungi, Kamuyu et al. 2013). However, we speculate that antibody titres at 5 months and beyond were lower than those previously reported to be protective (Høgh, Marbiah et al. 1995, Branch, Udhayakumar et al. 1998).

2.6 Conclusion

In conclusion, the present study did not find any evidence for an association between antibody titres to MSP3 and GLURP (R0 and R2) and protection against *P. falciparum* febrile malaria in children in their first two years of life. However the humoral immune response to malaria is expansive, directed to a broad repertoire of antigens and we cannot rule out a possible protective effect of antibodies. Sero-epidemiological studies are more informative when they include a wide range of immune targets and when they are standardized to allow comparisons across sites.
CHAPTER 3: Does transmission intensity affect young children’s antibody titres in relation to established protective threshold antibody concentrations in their first two years of life

3.1 Introduction

In the previous chapter, we have examined the relationship between antibodies to synthetic GLURP and MSP3 and the risk of febrile malaria in young children. We found associations between increasing antibody levels and increasing risk of malaria, and no evidence for protective antibody responses. Limitations of this previous study were the limited number of antigens that were tested, and the lack of standardized controls that would have allowed the estimation of relative antibody concentrations and a comparison with protective thresholds.

Indeed, in the search of immune correlates of protection to help disentangle conflicting results previously reported from sero-epidemiological studies, it has been recently shown that antibodies need to reach a threshold concentration to achieve protection against febrile malaria in children (Murungi, Kamuyu et al. 2013, Rono, Osier et al. 2013). Briefly, for each specific antibody response, they used a modified Poisson regression to model the association between the risk of clinical malaria and antibody concentration categorised into high vs low responders using a series of arbitrary cut-offs within the range of the levels measured in their study cohort. The protective threshold concentration was then selected based on the log pseudolikelihood of the regression model. These thresholds have then been validated in a
second independent cohort of lower transmission intensity in Kenya (Murungi, Kamuyu et al. 2013). The same methods were used to derive protective thresholds in a child cohort study in coastal Tanzania (Rono, Osier et al. 2013).

In this chapter, I describe our use of the sample and data set described in chapter 2, but with the following additions: a) access to an additional set of antigens, b) a quantification of antibodies using standards and c) a comparison of antibody levels with an additional sample set from Senegal.

As described in chapter 2, initial antigen selection was driven largely by availability. Here I selected 4 further antigens which are used in KEMRI-Wellcome, Kilifi, as reviewed above in 1.7.2.4.

Furthermore, I was able to compare antibody levels in two different sites with differing malaria transmission (i.e. Burkina Faso vs Senegal) with the hypothesis that transmission intensity might determine differences in the starting levels of maternally-derived antibodies, the antibody decay rate and the subsequent endogenous production. The study in Senegal was conducted by the Department of parasitology of Cheikh Anta Diop University in Keur Soce Health and demographic surveillance system to assess malaria morbidity in infancy and investigate the association between antibodies and the risk of malaria. This study in Keur Soce was conducted in collaboration with CNRFP and KEMRI-WTRP within the Malaria Vectored Vaccines Consortium (MVVC). Aliquots of the plasma samples in Banfora (Burkina Faso) and Keur Soce (Senegal) were then prepared and transferred, frozen in dry ice, to KEMRI-CGMRC laboratories (Kenya) where it was possible to use an external control allowing a comparison of the measured antibody levels with these previously established protective thresholds.
3.2 Study objectives

We conducted the present study with the following objectives.

Primary objective:

To investigate the impact of transmission intensity on the dynamics of antibody levels and kinetics in the first 18 months of life.

Secondary objectives:

- To expand the panel of *P. falciparum* antigens in the ELISA to test the hypothesis of an association between total IgG merozoite antigens and protection against clinical malaria
- To include an external control so that antibody levels could be estimated in comparison with established protective threshold concentrations.

3.3 Methods

3.3.1 Ethical statement

The ethical approval for the work in Burkina Faso was obtained from the Institutional Review Board of Centre National de Recherche et de Formation sur le Paludisme (CNRFP) in Burkina Faso. In Senegal, the study was approved by the National Ethics Committee. The parents of each child were informed and an individual written consent obtained prior to performing any study-specific procedure on the child. The studies in both settings were conducted according to the principles of the Declaration of Helsinki.
3.3.2 Study site and population

The study was conducted in Banfora (Burkina Faso) and Keur Soce (Senegal) in parallel. The Banfora site and study population have been described in detail in chapter two. In Keur Soce, the annual rainfall is less than Banfora, at 300 mm with rains from July to October. Transmission of malaria has previously been stable and seasonal, but has recently reduced (Trape, Tall et al.). The parasite rate in children aged less than ten years and living in Keur Soce was 0.3% in 2010 (Sylla, Tine et al. 2015). In all, 150 infants were recruited into the Keur Soce cohort.

3.3.3 Surveillance of malaria morbidity, parasitological examination and haemoglobin typing

The Keur Soce infant cohort was recruited at the same time and using the same criteria as described in chapter two for the Banfora cohort, and the children were monitored as their counterparts in Banfora. No haemoglobin typing was performed for the Keur Soce cohort.

3.3.4 Selection of samples for serological tests

For a subset of 40 children at each site (sampled randomly without replacement among all children with complete serum sample sets using Stata 13.1), samples at baseline, 2, 3, 4, 5, 6, 9, 12, 15 and 18 months were analysed to compare antibody dynamics. The samples of the remaining children in the Banfora cohort were analysed only at baseline, 3, 6, 9, 12 and 18 months and included in the analysis of the association between antibody levels and risk of clinical malaria in that cohort. The decision to select exactly 40 children at each site was arbitrary.
3.3.5 *P. falciparum* merozoite antigens tested in the assays

The following four recombinant merozoite antigens were tested in this study: the His-tagged AMA1 of the 3D7 allelic form (Osier, Weedall et al. 2010), the GST-tagged MSP1-19 of the Wellcome parasite line (Burghaus and Holder 1994), the GST-tagged MSP2 of the Dd2 parasite line (Taylor, Smith et al. 1995) and the MBP-tagged MSP3 of the 3D7 allelic form (Osier, Fegan et al. 2008). We have used all the antigens that were available to us at the time of the assays.

3.3.6 Antibody titres measurement by indirect ELISA

Plasma total IgG to AMA1-3D7, MSP1-19, MSP2-Dd2 and MSP3-3D7 were measured as described elsewhere (Osier, Polley et al. 2007, Murungi, Kamuyu et al. 2013) with samples diluted at 1:500. The indirect ELISA assays testing AMA1-3D7, MSP2-Dd2 and MSP3-3D7 were done in duplicates for the first 372 samples (representing 26% of the total number of samples to analyse) to estimate the variability (coefficient of variation, CV) between the duplicates. The coefficient of variation was calculated as follows: CV= (standard deviation/mean)*100. We met a pre-set criterion that <5% of sample pairs had a CV>20 (i.e. 0%, 0.81% and 1.88% respectively for responses to AMA1-3D7, MSP2-Dd2 and MSP3-3D7) and so proceeded with assays in singles rather than duplicates.

Ninety six wells microplates were coated with 200 µL/well of the *P. falciparum* merozoite antigens diluted at 50 ng/100 µL of coating buffer (15 mm Na₂CO₃, 35 mm NaHCO₃, pH 9.3) and incubated overnight at 4°C. The wells were then washed four times in washing buffer (Phosphate Buffered Saline + 0.05% Tween 20 (PBST)) to remove unbound proteins, and blocked for five hours at room temperature with 200 µL/well of blocking buffer (1%
skimmed milk in PBST) to reduce subsequent non-specific antibody binding. At the end of the blocking time, the plates were washed four times in PBST and the wells reacted overnight at 4°C with 100 µL/well of plasma samples diluted at 1:500 in blocking buffer. After washing the plates four times in PBST to remove unbound human antibodies, 100 µL of a secondary antibody (HRP-conjugated rabbit anti-human IgG / Dako Ltd, Buckinghamshire, UK) at 1:5000 dilution in blocking buffer was added to each wells and left for incubation at room temperature for three hours. The plates were then washed four times with PBST before a substrate solution (distilled water + 0.1M Citric acid + 0.2M Na₂HPO₄ + H₂O₂ + O-phenylenediamine (Sigma, St. Louis, MO, USA)) is reacted with the wells for fifteen minutes. The reaction was then stopped by addition of 25ul of 2M sulphuric acid and the absorbance read at 492 nm. Each plate included positive controls in two wells (Malaria Immune Globulin (MIG) reagent with total IgG concentration of 50mg/ml from the Central Laboratory Blood Transfusion Service SRC, Switzerland) and negative control sera from malaria-naïve UK donors.

All the samples were not assayed the same day. Day to day variation factors were calculated as the ratio of the average optical density (OD) of the positive control on a reference plate by the average OD of the positive control on each other plate. The day to day variation factor of the reference plate is 1. The ODs were then adjusted for day to day variation by multiplying the ODs of the samples on each plate by the corresponding variation factor. After adjusting ODs for day to day variation, for antigens that are GST or MBP-tagged, the actual OD of each sample was obtained by subtracting the OD of the tag. A four-parameter logistic regression was used to model the relationship between serial dilutions of a purified IgG standard (MIG) and the corresponding ODs, and therefore to allow conversion of the study samples ODs into antibody concentrations. Antibody concentrations were then
transformed into arbitrary units (AU) as previously described (Murungi, Kamuyu et al. 2013). Briefly, antibody concentrations were first transformed from log to normal scale, multiplied by the sample dilution factor (500) to get the concentration in µg/mL and finally divided by 1000 to get the concentration in mg/mL assuming that the MIG (50mg/mL) contained 50 AU of antigen-specific antibodies. In the study samples for which the OD could not be converted because they did not fall within the span of the standard curve, the missing concentrations were replaced by the lowest or the highest interpolated concentration for very low ODs and very high ODs, respectively.

3.3.7 Statistical methods

Analysis of the dynamics of antibody titres

We used Pearson correlation tests on log-transformed values to measure how strong the relationship is between antibody levels to a given antigen from one time point to the following one and between antigen-specific antibody titres. To estimate the overall decline of antibodies, we used a Random-Effects regression model of antibody titres on age to account for between and within-infant variability of antibody titres. We calculated the cut-off value for seropositivity as the mean OD of negative controls plus 3 standard deviations. A Fisher Exact test was used to compare seroprevalence of antibodies to the P.f. merozoite antigens between both the sites at baseline. Fractional polynomial regression models were constructed to fit the nonlinear relationship between anti-malaria antibody titre and age. The protective thresholds (for antibodies to AMA1-3D7, MSP1-19, MSP2-Dd2 and MSP3-3D7) displayed in the graphs have been taken from the results of studies conducted in the Kenyan Coast (Murungi, Kamuyu et al. 2013, Rono, Osier et al. 2013).
Analysis of correlates of protection

The outcome measure, febrile malaria, was defined in chapter two as the association of fever (axillary temperature $\geq 37.5^\circ C$ and/or reported fever in the past 24 hours) plus asexual parasitaemia $\geq 10000/\mu L$. An individual malaria exposure index was calculated as described in chapter one. We have investigated the relationship between the putative correlates of protection (seropositivity and antibody titres) and febrile malaria using two approaches. First, we used Cox proportional hazards regression to model the relationship between antibodies and time to first febrile malaria episode, and tested the proportional hazards assumption using Kaplan Meier Method and Schoenfeld residuals. The second approach consisted of modelling the relationship between antibodies and the number of febrile malaria episodes experienced using a negative binomial regression with the Huber-White Sandwich estimator to account for clustering by individual. In the latter approach, the analysis period was restricted to the three months following each time point for malaria serology to account for the short half-life of anti-malaria antibodies (Kinyanjui, Bejon et al. 2009). The antibody titres of the study samples were included in the models as time-changing covariates with the measured value at the beginning of each interval related to the febrile episodes recorded within this interval. The overall significance of categorical variables was estimated using a Wald test. All the antibody titres used in the data analysis are arbitrary units in $\log_{10}$ scale. The data were analysed using GraphPad Prism version 6.00 for Windows, GraphPad Software and Stata 13.1 for Windows, StataCorp LP.

3.4 Results
3.4.1 Malaria morbidity

The characteristics of the Banfora infant cohort, follow up and malaria morbidity are described in detail in chapter two. Briefly, 296 febrile malaria episodes were recorded over 249 child-years, with the number of cases peaking in October each year, giving an incidence rate of 1.2 episodes / child / year (95%CI, 1.06-1.33). In the first six months of life, five infections (two asymptomatic and three febrile) were detected, of which three occurred in the rains, in children aged above five months. In Keur Soce only 4 episodes of asymptomatic malaria were identified in the cohort of 150 infants, and no symptomatic episodes were identified. This is consistent with long-term trends of malaria described elsewhere in Northern Senegal (Trape, Tall et al. 2014).

3.4.2 Variability of antibody titres with time and transmission intensity

Overall, antibody levels were moderately correlated to each other as shown in Table 3.1.

<table>
<thead>
<tr>
<th></th>
<th>AMA1-3D7</th>
<th>MSP1-19</th>
<th>MSP2-Dd2</th>
<th>MSP3-3D7</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMA1-3D7</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSP1-19</td>
<td>0.364 (p&lt;0.001)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSP2-Dd2</td>
<td>0.466 (p&lt;0.001)</td>
<td>0.472 (p&lt;0.001)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>MSP3-3D7</td>
<td>0.392 (p&lt;0.001)</td>
<td>0.214 (p&lt;0.001)</td>
<td>0.413 (p&lt;0.001)</td>
<td>1</td>
</tr>
</tbody>
</table>

Antibody titres to AMA1-3D7, MSP1-19, MSP2-Dd2, MSP3-3D7 were strongly correlated from time point to time point during the first six months of life in both settings regardless of the transmission intensity (r, 0.86-0.98 in Keur Soce, 0.86-0.91 in Banfora for AMA-1) (Figure 3.1). After the first 6 months of life, antibody titres were weakly to moderately
correlated to each other in Keur Soce (r, 0.21-0.47) but not correlated in Banfora (-0.21 to 0.03).

At baseline, the seroprevalence of antibodies to merozoite antigens was significantly higher in the Banfora cohort compared with Keur Soce cohort except for antibodies to MSP1-19 (Table 3.2). After a steady decline in the first six months of life regardless of the transmission intensity, the seroprevalence for responses to all the antigens tested was below 20% for the remaining monitoring period in Keur Soce, the low transmission area. In Banfora where the transmission is higher, the seroprevalence peaked after six months for MSP1-19 and MSP3-3D7 corresponding approximately to the beginning of the first rainy season experienced by the children (Figure 3.2).

Table 3.2. Differences in seroprevalence of antibodies to merozoite antigens at baseline between Banfora (high transmission intensity) and Keur Soce (low transmission intensity). * One-sided Fisher’s Exact test.

<table>
<thead>
<tr>
<th></th>
<th>AMA1-3D7</th>
<th>MSP1-19</th>
<th>MSP2-Dd2</th>
<th>MSP3-3D7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banfora</td>
<td>0.97 (0.92, 1.02)</td>
<td>0.33 (0.19, 0.48)</td>
<td>0.95 (0.88, 1.02)</td>
<td>0.61 (0.46, 0.77)</td>
</tr>
<tr>
<td>Keur Soce</td>
<td>0.80 (0.68, 0.92)</td>
<td>0.25 (0.12, 0.38)</td>
<td>0.57 (0.42, 0.73)</td>
<td>0.23 (0.10, 0.35)</td>
</tr>
</tbody>
</table>

p=0.016* p=0.285* p<0.001* p<0.001*
Figure 3.1. Correlation matrix of antibodies to AMA1-3D7 between time points for antibody titres measurement from baseline to month 18. The X and Y axis indicate antibody titres at the respective timepoints.

Figure 3.2. Comparative dynamics of anti-merozoite antibody seroprevalences between Banfora (high transmission intensity) and Keur Soce (low transmission intensity). The next rainy season following the recruitment started approximately 5 months following the end of the recruitment. We then hypothesize that the sudden increase in the seroprevalence in Banfora is mostly explained by seasonality of malaria transmission.
No confidence interval was reported for the protective thresholds established by Murungi and colleagues (Murungi, Kamuyu et al. 2013); however, the antibody levels in our cohorts were mostly well below the protective thresholds throughout the follow up. Only anti-MSP2 antibodies increased appreciably from month 9 in the high transmission setting (Figure 3.3, Figure 3.4).

Figure 3.3. Comparative dynamics of individual antibody titres between Keur Soce and Banfora children. The dashed lines represent the protective thresholds established in children living in the Kenyan Coast. Each line represents an individual trajectory of antibody concentration.
Figure 3.4. Lines of best fit for the concentrations of antibodies to \textit{P. falciparum} merozoite antigens.

The average antibody decay rates were similar at both sites only for antibodies to MSP1-19, higher in Banfora for MSP2-Dd2 and MSP3-3D7 and moderately higher in Keur Soce for AMA1-3D7 (Table 3.3).

Table 3.3. Average antibody decay rates in Banfora (High transmission intensity) and Keur Soce (Low transmission intensity). Excepting MSP1-19, average antibody decay rates were different in both the sites.

<table>
<thead>
<tr>
<th>Antigens</th>
<th>Banfora cohort (site 1)</th>
<th>Keur Soce cohort (site 2)</th>
<th>Interaction Age vs site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Decay rate (log10 AU/month), 95 %</td>
<td>p value</td>
<td>Decay rate (log10 AU/month), 95 % CI</td>
</tr>
<tr>
<td>AMA1-3D7</td>
<td>-0.49 (-0.52, -0.45)</td>
<td>p&lt;0.001</td>
<td>-0.56 (-0.59, -0.53)</td>
</tr>
<tr>
<td>MSP1-19</td>
<td>-0.32 (-0.37, -0.27)</td>
<td>p&lt;0.001</td>
<td>-0.27 (-0.32, -0.22)</td>
</tr>
<tr>
<td>MSP2-Dd2</td>
<td>-0.47 (-0.50, -0.44)</td>
<td>p&lt;0.001</td>
<td>-0.23 (-0.27, -0.19)</td>
</tr>
<tr>
<td>MSP3-3D7</td>
<td>-0.23 (-0.26, -0.20)</td>
<td>p&lt;0.001</td>
<td>-0.12 (-0.15, -0.10)</td>
</tr>
</tbody>
</table>
3.4.3 Relationship between antibodies and incidence of febrile malaria

Univariate analysis

This analysis applies to Banfora only. In the time to event univariate analysis none of the antigen-specific antibodies, in terms of levels or seropositivity, was associated with protection. Antibodies to AMA1-3D7 (HR: 1.34, 95%CI: 1.11-1.62, p=0.002) and MSP1-19 (HR: 1.44, 95%CI: 1.19-1.74, p<0.001) were significantly associated with a higher risk of febrile malaria, but season was the strongest significant risk factor for febrile malaria (HR: 8.28, 95%CI: 2.18-31.44, p=0.002). The results of the event count analysis were similar except for anti-AMA1-3D7 antibodies that appeared as significantly associated with protection against febrile malaria episodes (HR: 0.89, 95%CI: 0.80-0.98, p=0.015). Children with haemoglobin CC type had a lower risk of febrile malaria compared with haemoglobin AA children. Age was significantly associated with risk of febrile malaria (Table 3.4). In both the models, the highest level of education was significantly associated with protection and children born in the last quarter of the year had significantly lower risk of febrile malaria compared to those born in January. The results of the univariate models of risk of malaria are shown in table 3.4.
Table 3.4. Univariate models of risk of *P. falciparum* febrile malaria.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Cox Proportional Hazards</th>
<th></th>
<th>Negative binomial models</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR 95% CI</td>
<td>p</td>
<td>IRR 95% CI</td>
<td>p</td>
</tr>
<tr>
<td>Age</td>
<td>-</td>
<td>-</td>
<td>1.14 [1.12, 1.17]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Female</td>
<td>0.84 [0.52, 1.35]</td>
<td>0.472</td>
<td>0.76 [0.50, 1.16]</td>
<td>0.205</td>
</tr>
<tr>
<td>Weight (baseline)</td>
<td>0.95 [0.64, 1.42]</td>
<td>0.817</td>
<td>1.07 [0.75, 1.51]</td>
<td>0.722</td>
</tr>
<tr>
<td>Length (baseline)</td>
<td>1.02 [0.92, 1.12]</td>
<td>0.715</td>
<td>1.04 [0.97, 1.12]</td>
<td>0.300</td>
</tr>
<tr>
<td>Fetal Hb rate (baseline)</td>
<td>0.98 [0.96, 1.001]</td>
<td>0.072</td>
<td>0.99 [0.97, 1.002]</td>
<td>0.084</td>
</tr>
<tr>
<td>Haemoglobin type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AS*</td>
<td>NA</td>
<td>-</td>
<td>NA</td>
<td>-</td>
</tr>
<tr>
<td>AC</td>
<td>1.81 [0.97, 3.38]</td>
<td>0.198</td>
<td>1.32 [0.81, 2.16]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CC</td>
<td>0.36 [0.05, 2.59]</td>
<td>0.54</td>
<td>0.54 [0.09, 3.11]</td>
<td></td>
</tr>
<tr>
<td>Anti-AMA1 titres</td>
<td>1.34 [1.11, 1.62]</td>
<td>0.002</td>
<td>0.89 [0.80, 0.98]</td>
<td>0.015</td>
</tr>
<tr>
<td>Anti-MSPI1 titres</td>
<td>1.44 [1.19, 1.74]</td>
<td>&lt;0.001</td>
<td>1.28 [1.16, 1.42]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anti-MSPI2 titres</td>
<td>1.27 [0.95, 1.70]</td>
<td>0.103</td>
<td>1.01 [0.85, 1.21]</td>
<td>0.911</td>
</tr>
<tr>
<td>Anti-MSPI3 titres</td>
<td>1.28 [0.70, 2.34]</td>
<td>0.428</td>
<td>0.73 [0.42, 1.26]</td>
<td>0.260</td>
</tr>
<tr>
<td>Seropositivity to AMA1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seronegative</td>
<td>1.62 [0.92, 2.87]</td>
<td>0.096</td>
<td>0.70 [0.47, 1.05]</td>
<td>0.087</td>
</tr>
<tr>
<td>Serumpositive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seropositivity to MSPI1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seronegative</td>
<td>1.24 [0.61, 2.50]</td>
<td>0.550</td>
<td>1.35 [0.75, 2.45]</td>
<td>0.320</td>
</tr>
<tr>
<td>Serumpositive</td>
<td>1.24 [0.61, 2.50]</td>
<td>0.550</td>
<td>1.35 [0.75, 2.45]</td>
<td>0.320</td>
</tr>
<tr>
<td>Month of birth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>January</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>October</td>
<td>0.19 [0.06, 0.64]</td>
<td>0.012</td>
<td>0.21 [0.07, 0.64]</td>
<td>0.002</td>
</tr>
<tr>
<td>November</td>
<td>0.44 [0.23, 0.83]</td>
<td>0.45</td>
<td>0.27 [0.17, 0.73]</td>
<td></td>
</tr>
<tr>
<td>December</td>
<td>0.53 [0.29, 0.96]</td>
<td>0.66</td>
<td>0.41 [0.24, 0.64]</td>
<td></td>
</tr>
<tr>
<td>EPI status (baseline)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Up to date</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not up to date</td>
<td>0.94 [0.45, 1.96]</td>
<td>0.825</td>
<td>1.06 [0.60, 1.86]</td>
<td>0.845</td>
</tr>
<tr>
<td>Age mother (baseline)</td>
<td>1.0 [1.00, 1.08]</td>
<td>0.042</td>
<td>1.02 [0.99, 1.06]</td>
<td>0.135</td>
</tr>
<tr>
<td>Gravidity status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primigravidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multigravidae</td>
<td>1.12 [0.61, 2.06]</td>
<td>1.08</td>
<td>1.08 [0.61, 1.89]</td>
<td>0.797</td>
</tr>
<tr>
<td>ITN use (pregnancy)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.24 [0.61, 2.50]</td>
<td>0.550</td>
<td>1.35 [0.75, 2.45]</td>
<td>0.320</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note that the analysis approach is similar to that taken in the previous chapter for table 2.5, except for slight variations in the individuals missing serological results and therefore there are slight variations in the coefficients presented between the tables.
### Cox Proportional Hazards vs. Negative binomial models

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Cox Proportional Hazards</th>
<th>Negative binomial models</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPTp courses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>1.47 [0.49, 4.41]</td>
<td>1.70 [0.71, 4.09]</td>
</tr>
<tr>
<td>2 or 3</td>
<td>1.15 [0.41, 3.19]</td>
<td>1.07 [0.47, 2.42]</td>
</tr>
<tr>
<td>Education level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Primary</td>
<td>1.52 [0.92, 2.51]</td>
<td>1.38 [0.91, 2.09]</td>
</tr>
<tr>
<td>Secondary or</td>
<td>0.45 [0.18, 1.15]</td>
<td>0.31 [0.15, 0.64]</td>
</tr>
<tr>
<td>Zone of residence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Urban</td>
<td>0.31 [0.16, 0.61] &lt;0.001</td>
<td>0.30 [0.16, 0.56] &lt;0.001</td>
</tr>
<tr>
<td>Mixed</td>
<td>1.32 [0.78, 2.23]</td>
<td>1.22 [0.80, 1.84]</td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry season</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Rains</td>
<td>8.28 [2.18, 31.44]</td>
<td>4.32 [2.88, 6.49]</td>
</tr>
<tr>
<td>Malaria Exposure</td>
<td>1.10 [1.05, 1.14] &lt;0.001</td>
<td>1.08 [1.03, 1.13]</td>
</tr>
</tbody>
</table>

### Multivariable analysis

There was only limited collinearity between the different antibody titres (VIFs<2; mean VIF=1.44) and no significant deviation from the proportional hazards assumption (Figure 3.5, Figure 3.6). In the multivariable time to event analysis, foetal haemoglobin rate was weakly but significantly associated with protection (HR: 0.97, 95%CI: 0.94-0.99, p=0.004). Season remained the strongest risk factor (HR: 9.39, 95%CI: 2.32-37.99, p=0.002), and exposure index was also associated with a risk of malaria (HR: 1.10, 95%CI: 1.05-1.15, p<0.001). There was a tendency of anti-MSP1-19 antibodies to be associated with higher risk of febrile malaria (HR: 1.40, 95%CI: 1.09-1.80, p=0.008), but overall the correlation between antibodies and increased risk of malaria was reduced by adjusting for exposure index and season. In the event count analysis, none of the antigen-specific antibodies was significantly associated with febrile malaria. Season was confirmed as the strongest risk factor. An interaction of weak effect size between season and individual exposure index was
observed (IRR=1.06, p=0.042) in the event count analysis. The results of the multivariable analysis of risk of malaria are shown in table 3.5.

Figure 3.5. Estimation of child survival in relation to anti-malaria antibody tertiles. (A) antibodies to AMA1-3D7, (B) antibodies to MSP1-19, (C) antibodies to MSP2-Dd2, (D) antibodies to MSP3-3D7.

Figure 3.6. Proportional Hazards assumption test. The lowess line shows the variation in hazard over time.
Table 3.5. Multivariable models of risk of *P. falciparum* febrile malaria.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Cox Proportional Hazards model</th>
<th>Negative Binomial model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age</td>
<td>1.14</td>
<td>[1.09, 1.19]</td>
</tr>
<tr>
<td>Anti-AMA1 titres</td>
<td>1.40</td>
<td>[1.09, 1.80]</td>
</tr>
<tr>
<td>Anti-MSP1 titres</td>
<td>0.89</td>
<td>[0.63, 1.27]</td>
</tr>
<tr>
<td>Anti-MSP2 titres</td>
<td>1.20</td>
<td>[0.63, 2.27]</td>
</tr>
<tr>
<td>Fetal Hb rate (baseline)</td>
<td>0.97</td>
<td>[0.94, 0.99]</td>
</tr>
<tr>
<td>Haemoglobin type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>AS*</td>
<td>NA</td>
<td>-</td>
</tr>
<tr>
<td>AC</td>
<td>1.75</td>
<td>[0.88, 3.51]</td>
</tr>
<tr>
<td>CC</td>
<td>0.28</td>
<td>[0.04, 2.19]</td>
</tr>
<tr>
<td>ITN use (pregnancy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.05</td>
<td>[0.47, 2.36]</td>
</tr>
<tr>
<td>Month of birth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>January</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>October</td>
<td>0.27</td>
<td>[0.07, 1.01]</td>
</tr>
<tr>
<td>November</td>
<td>0.71</td>
<td>[0.34, 1.50]</td>
</tr>
<tr>
<td>December</td>
<td>0.70</td>
<td>[0.36, 1.39]</td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry season</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Rains</td>
<td>9.39</td>
<td>[2.32, 37.99]</td>
</tr>
<tr>
<td>Malaria exposure index</td>
<td>1.10</td>
<td>[1.05, 1.15]</td>
</tr>
</tbody>
</table>

3.5 Discussion

The titres of antibodies to all the four antigens tested were below the protective thresholds except for a few outlying results. After a consistent decline up to six months of age, only antibodies to MSP2 showed a steady increase up to month 18 in the high transmission setting (Banfora). Overall, we did not find any protective effect in the investigation of the association between antibodies to *P. falciparum* merozoite antigens and febrile malaria. Antibody titres to some merozoite antigens (AMA1-3D7 and MSP1-19) were rather a maker of exposure to malaria, evidenced by the association with risk and the fact that this association was diminished after adjusting for the exposure index.
Average antibody decay rates were similar in both the settings only for antibodies to MSP1-19. Reduced exposure to infectious bites during the first six months in the high and seasonal transmission setting (Banfora) is a likely explanation of this similarity since only five malaria infections were detected during this period in Banfora, of which three occurred in the first rainy season in children aged above five months. However, infections were detected by microscopy in our study and we cannot rule out the possibility of submicroscopic infections in the high transmission setting (Banfora). The limited acquisition of antibodies and the strong correlation of antibody levels between time points in Keur Soce indicates reduced exposure throughout the monitoring period. In contrast, in Banfora antibody levels were more variable from time point to time point, indicating that exposure to malaria was stimulating antibody production in some children, and furthermore that increases in antibody titres were often transient. Indeed, it has been previously shown that antibody titres are short-lived in children (Kinyanjui, Conway et al. 2007).

In the first six months of life, antibody concentrations were mostly below protective levels in both sites regardless of the transmission intensity. We would have expected this in Keur Soce but not in Banfora where the transmission is high. The existence of a saturation point for transplacental antibody transfer from mothers with high antibody concentrations has been suggested by previous studies (Palmeira, Quinello et al. 2012). If such a saturation point was below the protective levels, that could explain these paradoxically low antibody levels in the infants born from the mothers living in the high transmission setting. Two other studies have reported similar findings in the sense that young children were seen to be slow in their endogenous antibody production following the decline of maternally-derived antibodies (Duah, Miles et al. 2010, Murungi, Sondén et al. 2016).
Differing intensity of exposure to malaria may or may not have an impact on the development of endogenous antibodies in infants. Singer and colleagues have even reported paradoxically higher antibody response in infants with lower malaria exposure for MSP1-19 (Singer, Mirel et al. 2003). In our study, we did not observe this and mean antibody levels were significantly higher or similar between high and low transmission settings throughout the time course of the study.

We found that anti-AMA1-3D7 antibodies appeared as a marker of exposure (positively associated with the risk of febrile malaria) in our univariate analysis, which is consistent with the conclusions of Riley et al. (Riley, Wagner et al. 2000). Furthermore, adjusting for exposure using the exposure index attenuated the positive association between anti-AMA1 antibodies and increasing risk, consistent with anti-AMA1 antibodies acting as a marker of exposure in our study. Our results are in keeping with the findings of previous studies in older children (Dodoo, Aikins et al. 2008, Nebie, Tiono et al. 2008). Similarly, in our study, antibody titres to MSP1-19, MSP2-Dd2 and MSP3-3D7 were not associated with protection as in other infant cohort studies (Kitua, Urassa et al. 1999, Riley, Wagner et al. 2000, Zhou, Xiao et al. 2002).

The apparent variation in results between infants and older children may be explained by the presence of a protective threshold. Murungi et al. and Rono et al. have analysed two independent cohorts of children and established protective threshold concentrations for some merozoite antigens (Murungi, Kamuyu et al. 2013, Rono, Osier et al. 2013). Even in a high transmission setting such as Burkina Faso, we found that antibody titres in the first six months of life for the four tested antigens were well below these protective threshold concentrations. Furthermore, there was no association with protection. We conclude that these maternally acquired antibodies are not the protective mechanism. If the antibody levels
are not protective in these infants, then what is the basis of their apparent early and short-lived protection period against clinical manifestations of malaria?

In the present study, we have investigated the role of antibodies (total IgG) in the resistance to the clinical manifestations of blood-stage malaria infection. However, there is evidence for an association between the cytophilic subclasses (IgG3 and IgG1) and clinical protection (Bouharoun-Tayoun and Druilhe 1992, Soe, Theisen et al. 2004, Lusingu, Vestergaard et al. 2005, Roussilhon, Oeuvray et al. 2007). We have not measured the titres of the cytophilic subclasses and therefore cannot rule out the existence of a protective effect of these cytophilic subclasses. Other possibilities are that other effector mechanisms or other factors are working in the background. Indeed, although we have tested few additional *P. falciparum* antigens in this study, hundreds of potential immune targets remain unexplored. Osier and colleagues have shown that some poorly investigated or untested *P. falciparum* proteins have similar and even superior potential protective efficacy against clinical malaria than the extensively studied current malaria vaccine candidates. Moreover, they have shown that the cumulative responses of some top-ranked antigens best correlate with protection (Osier, Mackinnon et al. 2014). Besides that, there is evidence that other antibody-mediated protective mechanisms not necessarily correlated with ELISA measurements may be operating. Indeed, Osier and colleagues have consistently shown that opsonic phagocytosis was strongly associated with protection when no significant protective effect was observed with total IgG or IgG sub-classes (Osier, Feng et al. 2014). Joos and colleagues, investigating another mechanism, found that antibody-dependent respiratory burst (ADRB) well correlated with protection against clinical malaria when anti-merozoite IgG levels did not (Joos, Marrama et al. 2010) although they recently reported high correlation of IgG responses with ADRB (Joos, Varela et al. 2015). Although evidence from observational or
experimental studies in humans is lacking, low para-aminobenzoic acid diet that would be achieved in exclusive breastfeeding has been associated with resistance to malaria in mice experiments (Kicska, Ting et al. 2003). The role of foetal haemoglobin in the reduced susceptibility of young infants to febrile malaria remains unclear. Initially believed to impair parasite growth in infected erythrocytes (Pasvol, Weatherall et al.), it was later suggested that it rather acts cooperatively with antibodies to impair cyto-adherence of infected erythrocytes (Amaratunga, Lopera-Mesa et al. 2011). Earlier entomological studies found an increasing biting rate with increasing age (therefore body size or surface), suggesting that the apparent early infancy resistance to clinical malaria is actually a reduced exposure to infectious mosquito bites (Muirhead-Thomson 1951, Carnevale, Frezil et al. 1978). Recent studies suggest an association between skin microbiome and attractiveness to malaria vectors (Verhulst and Takken 2014) and it might be worthy to investigate whether particular features of young infants’ microbiome makes them less attractive to mosquitoes in search of blood meals. Previous data on passive transfer of antibodies from cord blood suggest that antibodies are involved (Edozien, Gilles et al. 1962).

3.6 Conclusion

Even at high intensity of malaria transmission, antibody levels to the tested *P. falciparum* merozoite antigens (AMA1-3D7, MSP1-19, MSP2-Dd2 and MSP3-3D7) remained low compared to the previously established protective threshold concentrations. In addition, the antibodies were not significantly associated with a reduced risk of malaria raising more questions on the basis of the early apparent protection against febrile malaria.

However, these results should be interpreted with caution because of the limitations of this study. The protective thresholds used in the present study have been established in cohorts
including children more than 2 years old in addition to infants. In our cohort, the lack of any protective effect did not allow us to determine specific thresholds for this age group. Furthermore, the overlapping of the first few months of life with the low malaria transmission season made it practically impossible to assess the role of maternal antibodies at the higher titres seen in that period. However, if antibody thresholds are related to real biological phenomena than we would expect, the thresholds should be similar across study sites and age group. Hence the main contribution of our work is the demonstration that antibody levels in infants to the selected merozoite antigens are well below previously defined protective thresholds, and therefore that these are unlikely to be responsible for mediating protection in vivo. Further work to identify protective antibody responses might include assessment of antibodies to other targets, including red cell surface antigens, and functional assays in which the interaction of antibodies with immune cells is taken into account (Osier, Feng et al. 2014).
4. CHAPTER 4: Role of serology among different biomarkers used for malaria transmission hotspots detection

4.1 Introduction

The previous chapters have examined antibody responses as potential markers of protection. In both instances, we found that antibodies were in fact markers of exposure rather than protection. This raises the question of confounding in observational immunepidemiological studies due to heterogeneity of exposure. This present chapter examines in more detail the spatial heterogeneity of exposure to malaria and different metrics which can be used to characterize it.

Heterogeneity of the distribution of infectious disease seems to be a universal phenomenon. It has been well characterized for sexually transmitted and some vector-borne diseases with 80% of the disease burden tending to cluster in only 20% of the population at risk (Woolhouse, Dye et al. 1997). This phenomenon has been observed and reported for malaria (Gamage-Mendis, Carter et al. 1991, Mwangi, Fegan et al. 2008). Moreover, seasonality is observed in most malaria transmission settings and most malaria cases occur in rainy seasons during which conditions are suitable for the spread of malaria mosquito vectors (Roca-Feltrer, Schellenberg et al. 2009). This heterogeneity in distribution can be translated into spatial or space-time clusters of disease cases that have the practical advantage of being more accessible for disease surveillance and control interventions.

The concept of hotspot, sometimes termed cluster, has been applied to various disciplines such as criminology, forestry, wildlife research (Fei 2010, Maingi, Mukeka et al. 2012,
Uittenbogaard and Ceccato 2012) and can be defined as a sub-area of a main area with a concentration of an event of interest higher than what would be expected if that event was distributed randomly in the main area. Hotspots of malaria transmission have been observed and reported to occur at various scales ranging from regional to homestead level (Bejon, Williams et al. 2014).

The risk and spread of malaria are driven by various types of factors. Factors for which evidence exists include environmental factors such as temperature, altitude, distance to water bodies, wind direction and urbanization (Brooker, Clarke et al. 2004, De Silva and Marshall 2012, Midega, Smith et al. 2012). They also include intrinsic human characteristics such as red blood cell polymorphisms, differential host attractiveness to anopheline mosquitoes, foetal haemoglobin and dietary factors in early infancy, and extrinsic actors such as farming practices, socio-economic factors, housing design, level of education and behaviour (Pasvol, Weatherall et al., Kicska, Ting et al. 2003, Lacroix, Mukabana et al. 2005, Yadouléton, N'Guessan et al. 2010, Amoako, Asante et al. 2014, Sonko, Jaiteh et al. 2014, Fernández-Grandon, Gezan et al. 2015, Malaria Genomic Epidemiology Network 2015, Tusting, Ippolito et al. 2015).

The interest in detecting malaria transmission hotspots is that, as a hypothetical driving force of malaria infection spread, they represent an opportunity for targeted control interventions that are expected to be more efficient than untargeted interventions and ultimately benefit the whole community (Bousema, Griffin et al. 2012).

Challenges in targeting hotspots of transmission include the choice of the transmission marker to measure, the choice of the method of detection, the choice of the scale at which to detect them, when to detect them and how stable they are (Bousema, Griffin et al. 2012,
Asymptomatic parasite carriage, clinical malaria episodes, vector biting intensities or antibody responses to selected malaria antigens have been proposed as potential markers of malaria transmission in detecting hotspots in areas of low to moderate transmission intensity (Bousema, Drakeley et al. 2010).

4.2 Study objectives

Primary objective:

To detect malaria transmission hotspots using different biomarkers within the same study area and time period and examine the spatial associations between these hotspots.

Secondary objectives:

- To examine the spatial correlations of the different markers using a raster map of the study area.
- To examine the spatial stability of the detected hotspots.

4.3 Methods

4.3.1 Ethical approval

The study was approved by the Kenya Medical Research Institute (KEMRI) National Ethical Review Committee. The study procedures were explained and a written informed consent was sought and obtained from the parents/guardians of each individual child participating in the study prior to any study procedures. The study was conducted according to the Declaration of Helsinki.
4.3.2 Study area, population and surveillance method

The data used in the present study were collected from 2012 to 2013 taken from a study area in Ganze in Kilifi County on the Kenyan Coast (Figure 4.1). Two cohorts were monitored in this study: one for clinical episodes during two years of follow up and a second cohort monitored via cross-sectional surveys.

A total of 831 children aged 5-17 months residing in 633 homesteads were recruited into a vaccine trial in which longitudinal monitoring of malaria episodes was done (RTS 2015). Febrile malaria episodes were detected by passive case detection as previously described (RTS 2015). Clinical malaria was defined as the presence of fever (axillary temperature ≥ 37.5°C) or history of fever in the past 24 hours and parasitaemia ≥ 2500 µl (Mwangi, Ross et al. 2005).
The second cohort of children and adults was recruited by selecting homesteads at random based on enumeration areas. Twenty enumeration areas were selected at random and then 25 households were selected from each enumeration area. This provided a sample of 800 individuals living in the same study area as the children monitored for clinical malaria but in different homesteads. The surveys were used to measure asymptomatic parasitaemia, by microscopy of thick and thin blood smears and by PCR, and antibody responses to *P. falciparum* merozoite antigens (apical membrane antigen (AMA1) and merozoite surface protein 1 (MSP1-19) by indirect ELISA.

Figure 4.1. Study area. Spatial distribution of homesteads sampled for the different studies.
Data from an entomological study also conducted within the same study area were also available for 2012 and 2013. Mosquito captures using CDC light traps were conducted in 150 sampled houses, chosen at random, at six rounds covering the dry season, the long and the short rains. *Anopheles gambiae* and *Anopheles funestus* were the major human malaria vectors captured in the study area. Longitude and latitude data for each homestead involved in the clinical and entomological studies was recorded during the surveys.

4.3.3 Detection of hotspots

Using the scan statistic method by Kulldorff (Kulldorff 1997), we examined the study area for clusters of individuals positive for the biomarker of interest, at rates higher than what we would have expected if the distribution of positive cases was random in the study area. The following markers were examined: clinical malaria, positive blood films, positive PCR tests, seropositivity to AMA1 and MSP1 and densities of anopheline mosquitoes. The cut offs for seropositivity to AMA1 and MSP1 in normalized optical density were respectively 0.132 and -0.108 in 2012, and -0.091 and 0.13 in 2013, defined using previously described methods (Bousema, Youssef et al. 2010). The application of the Scan statistic by SaTScan has been described previously (Kulldorff 1997). Briefly, a scanning window (set to “circular” in our analysis) moves across the study area, and the maximum number of events that are captured by the window is recorded. The maximum window size was arbitrarily set to 30% of the population at risk. Though some attempts have been proposed to optimize maximum window size setting in scan statistic (Ma, Yin et al. 2016), none of these approaches have been validated and no clear guidelines exist to date. We have selected a window size below the default (and maximum size, 50%) in Kulldorf Scan statistics (implemented in SaTScan software (Kulldorff 2014)) because it has been argued that using a too large window size may result in a single large cluster covering multiple smaller cluster with lower rates (Ball,
LeFevre et al. 2008). Each of the different scanning windows is evaluated as a potential cluster by the calculation of a likelihood ratio test statistic based on the observed, expected and total number of cases. The corresponding p value is calculated using a Monte Carlo method (Dwass 1957).

To detect hotspots of clinical malaria cases, we used a discrete Poisson model where the cases were the clinical malaria cases detected at each homestead; the population was the population monitored in the corresponding homesteads.

A Bernoulli probability model was used to detect hotspots of positive blood films, hotspots of positive PCR tests and hotspots of individuals seropositive for AMA1 and MSP1. The cases were the individuals with a positive test (blood film, PCR or ELISA) in each homestead; the controls were defined as the individuals with negative tests in the corresponding homestead.

To detect hotspots of *Anopheles* mosquitoes, we used a discrete Poisson model in which the cases were the *Anopheles* mosquitoes captured in each house; the population was defined as the number of homesteads.

For each detected hotspot, a relative risk (RR) was computed. The RR is the magnitude of the risk of malaria for individuals residing within the hotspot compared with those residing outside the hotspot. It is calculated as the ratio of the estimated risk within the hotspot and the estimated risk in the surrounding area. The estimated risk is calculated as the number of observed cases divided by the number of expected cases if the null hypothesis was true i.e. if the distribution of cases was totally random. The threshold for statistical significance of the hotspots was set to 0.05.
Observations with missing coordinates data were 4.5%, 3% and < 1% for serological, entomological and clinical surveillance data respectively; they were dropped prior to any analysis. Observations with missing data for anopheline mosquito capture and AMA1/MSP1 serology were < 1% in the respective datasets.

4.3.4 Statistical analysis

The markers of malaria transmission were summarized at the homestead level by calculating the sum of clinical malaria cases, sum of positive blood films, sum of positive PCR tests and geometric mean antibody titre.

To aggregate the data, we have derived a raster map of the study area from a Kilifi County administrative map (the original shapefile was downloaded from http://www.wri.org/resources/data-sets/kenya-gis-data, accessed 04/08/2015). The resolution of the raster surface was set to 0.9 km x 0.9 km. At this resolution and for each marker, each homestead was assigned to a unique cell by computing the shortest distance between the index homestead and the surrounding grid points. The values of each of the markers summarized at the homestead level were then aggregated at the grid cell level. The statistics used to aggregate the markers were the mean for counts of positive blood films and positive PCR tests, the weighted mean for count of clinical malaria cases, the weighted geometric mean for antibody titres and the mean for vector numbers. This aggregation was repeated for each year. We then examined spatial correlations between these markers using Spearman Rank correlation coefficient on aggregated data.

SaTScanTM v9.4.1 was used to detect the hotspots and Stata 13.1 for Windows, StataCorp LP was used to perform data analysis and produce the maps.
4.4 Results

4.4.1 Malaria morbidity and transmission markers in the study area

The distribution of all the markers of malaria transmission is highly right skewed as shown in Figure 4.2 and Figure 4.3. Most individuals or homesteads have zero quantity of the measured transmission markers indicating a very low transmission intensity.

Figure 4.2. Distribution of clinical, parasitological and entomological markers of malaria transmission. The data are aggregated at homestead level.
The clinical, parasitological, serological and entomological markers measured in these studies are summarized in Table 4.1. Malaria transmission intensity was low in 2012 with parasite rate by microscopy at 2% in the general population, and underwent a tenfold decline, at 0.2% in 2013. Similarly, there was a decline in densities of malaria vectors in the study area in 2013 with anopheline mosquitoes captured in only 6% of the surveyed houses compared with 24% in 2012. Table 4.2 shows the detailed frequency distribution of seropositivity to AMA1 and MSP1.
Table 4.1. Yearly summary of clinical, parasitological, serological and entomological markers in the study area.

<table>
<thead>
<tr>
<th></th>
<th>Year</th>
<th>2012</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Longitudinal study</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homestead locations / Population</td>
<td></td>
<td>633 / 831</td>
<td>633 / 831</td>
</tr>
<tr>
<td>Locations with febrile malaria</td>
<td></td>
<td>65 (10.3%)</td>
<td>22 (3.5%)</td>
</tr>
<tr>
<td>Febrile malaria cases</td>
<td></td>
<td>112 (17.5%)</td>
<td>28 (3.4%)</td>
</tr>
<tr>
<td><strong>Cross-sectional studies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homestead locations / Population</td>
<td></td>
<td>211 / 779</td>
<td>183 / 797</td>
</tr>
<tr>
<td>Locations with positive blood films</td>
<td></td>
<td>8 (3.8%)</td>
<td>2 (1.1%)</td>
</tr>
<tr>
<td>Locations with positive PCR tests</td>
<td></td>
<td>31 (14.7%)</td>
<td>22 (12%)</td>
</tr>
<tr>
<td>Prevalence of infection (Microscopy), (95% CI)</td>
<td>2% (1.2-3.2)</td>
<td>0.2% (0.03-0.9)</td>
<td></td>
</tr>
<tr>
<td>Prevalence of infection (PCR), (95% CI)</td>
<td>6.2% (4.6-8)</td>
<td>3.3% (2.2-4.8)</td>
<td></td>
</tr>
<tr>
<td>Seroprevalence of antibodies to AMA1, (95% CI)</td>
<td>36.1% (32.7-39.5)</td>
<td>20.4% (17.7-23.4)</td>
<td></td>
</tr>
<tr>
<td>Seroprevalence of antibodies to MSP1, (95% CI)</td>
<td>19.9% (17.1-22.9)</td>
<td>10.5% (8.5-12.9)</td>
<td></td>
</tr>
<tr>
<td><strong>Entomological surveys</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>House locations</td>
<td>145</td>
<td>142</td>
<td></td>
</tr>
<tr>
<td>Locations with Anopheles</td>
<td>35 (24%)</td>
<td>8 (6%)</td>
<td></td>
</tr>
<tr>
<td>Range of Anopheles captured/house</td>
<td>0 – 17</td>
<td>0 - 6</td>
<td></td>
</tr>
<tr>
<td>Total Anopheles captured</td>
<td>101</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Total An. Gambiae captured</td>
<td>85</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Total An. Funestus captured</td>
<td>16</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.2. Contingency table of seropositivity to AMA1 and MSP1 in the cross-sectional studies in 2012 and 2013. Sero+ stands for seropositivity.

<table>
<thead>
<tr>
<th></th>
<th>2012</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sero+ to MSP1</td>
<td>Sero+ to AMA1</td>
<td>Sero+ to MSP1</td>
</tr>
<tr>
<td>Total</td>
<td>0   1     .   Total</td>
<td>0   1     .   Total</td>
</tr>
<tr>
<td>0</td>
<td>459 154 9 622</td>
<td>0   603 103 3 709</td>
</tr>
<tr>
<td>1</td>
<td>31 124 0 155</td>
<td>1   25 59 0 84</td>
</tr>
<tr>
<td>.</td>
<td>2 0 0 2</td>
<td>.   4 0 0 4</td>
</tr>
<tr>
<td>Total</td>
<td>492 278 9 779</td>
<td>Total</td>
</tr>
</tbody>
</table>

4.4.2 Malaria hotspots in the study area

For clinical malaria, asymptomatic parasitaemia determined by microscopy and anopheline mosquitoes captures, there were fewer positive cases in 2013 compared to 2012 and fewer
hotspots were identified (Figures 4.4, 4.5 and 4.11). The hotspots were also reduced in size (Figures 4.6 and 4.8) with some of them limited to single homesteads in 2013 (Figures 4.4 and 4.11). There was one stable hotspot of clinical malaria (Figure 4.4, Vitengeni area) in terms of position and size, limited to a single homestead, but the other hotspots identified in 2012 were not identified in 2013. There was some overlap between the hotspots of asymptomatic malaria detected by PCR in 2012 and 2013 (Figure 4.7) and no hotspots of asymptomatic malaria detected by microscopy in 2013 (Figure 4.5).

Figure 4.4. Hotspots of clinical malaria cases. Each blue circle represents a statistically significant hotspot with its relative risk (RR) and p value (p) displayed beside the circles.
Figure 4.5. Hotspots of asymptomatic malaria infections detected by light microscopy. Each blue circle represents a statistically significant hotspot with its relative risk (RR) and p value (p) displayed beside the circles. No hotspots were identified in 2013.

Figure 4.6. Hotspots of asymptomatic malaria infections detected by polymerase chain reaction (PCR). Each blue circle represents a statistically significant hotspot with its relative risk (RR) displayed beside the circles.
The sampling of homesteads for malaria serology differed between 2012 and 2013 (Figure 4.1). However, we observed, within the same year, a consistent overlapping of hotspots of seropositive children to AMA1 and MSP1. When we scanned for hotspots of children seropositive to both AMA1 and MSP1, we detected hotspots that substantially overlapped hotspots of children seropositive to AMA1 or MSP1. There was one location in 2012 where the three types of serology hotspots (i.e. seropositive to AMA1, seropositive to MSP1 and seropositive to both AMA1 and MSP1) exactly overlapped each other (Figure 4.10).

Figure 4.7. Dynamics of hotspots of asymptomatic parasite carriers detected by PCR. Each green dot represents a homestead.
Figure 4.8. Hotspots of individuals seropositive to *Plasmodium falciparum* AMA1. Each green dot is a homestead. Each blue circle represents a hotspot with its relative risk (RR) displayed beside the circles. The homesteads sampled in 2013 were different from those sampled in 2012.

Figure 4.9. Hotspots of individuals seropositive to *Plasmodium falciparum* MSP1. Each green dot is a homestead. Each blue circle represents a hotspot with its relative risk (RR) displayed beside the circles. The homesteads sampled in 2013 were different from those sampled in 2012.
Figure 4.10. Spatial overlapping of hotspots of serological markers of malaria transmission. Each green dot is a homestead. The homesteads sampled in 2013 were different from those sampled in 2012.

Figure 4.11. Hotspots of Anopheles mosquitoes. *Anopheles gambiae* and *Anopheles funestus* were the only human malaria vector species captured during the survey. The orange and black circles represent the statistically significant hotspots of anopheline mosquitoes in 2012 and 2013 respectively. Each hotspot is displayed with its malaria relative risk (RR) and p value beside the circles.
Variable overlapping of hotspots of clinical and parasitological markers of transmission occurred in 2012 (Figure 4.12). Only the two single-homestead hotspots of clinical malaria did not overlap the hotspot determined by positive PCR tests. The hotspot of asymptomatic malaria detected by microscopy was totally contained within the hotspot determined by positive PCR tests.

![Figure 4.12. Spatial overlapping of hotspots of clinical and parasitological markers of malaria transmission. The homesteads sampled in 2012 were the same as those sampled in 2013 for the cross-sectional study.](image)

Although the data was collected from different sets of homesteads for each study, these homesteads were all contained within a single study area. We visualized the different hotspots to examine the extent of overlapping between them (Figure 4.13). Most hotspots concentrated in the southern part of the study area and moved towards the northern part in 2013. There were considerable overlapping between hotspots of all the types of the transmission markers examined. The substantial overlapping of the hotspots of serological...
markers with those of asymptomatic infections detected by PCR observed in 2012 was also observed in 2013 when the transmission declined further.

Figure 4.13. Overlapping hotspots of malaria transmission markers. Each green spot is a homestead. All homesteads involved in the clinical surveillance, serology and entomology studies and the specific hotspots are overlaid on the same map for each year.
4.4.3 Spatial correlations between markers of malaria transmission

The study area has been subdivided into 0.9 x 0.9 km square-shaped cells as shown in Figure 4.14. All markers are expressed as an average per cell.

Figure 4.14. Tessellation of the study area and densities of homesteads. Each square measures 0.9 by 0.9 km. In A the green dots indicate the homesteads. In B the shades of green colour are proportional to the densities of homesteads with darker shades representing higher densities.

With the exception of Anopheles mosquito densities, weak to moderate statistically significant correlations were found between the other markers of transmission in 2012 when the markers were averaged at grid cell level (Table 4.3, Figure 4.15). Clinical malaria was moderately correlated with positive blood films, but not with any other marker. Serological markers were better correlated with asymptomatic parasitaemia detected by PCR than with asymptomatic parasitaemia detected by microscopy. Asymptomatic parasitaemia detected by microscopy was correlated with asymptomatic parasitaemia detected by PCR and serological markers well correlated with each other. However, in 2013, when the transmission intensity declined further, only asymptomatic parasitaemia detected by microscopy correlated with positive PCR tests (Table 4.4). When correlations were analysed
between 2012 and 2013 for each marker, only clinical malaria and PCR showed significant but weak correlation (rho < 0.21) (Figure 4.16).

Table 4.3. Correlations between malaria transmission markers in 2012 at 0.9 x 0.9 km resolution. Each table cell reports from top to bottom the correlation coefficient (rho), the number of grid cells (n) and the p value for r.

<table>
<thead>
<tr>
<th></th>
<th>Clinical malaria</th>
<th>Positive blood</th>
<th>Positive PCR</th>
<th>Anti-AMA1</th>
<th>Anti-MSP1</th>
<th>Anopheles mosquitoes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical malaria</td>
<td>1</td>
<td>266</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive Blood</td>
<td>0.2672</td>
<td>1</td>
<td>266</td>
<td>292</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Positive PCR tests</td>
<td>0.0328</td>
<td>0.5114</td>
<td>1</td>
<td>266</td>
<td>292</td>
<td>292</td>
</tr>
<tr>
<td>Anti-AMA1</td>
<td>0.0215</td>
<td>0.2927</td>
<td>0.5413</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>41</td>
<td>42</td>
<td>42</td>
<td>43</td>
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<td></td>
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<td>0.06</td>
<td>0.0002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-MSP1 Abs</td>
<td>-0.0444</td>
<td>0.3509</td>
<td>0.5745</td>
<td>0.6338</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>41</td>
<td>42</td>
<td>42</td>
<td>43</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>Anopheles mosquitoes</td>
<td>0.7828</td>
<td>0.0227</td>
<td>0.0001</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>87</td>
<td>87</td>
<td>20</td>
<td>20</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3923</td>
<td>0.5513</td>
<td>0.3086</td>
<td>0.9272</td>
<td>0.06</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.4. Correlations between malaria transmission markers in 2013 at 0.9 x 0.9 km resolution. Each table cell reports from top to bottom the correlation coefficient (r), the number of grid cells (n) and the p value for r.

<table>
<thead>
<tr>
<th></th>
<th>Clinical malaria</th>
<th>Positive blood</th>
<th>Positive PCR</th>
<th>Anti-AMA1</th>
<th>Anti-MSP1</th>
<th>Anopheles mosquitoes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical malaria</td>
<td>1</td>
<td>266</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive Blood</td>
<td>-0.0165</td>
<td>1</td>
<td>266</td>
<td>292</td>
<td>0.7884</td>
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<tr>
<td>Positive PCR tests</td>
<td>0.057</td>
<td>0.3295</td>
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<td>266</td>
<td>292</td>
<td>292</td>
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<tr>
<td>Anti-AMA1</td>
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<td>0.0516</td>
<td>0.002</td>
<td>1</td>
<td></td>
<td></td>
</tr>
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<td>22</td>
<td>22</td>
<td>22</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.8808</td>
<td>0.8196</td>
<td>0.9931</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-MSP1 Abs</td>
<td>-0.2376</td>
<td>0.0172</td>
<td>-0.0773</td>
<td>-0.0627</td>
<td>1</td>
<td></td>
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<td>22</td>
<td>22</td>
<td>22</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Anopheles mosquitoes</td>
<td>0.2869</td>
<td>0.9394</td>
<td>0.7323</td>
<td>0.7817</td>
<td></td>
<td></td>
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<tr>
<td></td>
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<td>12</td>
<td>12</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>0.1169</td>
<td>0.7527</td>
<td>0.2846</td>
<td>0.5451</td>
<td>0.0467</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4.15. Correlations between clinical, parasitological, serological and entomological malaria transmission markers. All markers are expressed as an average per cell of a 0.9 x 0.9 km resolution grid superimposed on the study area.

Figure 4.16. Correlations between malaria transmission markers measured in 2012 and 2013. All markers are expressed as an average per cell of a 0.9 x 0.9 km resolution grid superimposed on the study area.
4.5 Discussion

The present study describes the fine-scale spatial distribution of \textit{P. falciparum} malaria and examines the relationships between different markers of malaria transmission in a low malaria transmission area on the coast of Kenya. The levels of the malaria transmission markers seen in this study were lower in 2013 compared with 2012. The low number of asymptomatic infections detected by microscopy prevented us having enough power to detect hotspots in 2013, but we could still detect hotspots with the larger number of positives seen by PCR. Most of the hotspots were unstable (i.e. inconsistent in location between 2012 and 2013), but one hotspot of clinical malaria was maintained in its position and size over the two years. When the transmission intensity declined, the spatial correlations observed between the markers were reduced to correlations between parasitological markers on the one hand, and between serological and entomological markers on the other hand.

The decline of malaria transmission observed in the present study appears opposite to the observed trends in the study of Snow and colleagues who reported rising \textit{P. falciparum} parasite rates along the Coast since 2010 following a steady decline from 1998 (Snow, Kibuchi et al. 2015). However, our study was conducted in a restricted area and time window, and previous studies have reported high rates of heterogeneity in malaria transmission in the region, and that differing trends can be observed in sub-locations within the same area (Bejon, Williams et al. 2014).

From 2012 to 2013, most of the malaria transmission hotspots disappeared or shrank to single homestead hotspots except for PCR and serological markers. Hotspots of asymptomatic carriers detected by microscopy disappeared in 2013 despite ongoing transmission evidenced by clinical malaria cases. This suggests that cross-sectional surveys
using microscopy may not be ideal when transmission intensity declines to very low levels.

The hotspots that persisted were asymptomatic parasite carriers detected by molecular and serological methods, suggesting these markers as good candidates for sensitive hotspot detection in settings with declining malaria transmission.

In 2012, there was a total overlap of hotspots of asymptomatic parasite carriers detected by microscopy with hotspots of asymptomatic parasite carriers detected by PCR, as expected given that the sensitivity of PCR is higher than that of light microscopy (Bejon, Andrews et al. 2006). This is consistent with the statistically significant spatial correlation observed between these markers when the data was analysed at the grid cell level. On the other hand epidemiological studies have shown that parasite density can be inversely proportional to the intensity of transmission at a micro-epidemiological scale (Mosha, Sturrock et al. 2013), and this might have led microscopy-defined hotspots to be differently located from PCR-defined hotspots. In our study the transmission intensity was much lower and we did not observe this phenomenon. The hotspots of asymptomatic parasite carriers detected by PCR overlapped the hotspots of AMA1 and MSP1-seropositive individuals in 2012 and 2013, supporting the idea of the use serological markers as an alternative to PCR in the detection of hotspots. It has been previously shown that children living in hotspots of asymptomatic parasitaemia have higher antibody titres compared with those living in clinical malaria hotspots (Bejon, Williams et al. 2010), and antibody titres have been described as a marker of exposure (Badu, Gyan et al. 2015).

One clinical malaria hotspot was found to be stable across the two years, which is not consistent with previous reports in which hotspots of clinical malaria were found to be unstable compared with hotspots of asymptomatic parasitaemia (Bejon, Williams et al. 2010). However, the short period of observation in the present study and the low age of
children assessed (i.e. 5 to 17 months olds) in a low transmission setting is a likely explanation of this observation since immunity to clinical malaria builds up over a much longer period at lower transmission intensities (Langhorne, Ndungu et al. 2008, Griffin, Hollingsworth et al. 2015).

The relative simplicity and lower cost of serology compared with PCR may make the use of serological markers more attractive for large-scale surveillance. However the fact that serological surveys may not distinguish recent from medium-term exposure may be a disadvantage, since the location of hotspots may vary from year to year. This could be overcome by including only young children in surveys (Singer, Mirel et al. 2003) whose antibody responses have been attributed to short-lived plasma cells (Kinyanjui, Conway et al. 2007, Weiss, Traore et al. 2010) or by measuring responses to antigens for which evidence suggests that they have limited capacity to induce long-lived plasma cells (Proietti, Verra et al. 2013). Helb and colleagues have recently identified Plasmodium falciparum antigens whose specific antibody responses reflect very recent exposure i.e. within the last 30 days (Helb, Tetteh et al. 2015).

Regardless of the marker used to detect transmission hotspots, the analysis output in Satscan enables a very precise mapping of the detected hotspots. However, hotspot detection in Satscan is limited to regular shapes. The detected hotspots will always be an approximation of the true underlying hotspots and applying different detection methods may help increase confidence in the boundaries of the hotspots (Ward and Carpenter 2000, Hu, Xiong et al. 2014, Xia, Cai et al. 2015).

In chapter 2, we have been able to detect hotspots in a high transmission setting using clinical markers as has been done in previous studies (Gaudart, Poudiougou et al. 2006, Kreuels,
Kobbe et al. 2008, Cook, Kleinschmidt et al. 2011, Sissoko, van den Hoogen et al. 2015). Serological markers have been used too (Sissoko, van den Hoogen et al. 2015). Malaria transmission usually seasonal and it would be easier and advantageous, in high transmission settings, to detect and at least start control interventions during the low transmission season (generally the dry season) and before the next high transmission season.

Where control programmes opt for targeted control interventions, this should not be taken to mean an absence of interventions outside hotspots. Rather, targeting should consist of intensification of activities within hotspots and the addition of less commonly used measures like indoor residual spraying and larviciding. This approach in high transmission settings may bring malaria prevalence to lower levels faster than the untargeted approach. (Bejon, Williams et al. 2010, Bousema, Griffin et al. 2012).

In low transmission settings, unless antigens with antibody responses reflecting recent exposure are available, it would be useful to confirm ongoing transmission by appropriate parasitological tests (PCR). What makes targeted interventions attractive is that in theory, hotspots are believed to act a reservoir of parasite maintaining transmission during the dry season when conditions are not suitable for vector breeding and spreading infection in the surrounding community, and during this period it may be tractable to interrupt transmission (Bousema, Griffin et al. 2012). Additional research in various settings of differing transmission intensity is needed to better characterize the dynamics of malaria transmission around hotspots and verify this theory.

Our study was opportunistic based on the datasets available and has some limitations. The sampled homesteads were not the same for the entomology, serology and clinical surveillance studies and this prevented us from examining homestead-level correlations in
more detail. Our sample size was not large and the limited period of observation does not allow a definitive assessment of temporal stability.

4.6 Conclusion

The global decline of malaria transmission and plans for elimination have led to increased interest in the fine-scale epidemiology of malaria. One of the challenges in targeted interventions is the appropriate detection of residual transmission foci at the pre-elimination stage. The choice of a cost-effective marker that can be logistically feasible and readily implemented across sites by malaria control programs would be decisive in the elimination efforts as well as the post-elimination surveillance. Our findings may support the choice of serology or of PCR as markers in detecting the hotspots to which malaria control interventions should be targeted.
5. CHAPTER 5: General discussion

5.1 The key findings

Malaria transmission is markedly seasonal in Banfora, south-western Burkina Faso, with 93% of febrile episodes in children occurring between June and November. The children with higher exposure indexes and the febrile malaria cases tend to cluster in the northern part of the study area. Indeed, this part of the study area is rural and marshy with a few rice fields compared to the southern part that overlaps Banfora town.

In the present study conducted in Banfora, there was no evidence of protective effect in any of the antibodies to the six synthetic or recombinant merozoite antigens tested (GLURP R0, GLURP R2, AMA1, MSP1-19, MSP2, MSP3). In the published literature, only antibodies to MSP1-19 have been associated with protection against clinical malaria in young infants (Høgh, Marbiah et al. 1995, Branch, Udhayakumar et al. 1998). In contrast, there are a number of reports of a protective effect of antibodies to these merozoite antigens in older children (Fowkes, Richards et al. 2010, Rono, Osier et al. 2013, Tran, Ongoiba et al. 2014, Dent, Nakajima et al. 2015, Irani, Ramsland et al. 2015).

When high and low malaria transmission settings were compared, we found that the antibody titres in the young children were well below the previously established protective levels during the first two years of life regardless of the transmission intensity. That was expected in the low transmission setting, but not in the high transmission one. In addition to the lack of protective effect, these findings suggest that the antibody responses to these six *P. falciparum* merozoite antigens are not responsible of the early and short-lived resistance of
young infants to febrile malaria. It is noteworthy that the recruitment in this study started well before the rains and therefore the strongest malaria challenge occurred at a time when the maternally-derived antibody levels had already fallen. However, this limitation is unlikely to be the sole explanation of the lack of association we found in our analysis since the antibody titres were well below previously defined protective concentrations. The question remains unsolved but, at least, we have shown that the antibody titres are below the established protective threshold concentrations.

There was a steady decline of the antibody titres in the first six months of life. The rates of decay were variable from antigen to antigens and were not necessarily higher in the high transmission area. The effect of malaria transmission intensity on antibody responses was more apparent when looking at the dynamics of seroprevalences and the individual trajectories of antibody concentrations. Spikes in antibody concentrations approximately coinciding with the rainy season were more noticeable in magnitude and frequency in the high transmission setting when no spike was observed for some antigens (MSP1-19) in the low transmission setting. However, overall, only antibodies to MSP2 showed a moderate and steady increase until the end of the monitoring period in the high transmission setting following the decline of the presumably maternally-derived antibodies at the end of the first six months of life. This suggests that the endogenous antibody production in young children might require a much longer period to reach protective levels.

Foetal haemoglobin is believed to play a role in the protection of young infants against febrile malaria (Pasvol, Weatherall et al. 1976, Amaratunga, Lopera-Mesa et al. 2011) but there is limited evidence from field studies of this putative protective effect. In our study, although of small effect size, we found that foetal haemoglobin was significantly associated with protection against febrile malaria after adjusting for exposure in the multivariable
analysis. The Kaplan Meier estimates analysis showed that this protective effect was apparent after six months of age when we expect foetal haemoglobin to have reached its lowest levels. We make the hypothesis that this delayed protective effect could have resulted from an interaction between malaria exposure and foetal haemoglobin where initial low parasitaemia in the presence of high levels of foetal haemoglobin would have led to a more rapid acquisition of immunity (Pombo, Lawrence et al. 2002).

Antibody responses have been proposed as a marker for the detection of fine scale spatial variation in malaria transmission (Bousema, Drakeley et al. 2010). However, few studies have compared the suitability of different possible markers for the detection of malaria hotspots in a context of declining malaria transmission. In the study conducted in Ganze, we found that hotspots of positive PCR tests partially or totally overlapped with at least one of the other types of hotspots. On the one hand, the critical role of asymptomatic parasites carriers in sustaining malaria transmission and the high sensitivity of the test advocate for the choice of PCR for the detection of hotspots of malaria transmission. On the other hand, the simplicity and low cost of serology compared with PCR advocate for the choice of serological markers for the detection of hotspots.

5.2 The future directions

At least 1.3% of the predicted 5268 proteins coded by the genes of *P. falciparum* are believed to be involved in RBCs invasion (Gardner, Hall et al. 2002) and we have only tested six. The findings that the endogenous antibody production in infants is low in the first two years of life in our cohort study needs to be validated in larger cohort studies in settings of variable
malaria transmission intensity and testing a much wider repertoire of antigens for which protective concentrations would need to be estimated. High throughput ELISA technologies would be ideal for large scale testing.

The high concentrations of immunoglobulin preparations used in successful malaria immuno-therapeutic experiments suggest that the quantity of antibody matters (Cohen, McGregor et al. 1961). However, other studies suggest that the quality of antibody response is also critical. Indeed, it has been shown that passive transfer antibodies that are protective in vivo do not necessarily interfere with asexual parasites invasion and growth in vitro, and that sera from unprotected individuals fail to exert in vitro anti-parasitic effect in presence of monocytes (Bouharoun-Tayoun, Attanath et al. 1990). Though the antibody levels were low in infants in our studies, we speculate that they may be protected by antibodies not directly, but through cooperation with other immune cells such as monocytes or neutrophils and/or with other immune factors such as the complement.

Young children do get infected by malaria parasites (Wagner, Koram et al. 1998) despite the circulating maternally-derived antibodies, but fever is generally absent. Moreover, there is evidence that the pyrogenic threshold parasitaemia is much higher in children compared with adults (Miller 1958) suggesting the existence of an immune tolerance (of the parasite presence) mechanism. An anti-toxic immunity more efficient than the anti-parasite immunity might then be preventing the clinical manifestations in young infants. The transplacental passage of soluble antigens has been linked to foetal T-cells priming and immunosuppression (immune tolerance) (Brustoski, Möller et al. 2006). Haemozoin, a byproduct of haemoglobin metabolism by malaria parasites and glycosylphosphatidylinositol (GPI), a membrane anchor for a number of malaria parasite surface antigens, have been shown to promote the production of pro-inflammatory cytokines
by monocytes and proposed as putative malaria toxins (Schofield and Hackett 1993, Sherry, Alava et al. 1995). On the one hand, anti-GPI antibodies have been associated to protection against the clinical manifestations of malaria (Naik, Branch et al. 2000) and other studies suggest that anti-haemozoin antibodies may have an inhibitory effect on pro-inflammatory cytokine production (Biswas, Karmarkar et al. 2001). On the other hand, an increased production of nitric oxide (a molecule that has anti-inflammatory effects) by peripheral mononuclear cells has been proposed as a mechanism of the anti-toxic immunity (Boutlis, Tjitra et al. 2003). However, subsequent studies yielded conflicting results and it is still unclear which mechanisms support the anti-toxic immunity (Boutlis, Gowda et al. 2002, de Souza, Todd et al. 2002, Boutlis, Weinberg et al. 2004). Anti-GPI antibodies are mainly IgG (Boutlis, Gowda et al. 2002) and there is evidence that IgG are the most efficiently transferred antibodies across the placenta (Gitlin, Kumate et al. 1964). The fact that this proposed anti-toxic immunity ends with the decline of maternal antibodies and foetal haemoglobin at around four months of age supports the hypothesis of the presence of anti-toxin antibodies among in the pool of maternally-derived antibodies. The kinetics of foetal haemoglobin is almost parallel to that of maternally-derived antibodies and we cannot rule out a protective effect of an interaction between both factors.

In the current context of high-level and global commitment to malaria elimination/eradication, some successes have been achieved over the last fifteen years with four countries certified as having completed elimination, twenty in pre-elimination or elimination phase and nine working to prevent re-introduction (WHO 2015). In areas where malaria transmission has already declined to moderate to low intensity or where malaria has been eliminated, the micro-epidemiological patterns of malaria take a critical importance since targeted interventions are likely to override the large-scale ones for cost and efficiency.
reasons (Woolhouse, Dye et al. 1997). Methods for the identification of malaria transmission hotspots that are cost-effective and appropriate to the transmission intensity need to be validated for large scale use and for comparability purposes. Vaccines are predicted to contribute substantially to malaria control and elimination (Nunes, Cardenas et al. 2013). In the current race to the development of a malaria vaccine of high and long-lasting efficacy, a number of studies advocate for multicomponent vaccines (Hill, Biswas et al. 2014, Osier, Mackinnon et al. 2014). Sero-epidemiological studies and immunological correlates of protection if validated, would provide useful guidance to these antigen combinations as it has been done for other infectious diseases (O’Ryan, Stoddard et al. 2014).

The present work aimed at contributing to fill the gap of knowledge in the role of antibodies in the immunity to malaria in infancy and early childhood by examining antibody responses to *P. falciparum* merozoite antigens, the blood stage form responsible of the clinical manifestations of the disease. Especially, we have analysed the dynamics of antibody responses in relation to previously established protective threshold antibody concentrations. In addition, we have examined the place of serological markers in the detection of hotspots of malaria transmission among other clinical, parasitological and entomological markers.


the extent of malaria transmission and challenges facing pre-elimination in the Republic of Djibouti." BMC Infectious Diseases 11: 121-121.


the Mosquitocidal Drug Ivermectin to Prevent Malaria Transmission After Treatment: A Double-Blind, Randomized, Clinical Trial." Clinical Infectious Diseases 60(3): 357-365.


7. Appendices

7.1 ELISA reagents and laboratory equipment

7.1.1 ELISA conducted in CNRFP laboratories

<table>
<thead>
<tr>
<th>REAGENTS</th>
<th>SOURCES</th>
</tr>
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<tbody>
<tr>
<td>Synthetic Merozoite surface protein 3 (MSP3-LSP)</td>
<td>Statens Serum Institute, Copenhagen</td>
</tr>
<tr>
<td>Synthetic Glutamate Rich Protein R0 (GLURP-R0)</td>
<td>Statens Serum Institute, Copenhagen</td>
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<tr>
<td>Synthetic Glutamate Rich Protein R2 (GLURP-R2)</td>
<td>Statens Serum Institute, Copenhagen</td>
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<tr>
<td>Positive control serum</td>
<td>Adults sera from Saponé donors</td>
</tr>
<tr>
<td>Negative control sera</td>
<td>Adults sera from UK donors</td>
</tr>
<tr>
<td>Distilled water</td>
<td>CNRFP laboratories</td>
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<tr>
<td>Phosphate buffered saline tablets (salt)</td>
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<tr>
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<td>Sigma Aldrich</td>
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<td>Skimmed milk</td>
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<td>Microplate reader</td>
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7.1.2 ELISA conducted in KEMRI-WTRP laboratories

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</tr>
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</tr>
<tr>
<td>Recombinant Merozoite surface protein 1 (MSP1-19)</td>
<td>KEMRI-WTRP Immunology Lab</td>
</tr>
<tr>
<td>Recombinant Merozoite surface protein 2 (MSP2-Dd2)</td>
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</tr>
<tr>
<td>Recombinant Merozoite surface protein 3 (MSP3-3D7)</td>
<td>KEMRI-WTRP Immunology Lab</td>
</tr>
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<td>Malaria Immune Globuline (MIG)</td>
<td>Central Laboratory Blood Transfusion Service</td>
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<tr>
<td>Positive control serum</td>
<td>Adult sera samples from Kilifi donors</td>
</tr>
<tr>
<td>Negative control sera</td>
<td>Adult sera samples from UK donors</td>
</tr>
<tr>
<td>Distilled water</td>
<td>KEMRI-WTRP Immunology Lab</td>
</tr>
<tr>
<td>Phosphate buffered saline tablets (salt)</td>
<td>Oxoid Limited</td>
</tr>
<tr>
<td>REAGENTS</td>
<td>SOURCES</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>Tween 20 (detergent)</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Skimmed milk</td>
<td>Marvel® Premier Foods Group, UK</td>
</tr>
<tr>
<td>Na$_2$CO$_3$ (Sodium carbonate)</td>
<td>Sigma</td>
</tr>
<tr>
<td>NaHCO$_3$ (Sodium hydrogen carbonate)</td>
<td>Sigma</td>
</tr>
<tr>
<td>HRP-conjugated rabbit anti-human IgG</td>
<td>Dako</td>
</tr>
<tr>
<td>C$_6$H$_8$O$_7$ (Citric acid)</td>
<td>AnalalR®</td>
</tr>
<tr>
<td>Na$_3$HPO$_4$ (Sodium hydrogen phosphate)</td>
<td>Sigma</td>
</tr>
<tr>
<td>H$_2$O$_2$ (Hydrogen peroxide)</td>
<td>AnalalR®</td>
</tr>
<tr>
<td>C$_6$H$_4$N$_2$ (O-phenylenediamine)</td>
<td>Sigma</td>
</tr>
<tr>
<td>H$_2$SO$_4$ (Sulfuric acid)</td>
<td>Sigma</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EQUIPEMENTS</th>
<th>SOURCES (trademark, manufacturer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-well Microtiter Microplates</td>
<td>IMMULON 4HBX – Thermo Scientific</td>
</tr>
<tr>
<td>Microplates washer</td>
<td>BIoTEK</td>
</tr>
<tr>
<td>Microplate reader</td>
<td>BIoTEK</td>
</tr>
</tbody>
</table>
clear all
macro drop _all
capture log close
set more off
log using chap2_coxreg,replace text
* chap2_coxreg.do: survival analysis of bics data
*david kangoye,PhD student,Open University/KEMRI-WTRP
version 11.2
set linesize 80
*===============================================================
cd "C:\Users\dkangoye\Dropbox\projects\phdprogram_ou\work\data\data_bics\elisa_bics"
use bicsV0,clear
*combine morbidity data with exposure data
*=====================================
joinby code using dist1.5ei,unm(both)
tab _merge, m
list code if _merge==2
drop if _merge==2
drop _merge
codebook mob
recode mob (10=1) (11=2) (12=3) (1=4)
codebook mob
order mob,after(dob)
lab def cald 1"October" 2"November" 3"December" 4"January"
lab val mob cal
tab mob
tab mob,nolabel
foreach i in logmsp3 logr0 logr2{
gen b`i'=`i' if months=="M00"
sort code datevisit
replace b`i'=b`i'[_n-1] if code==code[_n-1]
lab var b`i' "baseline anti-`i' titer"
order b`i',after(`i')
replace b`i'=round(b`i',.01)
sum b`i',d
}
codebook hb_type
recode hb_type (1=1) (2=. ) (3=2) (4=3)
lab def types 1"AA" 2"AC" 3"CC"
lab val hb_type types
codebook hb_type
stset  datevisit, failure(malar10) id(code) origin(datscren) scale(28)
*univariate cox regression
*=====================================
*create local macros for explanatory variables
local envfac i.season i.mob i.zone ei
local socufac i.educ
local matfac agem i.primgrav i.sg_birth i.itn_use i.iptp_n
local infac1 i.sex  muac height_scr weight_scr i.del_way i.rea i.epi_stat i.neo_inf
local infac2 i.hb_type hbf logmsp3 logr0 logr2
foreach i in `infac1' `infac2' `envfac' `socufac' `matfac' {
    stcox `i'
}
*perform collinearity diagnostic
*==-------------------------------*

corr season ei hbf logmsp3 logr0 logr2 mob hb_type itn_use educ muac_scr
collin season ei hbf logmsp3 logr0 logr2 mob hb_type itn_use educ muac_scr

multivariate cox regression

*include in baseline multivariable model if p<0.2 or high importance variable

xi:stcox i.season ei hbf logmsp3 logr0 logr2 i.mob i.hb_type i.itn_use i.educ muac_scr
est store model0

*drop variables with highest non significant p values one by one

*drop educ

xi:stcox i.season ei hbf logmsp3 logr0 logr2 i.mob i.hb_type i.itn_use muac_scr
est store model1

est table model0 model1,b(%5.3f) p(%4.3f) stats(N ll aic bic)
lrtest model0 model1,stats

*drop mob

xi:stcox i.season ei hbf logmsp3 logr0 logr2 i.hb_type i.itn_use muac_scr
est store model2

est table model0 model1 model2,b(%5.3f) p(%4.3f) stats(N ll aic bic)
lrtest model0 model2,stats

*drop muac_scr

xi:stcox i.season ei hbf logmsp3 logr0 logr2 i.hb_type i.itn_use
est store model3

est table model0 model1 model2 model3,b(%5.3f) p(%4.3f) stats(N ll aic bic)
lrtest model0 model3,stats

*test for interaction between foetal haemoglobin and exposure index

xi:stcox i.season ei hbf logmsp3 logr0 logr2 i.hb_type i.itn_use c.ei#c.hbf

*test of proportional hazards assumption

*==-------------------------------*

estat phtest,d
local grafreg "graphregion(fcolor(white) lcolor(white) ifcolor(white) ilcolor(white))"
local plotreg "plotregion(fcolor(white) lcolor(white) ifcolor(white) ilcolor(white))"
estat phtest.plot(logmap3) yline(0) `grafreg' `plotreg' title(D)
graph save 4.replace
estat phtest.plot(logr0) yline(0) `grafreg' `plotreg' title(E)
graph save 5.replace
estat phtest.plot(logr2) yline(0) `grafreg' `plotreg' title(F)
graph save 6.replace
estat phtest.plot(ei) yline(0) `grafreg' `plotreg' title(G)
graph save 7.replace
estat phtest.plot(hbf) yline(0) `grafreg' `plotreg' title(H)
graph save 8.replace

graph combine 4.gph 5.gph 6.gph 7.gph 8.gph,`grafreg' `plotreg'
graph export phtest.tif,width(2049) replace

log close
exit

7.2.2 Cox regression output log / Chaper 2
*david kangoye, PhD student, Open University/KEMRI-WTRP

version 11.2

set linesize 80

> ==

. cd "C:\Users\dkangoye\Dropbox\projects\phdprogram_ou\work\data\data_bics\elisa_bics"

. use bicsV0, clear
(combination of all data sets {morb+geo+sero})

. *combine morbidity data with exposure data
.*----------------------------------------
. joinby code using dist1.5ei, unm(both)
.
. tab _merge,m

<table>
<thead>
<tr>
<th>_merge</th>
<th>Freq.</th>
<th>Percent</th>
<th>Cum.</th>
</tr>
</thead>
<tbody>
<tr>
<td>both in master and using data</td>
<td>14,148</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Total</td>
<td>14,148</td>
<td>100.00</td>
<td></td>
</tr>
</tbody>
</table>

. list code if _merge==2

. drop if _merge==2
(0 observations deleted)

. drop _merge
.
. codebook mob

--------------------------------------------------------------------------------
 mob                                                                   month of birth
--------------------------------------------------------------------------------
type: numeric (float)                                                  range: [1,12]       units: 1
unique values: 4                     range: [1,4]       units: 1
 missing .: 0/14148                                      missing .: 0/14148
tabulation: Freq.  Value
2906  1
1273 10
4435 11
5534 12

. recode mob (10=1) (11=2) (12=3) (1=4)
(mob: 14148 changes made)

. codebook mob

--------------------------------------------------------------------------------
 mob                                                                   month of birth
--------------------------------------------------------------------------------
type: numeric (float)                                                  range: [1,4]       units: 1
unique values: 4                     range: [1,12]       units: 1
 missing .: 0/14148                                      missing .: 0/14148
tabulation: Freq.  Value
1273 1
4435 2
5534 3
2906 4
. order mob, after(dob)

. lab def cald "October" 2"November" 3"December" 4"January"

. lab val mob cald

. tab mob

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>October</td>
<td>1,273</td>
<td>9.00</td>
<td>9.00</td>
</tr>
<tr>
<td>November</td>
<td>4,435</td>
<td>31.35</td>
<td>40.34</td>
</tr>
<tr>
<td>December</td>
<td>5,534</td>
<td>39.12</td>
<td>79.46</td>
</tr>
<tr>
<td>January</td>
<td>2,906</td>
<td>20.54</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Total | 14,148 | 100.00 |

. tab mob, nolabel

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,273</td>
<td>9.00</td>
<td>9.00</td>
</tr>
<tr>
<td>2</td>
<td>4,435</td>
<td>31.35</td>
<td>40.34</td>
</tr>
<tr>
<td>3</td>
<td>5,534</td>
<td>39.12</td>
<td>79.46</td>
</tr>
<tr>
<td>4</td>
<td>2,906</td>
<td>20.54</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Total | 14,148 | 100.00 |

. foreach i in logmsp3 logr0 logr2{
  2.         gen b`i'=`i' if months="M00"
  3.         sort code datevisit
  4.         replace b`i'=b`i'[_n-1] if code==code[_n-1]
  5.         lab var b`i' "baseline anti-`i' titer"
  6.         order b`i', after(`i')
  7.         replace b`i'=round(b`i',.01)
  8.         sum b`i', d
  9. }

(14025 missing values generated)
(13824 real changes made)
(13947 real changes made)

baseline anti-logmsp3 titer

<table>
<thead>
<tr>
<th>Percentiles</th>
<th>Smallest</th>
<th>Largest</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>.29</td>
<td>Mean</td>
</tr>
<tr>
<td>5%</td>
<td>.8</td>
<td>Std. Dev.</td>
</tr>
<tr>
<td>10%</td>
<td>2.05</td>
<td>Variance</td>
</tr>
<tr>
<td>25%</td>
<td>2.84</td>
<td>Skewness</td>
</tr>
<tr>
<td>50%</td>
<td>4.33</td>
<td>Kurtosis</td>
</tr>
</tbody>
</table>

(14025 missing values generated)
(13824 real changes made)
(13947 real changes made)

baseline anti-logr0 titer

<table>
<thead>
<tr>
<th>Percentiles</th>
<th>Smallest</th>
<th>Largest</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>.52</td>
<td>Mean</td>
</tr>
<tr>
<td>5%</td>
<td>2.01</td>
<td>Std. Dev.</td>
</tr>
<tr>
<td>10%</td>
<td>2.13</td>
<td></td>
</tr>
<tr>
<td>25%</td>
<td>2.96</td>
<td></td>
</tr>
<tr>
<td>50%</td>
<td>3.86</td>
<td></td>
</tr>
<tr>
<td>75%</td>
<td>4.91</td>
<td>7.82</td>
</tr>
</tbody>
</table>
Baseline anti-log2 titer

Percentiles Smallest
1% .97 .52
5% 2.71 .52
10% 2.92 .52 Obs 13947
25% 4.31 .52 Sum of Wgt. 13947
50% 5.34 Mean 5.337898
75% 6.51 9
90% 7.29 9 Variance 2.734306
99% 8.82 9 Skewness -.3830149

Kurtosis 3.218267.

Type: numeric (float)
Label: types
Range: [1,3]
Units: 1
Unique values: 3
Missing.: 212/14148

Tabulation:

```
Freq. Numeric Label
11595 1 AA
1839 2 AC
502 3 CC
212 .
```

Stset datevisit, failure(malar10) id(code) origin(datscren) scale(28)

Id: code
Failure event: malar10 != 0 & malar10 < .
Obs. time interval: (datevisit[_n-1], datevisit]
Exit on or before: failure
* univariate cox regression
. * create local macros for explanatory variables
. local envfac i.season i.mob i.zone ei
. local socufac i.educ
. local matfac agem i.primgrav i.sg_birth i.itn_use i.iptp_n
. local infac1 i.sex muac height_scr weight_scr i.del_way i.rea i.epi_stat i.ne > o_inf
. local infac2 i.hb_type hbf logmsp3 logr0 logr2
.
. foreach i in `infac1' `infac2' `envfac' `socufac' `matfac'
. 2.         stcox `i'
. 3. 

failure d: malar10
analysis time t: (datevisit-origin)/28
origin: time dat scren
id: code

Iteration 0: log likelihood = -386.0048
Iteration 1: log likelihood = -385.74996
Iteration 2: log likelihood = -385.74996

Refining estimates:
Iteration 0: log likelihood = -385.74996

Cox regression -- Breslow method for ties

|             | Haz. Ratio | Std. Err. | z   | P>|z| | 95% Conf. Interval |
|-------------|-----------|-----------|-----|-----|-------------------|
| 2.sex       | .8599306  | .1817821  | -0.71 | 0.475 | .5682332 - 1.301368 |

failure d: malar10
analysis time t: (datevisit-origin)/28
origin: time dat scren
id: code

Iteration 0: log likelihood = -386.0048
Iteration 1: log likelihood = -385.14403
Iteration 2: log likelihood = -385.14197
Iteration 3: log likelihood = -385.14197

Refining estimates:
Iteration 0: log likelihood = -385.14197
Cox regression -- Breslow method for ties

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>125</td>
<td>Number of obs</td>
<td>9346</td>
<td></td>
</tr>
<tr>
<td>No. of failures</td>
<td>90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time at risk</td>
<td>2112.928571</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log likelihood</td>
<td>-385.14197</td>
<td>LR chi2(1)</td>
<td>1.73</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prob &gt; chi2</td>
<td>0.1890</td>
<td></td>
</tr>
</tbody>
</table>

| t  | Haz. Ratio | Std. Err. | z    | P>|z|    | [95% Conf. Interval] |
|----|------------|-----------|------|--------|---------------------|
| muac_scr | 1.127406 | .1012997 | 1.33 | 0.182  | .945362    1.344505 |

failure _d_: malar10
analysis time _t_: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0: log likelihood = -384.65069
Iteration 1: log likelihood = -384.35241
Iteration 2: log likelihood = -384.35234
Refining estimates:
Iteration 0: log likelihood = -384.35234

Cox regression -- Breslow method for ties

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>124</td>
<td>Number of obs</td>
<td>9232</td>
<td></td>
</tr>
<tr>
<td>No. of failures</td>
<td>90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time at risk</td>
<td>2086.892857</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log likelihood</td>
<td>-384.35234</td>
<td>LR chi2(1)</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prob &gt; chi2</td>
<td>0.4398</td>
<td></td>
</tr>
</tbody>
</table>

| t  | Haz. Ratio | Std. Err. | z    | P>|z|    | [95% Conf. Interval] |
|----|------------|-----------|------|--------|---------------------|
| height_scr | 1.034446 | .045581 | 0.77 | 0.442  | .9488578   1.127754 |

failure _d_: malar10
analysis time _t_: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0: log likelihood = -381.24858
Iteration 1: log likelihood = -381.19957
Iteration 2: log likelihood = -381.19957
Refining estimates:
Iteration 0: log likelihood = -381.19957

Cox regression -- Breslow method for ties

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>124</td>
<td>Number of obs</td>
<td>9252</td>
<td></td>
</tr>
<tr>
<td>No. of failures</td>
<td>89</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time at risk</td>
<td>2090.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log likelihood</td>
<td>-381.19957</td>
<td>LR chi2(1)</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prob &gt; chi2</td>
<td>0.7542</td>
<td></td>
</tr>
</tbody>
</table>

| t  | Haz. Ratio | Std. Err. | z    | P>|z|    | [95% Conf. Interval] |
|----|------------|-----------|------|--------|---------------------|
| weight_scr | 1.057692 | .1890977 | 0.31 | 0.754  | .7450378  1.50155  |

failure _d_: malar10
analysis time _t_: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0: log likelihood = -376.47846
Iteration 1: log likelihood = -375.61234
Iteration 2: log likelihood = -375.54215
Iteration 3: log likelihood = -375.54132
Iteration 4: log likelihood = -375.54132
Refining estimates:
Iteration 0: log likelihood = -375.54132

Cox regression -- Breslow method for ties

No. of subjects = 123
No. of failures = 88
Time at risk = 2070.071429
Log likelihood = -375.54132

LR chi2(1) = 1.87
Prob > chi2 = 0.1710

| t | Haz. Ratio | Std. Err. | z    | P>|z|     | [95% Conf. Interval] |
|---|------------|-----------|------|---------|---------------------|
| del_w way | cesarean  | .4272051 | .3058909 | -1.19   | 0.235 | .104991 | 1.738284 |

failure _d: malar10
analysis time _t: (datevisit-origin)/28
origin: time datscreen
id: code

Iteration 0: log likelihood = -386.0048
Iteration 1: log likelihood = -385.95161
Iteration 2: log likelihood = -385.95146
Iteration 3: log likelihood = -385.95146
Refining estimates:
Iteration 0: log likelihood = -385.95146

Cox regression -- Breslow method for ties

No. of subjects = 125
No. of failures = 90
Time at risk = 2112.928571
Log likelihood = -385.95146

LR chi2(1) = 0.11
Prob > chi2 = 0.7439

| t | Haz. Ratio | Std. Err. | z    | P>|z|     | [95% Conf. Interval] |
|---|------------|-----------|------|---------|---------------------|
| rea | no  | .8838838 | .3284886 | -0.33   | 0.740 | .4266313 | 1.831208 |

failure _d: malar10
analysis time _t: (datevisit-origin)/28
origin: time datscreen
id: code

Iteration 0: log likelihood = -386.0048
Iteration 1: log likelihood = -385.76567
Iteration 2: log likelihood = -385.76389
Iteration 3: log likelihood = -385.76389
Refining estimates:
Iteration 0: log likelihood = -385.76389

Cox regression -- Breslow method for ties

No. of subjects = 125
No. of failures = 90
Time at risk = 2112.928571
Log likelihood = -385.76389

LR chi2(1) = 0.48
Prob > chi2 = 0.4876

| t | Haz. Ratio | Std. Err. | z    | P>|z|     | [95% Conf. Interval] |
|---|------------|-----------|------|---------|---------------------|
| epi_stat | no  | 1.238263 | .3716989 | 0.71   | 0.476 | .6875455 | 2.230102 |

failure _d: malar10
analysis time _t: (datevisit-origin)/28
Cox regression -- Breslow method for ties

No. of subjects = 125  Number of obs = 9346
No. of failures = 90
Time at risk = 2112.928571
Log likelihood = -384.4845

------------------------------------------------------------------------------
_t | Haz. Ratio   Std. Err.   z    P>|z|     [95% Conf. Interval]
-------------
neo_inf |       no |   .0840198   .0881232   -2.36   0.018     .010755    .6563778
-------------

Cox regression -- Breslow method for ties

No. of subjects = 123  Number of obs = 9135
No. of failures = 90
Time at risk = 2061.071429
Log likelihood = -382.27458

------------------------------------------------------------------------------
_t | Haz. Ratio   Std. Err.   z    P>|z|     [95% Conf. Interval]
-------------
hb_type |     AC  |   1.299099   .4045907   0.84   0.401     .7055772    2.391883
         CC  |   .4972872   .356377   -0.97   0.330     .1220676    2.025883
-------------

Cox regression -- Breslow method for ties

No. of subjects = 124  Number of obs = 9246
No. of failures = 90
Time at risk = 2087
Log likelihood = -383.09965

------------------------------------------------------------------------------
_t | Haz. Ratio   Std. Err.   z    P>|z|     [95% Conf. Interval]
-------------
```plaintext
--
hbf | .9848312 .0084409 -1.78 0.075 .9684256 1.001515
--

failure _d: malar10
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0:   log likelihood = -386.0048
Iteration 1:   log likelihood = -385.91068
Iteration 2:   log likelihood = -385.91068
Refining estimates:
Iteration 0:   log likelihood = -385.91068

Cox regression -- Breslow method for ties

No. of subjects = 125                     Number of obs   = 9346
No. of failures = 90                     Time at risk    = 2112.928571
Log likelihood  = -385.91068
LR chi2(1)      = 0.19                     Prob > chi2     = 0.6644

| _t | Haz. Ratio   Std. Err.   z     P>|z|     [95% Conf. Interval] |
|----|-------------|-----------------|-----|--------|--------------------------|
|logr0 | 1.109708   .1176061  0.98 0.326  .9015686   1.365898 |
```

---

```plaintext
--
hbf | .9848312 .0084409 -1.78 0.075 .9684256 1.001515
--

failure _d: malar10
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0:   log likelihood = -386.0048
Iteration 1:   log likelihood = -385.91068
Iteration 2:   log likelihood = -385.91068
Refining estimates:
Iteration 0:   log likelihood = -385.91068

Cox regression -- Breslow method for ties

No. of subjects = 125                     Number of obs   = 9346
No. of failures = 90                     Time at risk    = 2112.928571
Log likelihood  = -385.91068
LR chi2(1)      = 0.19                     Prob > chi2     = 0.6644

| _t | Haz. Ratio   Std. Err.   z     P>|z|     [95% Conf. Interval] |
|----|-------------|-----------------|-----|--------|--------------------------|
|logr0 | 1.109708   .1176061  0.98 0.326  .9015686   1.365898 |
```
| _t | Haz. Ratio   | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|----|--------------|-----------|------|-------|----------------------|
| logr2 | 1.03344     | 0.0783198 | 0.43 | 0.664 | .8907923   1.19893 |

Cox regression -- Breslow method for ties

No. of subjects = 125                     Number of obs = 9346
No. of failures = 90
Time at risk = 2112.928571
Log likelihood = -377.14563
LR chi2(1) = 17.72
Prob > chi2 = 0.0000

| _t | Haz. Ratio   | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|----|--------------|-----------|------|-------|----------------------|
| season |  |             |     |       |                     |
| high_ts | 10.09555     | 6.548771  | 3.56 | 0.000 | 2.831221  35.99867 |

Cox regression -- Breslow method for ties

No. of subjects = 125                     Number of obs = 9346
No. of failures = 90
Time at risk = 2112.928571
Log likelihood = -382.69276
LR chi2(3) = 6.62
Prob > chi2 = 0.0849

| _t | Haz. Ratio   | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|----|--------------|-----------|------|-------|----------------------|
| mob |              |           |      |       |                     |
| November  | 1.408442     | 0.5631323 | 0.86 | 0.392 | 0.6432892  3.083698 |
| December  | 1.325864     | 0.5250798 | 0.71 | 0.476 | 0.6100973  2.88137  |
| January   | 2.489854     | 1.03978   | 2.18 | 0.029 | 1.098263   5.644708 |

Cox regression -- Breslow method for ties

No. of subjects = 125                     Number of obs = 9346
No. of failures = 90
Time at risk = 2112.928571
Log likelihood = -376.20795
LR chi2(1) = 17.72
Prob > chi2 = 0.0000

| _t | Haz. Ratio   | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|----|--------------|-----------|------|-------|----------------------|
| logr2 | 1.03344     | 0.0783198 | 0.43 | 0.664 | .8907923   1.19893 |

Cox regression -- Breslow method for ties

No. of subjects = 125                     Number of obs = 9346
No. of failures = 90
Time at risk = 2112.928571
Log likelihood = -376.20795
LR chi2(1) = 17.72
Prob > chi2 = 0.0000

| _t | Haz. Ratio   | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|----|--------------|-----------|------|-------|----------------------|
| mob |              |           |      |       |                     |
| November  | 1.408442     | 0.5631323 | 0.86 | 0.392 | 0.6432892  3.083698 |
| December  | 1.325864     | 0.5250798 | 0.71 | 0.476 | 0.6100973  2.88137  |
| January   | 2.489854     | 1.03978   | 2.18 | 0.029 | 1.098263   5.644708 |
Cox regression -- Breslow method for ties

|                | Haz. Ratio | Std. Err. | z       | P>|z|    | [95% Conf. Interval] |
|----------------|------------|-----------|---------|--------|----------------------|
| zone           |            |           |         |        |                      |
| urban          | .4270694   | .1166378  | -3.12   | 0.002  | .2500491 .7294101    |
| mixed          | 1.324263   | .3245323  | 1.15    | 0.252  | .8191688 2.140795    |

failure _d: malar10
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0: log likelihood = -386.0048
Iteration 1: log likelihood = -383.49799
Iteration 2: log likelihood = -383.45047
Iteration 3: log likelihood = -383.45046
Refining estimates:
Iteration 0: log likelihood = -383.45046

Cox regression -- Breslow method for ties

|                | Haz. Ratio | Std. Err. | z       | P>|z|    | [95% Conf. Interval] |
|----------------|------------|-----------|---------|--------|----------------------|
| educ           |            |           |         |        |                      |
| primary sc.    | 1.385446   | .3182677  | 1.42    | 0.156  | .8831812 2.173349    |
| secondary     | .642815    | .2233491  | -1.27   | 0.203  | .3263359 1.270106    |

failure _d: malar10
analysis time _t: (datevisit-origin)/28
origin: time datscren

Iteration 0: log likelihood = -386.0048
Iteration 1: log likelihood = -383.45047
Iteration 3: log likelihood = -383.45046
Refining estimates:
Iteration 0: log likelihood = -383.45046
id: code

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Refining estimates:

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Cox regression -- Breslow method for ties

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<tr>
<th>No. of subjects</th>
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<th>Log likelihood</th>
<th>LR chi2(1)</th>
<th>Prob &gt; chi2</th>
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| _t | Haz. Ratio | Std. Err. | z | P>|z| | [95% Conf. Interval] |
|-----|------------|-----------|---|-------|---------------------|
| agem | 1.016155   | 0.0173533 | 0.94 | 0.348 | 0.9827059 - 1.050742 |

failure _d: malar10

analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

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Refining estimates:

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Cox regression -- Breslow method for ties

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| _t | Haz. Ratio | Std. Err. | z | P>|z| | [95% Conf. Interval] |
|-----|------------|-----------|---|-------|---------------------|
| primgrav | .9860174 | .2551392 | -0.05 | 0.957 | .5937861 - 1.637341 |

failure _d: malar10

analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

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Refining estimates:

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Cox regression -- Breslow method for ties

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| _t | Haz. Ratio | Std. Err. | z | P>|z| | [95% Conf. Interval] |
|-----|------------|-----------|---|-------|---------------------|
| sg_birth | .3834371 | .2746089 | -1.34 | 0.181 | .094207 - 1.560649 |

failure _d: malar10

analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code
failure _d: malar10
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0: log likelihood = -381.19
Iteration 1: log likelihood = -381.11466
Iteration 2: log likelihood = -381.11448
Iteration 3: log likelihood = -381.11448
Refining estimates:
Iteration 0: log likelihood = -381.11448

Cox regression -- Breslow method for ties

No. of subjects =          124                     Number of obs   =      9289
No. of failures =           89
Time at risk    =  2100.285714
LR chi2(1)      =      0.15
Log likelihood  = -381.11448                     Prob > chi2     =    0.6975

_t | Haz. Ratio   Std. Err.   z    P>|z|     [95% Conf. Interval]
 -------------
+----------------------
itrn_use |
no |   .8742819   .3076698  -0.38   0.703     .4386357    1.742605

failure _d: malar10
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0: log likelihood = -386.0048
Iteration 1: log likelihood = -384.80566
Iteration 2: log likelihood = -384.77529
Iteration 3: log likelihood = -384.77516
Iteration 4: log likelihood = -384.77516
Refining estimates:
Iteration 0: log likelihood = -384.77516

Cox regression -- Breslow method for ties

No. of subjects =          125                     Number of obs   =      9346
No. of failures =           90
Time at risk    =  2112.928571
LR chi2(3)      =      2.46
Log likelihood  = -384.77516                     Prob > chi2     =    0.4827

_t | Haz. Ratio   Std. Err.   z    P>|z|     [95% Conf. Interval]
 -------------
+----------------------------------
iptp_n |
1  |   1.705241   .8494711   1.07   0.284   .6423284    4.527041
2  |   1.260928   .585985    0.50   0.618   .5071253    3.135199
3  |   .6471596   .7090459  -0.40   0.691  .0755808    5.541295

*perform collinearity diagnostic
*================================
corr season ei hbf logmsp3 logr0 logr2 mob hb_type itn_use educ muac_scr
(obs=13830)

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Mean VIF 1.17

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Condition Number 56.8802

Det(correlation matrix) 0.4198

*multivariate cox regression

*include in baseline multivariable model if p<0.2 or high importance variable

*xi:stcox i.season ei hbf logmsp3 logr0 logr2 mob i hb_type i.itn_use i.educ
> muac_scr

failure _d: malar10
analysis time _t: (datevisit-origin)/28
origin: time datscreen
id: code
Iteration 0:   log likelihood =  -378.47353
Iteration 1:   log likelihood =  -346.40614
Iteration 2:   log likelihood =  -344.88137
Iteration 3:   log likelihood =  -344.85786
Iteration 4:   log likelihood =  -344.85784
Refining estimates:
Iteration 0:   log likelihood =  -344.85784

Cox regression
Breslow method for ties

No. of subjects =          122                     Number of obs   =      9078
No. of failures =           89
Time at risk    =  2048.428571
LR chi2(15)     =     67.23
Log likelihood  =  -344.85784                     Prob > chi2     =    0.0000

------------------------------------------------------------------------------
   _t | Haz. Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
  _Iseason_1 |    9.95621   6.806982     3.36   0.001     2.606959    38.02365
   ei |    1.07433   0.231541     3.33   0.001     1.029894    1.120683
  hbf |    0.96647   0.012502   -3.22    0.001     0.946592    0.987752
logmsp3 |    1.38253   0.150887     2.89   0.004     1.109663    1.722511
logr0 |    1.12778   0.137890     0.98   0.325     0.887469    1.433178
  logr2 |    1.03376   0.07095     0.70   0.478     0.865219    1.230287
 _Imob_2 |    1.99559   0.867481    1.59    0.112     0.851245    4.678298
 _Imob_3 |    1.71792   0.756239    1.23    0.219     0.724937    4.071056
 _Imob_4 |    2.60388   1.253705    1.99    0.047     1.013418    6.690426
 _Ihb_type_2 |    1.11311   0.379267    0.31    0.753     0.570838    2.170533
 _Ihb_type_3 |    0.57291   0.429874   -0.74    0.458     0.131641    2.453229
 _Iitn_use_2 |    0.84152   0.339804   -0.43    0.669     0.381369    1.856852
 _Ieduc_1 |    1.83774   0.472923    1.99    0.047     1.013418    6.690426
 _Ieduc_2 |    0.93398   0.429874   -0.74    0.458     0.131641    2.453229
 muac_scr |    1.22096   0.139359     1.75    0.080     0.976221    1.527064
------------------------------------------------------------------------------
. est store model0
.*drop variables with highest non significant p values one by one
.*drop educ
  xi:stcox i.season ei  hbf  logmsp3  logr0 logs2 i.mob i.hb_type i.itn_use muac_sc
> r
  i.season          _Iseason_0       (naturally coded; _Iseason_0 omitted)
i.mob             _Imob_1         (naturally coded; _Imob_1 omitted)
i.hb_type         _Ihb_type_1      (naturally coded; _Ihb_type_1 omitted)
i.itn_use         _Iitn_use_1      (naturally coded; _Iitn_use_1 omitted)
  failure _d:  malar10
  analysis time _t:  (datevisit-origin)/28
  origin:  time datscren
  id: code

Iteration 0:   log likelihood =  -378.47353
Iteration 1:   log likelihood =  -349.3221
Iteration 2:   log likelihood =  -347.81266
Iteration 3:   log likelihood =  -347.78781
Iteration 4:   log likelihood =  -347.78778
Refining estimates:
Iteration 0:   log likelihood =  -347.78778

Cox regression
Breslow method for ties

No. of subjects =          122                     Number of obs   =      9078
No. of failures =           89
Time at risk    =  2048.428571
LR chi2(13)     =     61.37
Log likelihood  =  -347.78778                     Prob > chi2     =    0.0000
------------------------------------------------------------------------------
   _t | Haz. Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
  _Iseason_1 |    9.87688   6.663235     3.39    0.001     2.632533    37.05659
. est store model1
. est table model0 model1,b(%5.3f) p(%4.3f) stats(N ll aic bic)

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<th>model0</th>
<th>model1</th>
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<tr>
<td>bic</td>
<td>826.420</td>
<td>814.052</td>
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. lrtest model0 model1,stats

Likelihood-ratio test: LR chi2(2) = 5.86
(Assumption: model1 nested in model0) Prob > chi2 = 0.0534

Akaike's information criterion and Bayesian information criterion

<table>
<thead>
<tr>
<th>Model</th>
<th>Obs</th>
<th>ll(null)</th>
<th>ll(model)</th>
<th>df</th>
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<th>BIC</th>
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<td>826.4198</td>
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</table>

Note: N=Obs used in calculating BIC; see [R] BIC note
. *drop mob
. xi: stcox i.season ei hbf logmsp3 logr0 logr2 i hb_type i itn_use muac_scr
i.season     _Iseason_0-1 (naturally coded; _Iseason_0 omitted)
i hb_type     _Ihb_type_1-3 (naturally coded; _Ihb_type_1 omitted)
i itn_use     _Iitn_use_1-2 (naturally coded; _Iitn_use_1 omitted)

failure_d: malar10
analysis time_t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0:  log likelihood =  -378.47353
Iteration 1:  log likelihood =  -349.96533
Iteration 2:  log likelihood =  -349.09659
Iteration 3:  log likelihood =  -349.07859
Iteration 4:  log likelihood =  -349.07857
Refining estimates:
Iteration 0:  log likelihood =  -349.07857

Cox regression -- Breslow method for ties

No. of subjects =          122                     Number of obs   =      9078
No. of failures =           89                     Time at risk    =  2048.428571
LR chi2(10)     =     58.79                     Log likelihood  =  -349.07857                     Prob > chi2     =    0.0000

------------------------------------------------------------------------------
  _t | Haz. Ratio   Std. Err.        z    P>|z|   [95% Conf. Interval]
-------------
+----------------------------------------------------------------
 _Iseason_1 |   10.41847   7.163339     3.41   0.001     2.707391    40.09196
 ei |   1.083885   0.0213378     4.09   0.000     1.04286    1.126523
 hbf |   .9736989   0.0091795    -2.83   0.005     .9558726    .9918578
 logmsp3 |   1.363777   0.1494158     2.83   0.005     1.100235    1.690445
 logr0 |   1.163793   0.0091795    -2.83   0.005     1.100235    1.690445
 logr2 |   1.078476   0.0522807     2.07   0.039     1.007879    1.151727
 _Ihb_type_2 |   1.292225   0.4262081     0.78   0.437     .6770028    2.466526
 _Ihb_type_3 |   .4686875   0.3454411    -1.03   0.304     .1105387    1.987249
 _Iitn_use_2 |   .9727559   0.3728144    -0.07   0.943     .4589612    2.061733
 muac_scr |   1.172246   0.1212585     1.54   0.124     .9571266    1.435714

--------------------------------------
----------------------------------------

. est store model2
. est table model0 model1 model2,b(%5.3f) p(%4.3f) stats(N ll aic bic)

-----------------------------------------------------------------------------
Variable | model0   model1   model2
-------------
+-------------
 _Iseason_1 |    2.298    2.290    2.344
 ei |    0.001    0.001    0.001
 hbf |    0.072    0.078    0.081
 logmsp3 |    0.001    0.000    0.000
 logr0 |   -0.034   -0.028   -0.027
 logr2 |    0.001    0.006    0.005
 _Ihb_type_2 |    0.120    0.139    0.152
 _Ihb_type_3 |    0.325    0.248    0.201
 _Iitn_use_2 |    0.033   -0.000   -0.012
 muac_scr |    0.079    0.098    0.892

-----------------------------------------------------------------------------

. est store model2
. est table model0 model1 model2,b(%5.3f) p(%4.3f) stats(N ll aic bic)
. lrtest model0 model2, stats
Likelihood-ratio test         LR chi2(5) = 8.44
(Assumption: model2 nested in model0)     Prob > chi2 = 0.1335

Akaike's information criterion and Bayesian information criterion

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<tr>
<th>Model</th>
<th>Obs</th>
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<th>ll(model)</th>
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<th>BIC</th>
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<td>-344.8578</td>
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<td>719.7157</td>
<td>826.4198</td>
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</table>

Note: N=Obs used in calculating BIC; see [R] BIC note

-xdrop muac_scr-

.xistcox i.season ei hbf logmsp3 logr0 logr2 i.hb_type i.itn_use

failure _d: malar10
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Cox regression -- Breslow method for ties

No. of subjects =    122    Number of obs =    9078
No. of failures =       89
Time at risk =   2048.428571
Log likelihood =  -350.21669
LR chi2(9) = 56.51
Prob > chi2 = 0.0000

LOGISTIC REGRESSION

_beduc_1 |   0.609
_beduc_2 |  -0.068
muac_scr |   0.200
-------------
N |    9078      9078      9078
ll |  -344.858     -347.788    -349.079
aic |   719.716     721.576     718.157
bic |  826.420     814.052     789.293

Legend: b/p

Note: N=Obs used in calculating BIC; see [R] BIC note

*drop muac_scr*

.xistcox i.season ei hbf logmsp3 logr0 logr2 i.hb_type i.itn_use

failure _d: malar10
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0:   log likelihood =  -378.47353
Iteration 1:   log likelihood =  -351.08804
Iteration 2:   log likelihood =  -350.23558
Iteration 3:   log likelihood =  -350.21671
Iteration 4:   log likelihood =  -350.21669
Refining estimates:
Iteration 0:   log likelihood =  -350.21669

Cox regression -- Breslow method for ties

|   _t | Haz. Ratio Std. Err. | z    | P>|z|   [95% Conf. Interval] |
|------|-----------------------|------|------|-------------------------|
|   _Iseason_1 |       10.85486   |  7.513924 |  3.44   | 0.001       | 2.795168  | 42.15419 |
|      ei |       1.084239   |  0.021520 |  4.07   | 0.000       | 1.04287  | 1.127249 |
|      hbf |        .9727917  |  0.009551 | -2.96   | 0.003       | .955205  | .990722 |
|    logmsp3 |       1.340013   |  1.450433 |  2.70   | 0.007       | 1.083964 | 1.656698 |
|     logr0 |        1.149006   |  1.337748 |  1.19   | 0.233       | .9145765 | 1.443527 |
|    logr2 |        .9849329   |  0.084189 | -0.18   | 0.859       | .8330071 | 1.164567 |
|   _Ihb_type_2 |      1.294033   |  0.4264765 |  0.78  | 0.434       | .678267 | 2.46675 |
|   _Ihb_type_3 |        .517231   |  0.3805478 | -0.90  | 0.370       | .1222985 | 2.187499 |
|   _Iitn_use_2 |       1.8602875  |  3.216058 |  0.40   | 0.687       | .4134609 | 1.789999 |

.est store model3
.est table model0 model1 model2 model3,b(%5.3f) p(%4.3f) stats(N ll aic bic)
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Note: N=Obs used in calculating BIC; see [R] BIC note

*test for interaction between foetal haemoglobin and exposure index
. xi:stcox i.season ei hbf logmsp3 logr0 logr2 i.hb_type i.itn_use c.eic#chbf
i.season          _Iseason_0-1 (naturally coded; _Iseason_0 omitted)
i.hb_type         _Ihb_type_1-3 (naturally coded; _Ihb_type_1 omitted)
i.itn_use         _Iitn_use_1-2 (naturally coded; _Iitn_use_1 omitted)
failure _d:  malar10
analysis time _t:  (datevisit-origin)/28
origin: time datscreen
id:  code

Iteration 0:  log likelihood = -378.47353
Iteration 1:  log likelihood = -350.16068
Iteration 2:  log likelihood = -348.43686
Iteration 3:  log likelihood = -348.38439
Iteration 4:  log likelihood = -348.38439
Iteration 5: log likelihood = -348.38439
Refining estimates:
Iteration 0: log likelihood = -348.38439

Cox regression -- Breslow method for ties

No. of subjects = 122 Number of obs = 9078
No. of failures = 89
Time at risk = 2048.428571

LR chi2(10) = 60.18
Log likelihood = -348.38439 Prob > chi2 = 0.0000

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* test of proportional hazards assumption
.*---------------------------------------
. estat phtest,d

Test of proportional-hazards assumption

Time: Time

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<th>df</th>
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<td>0.56</td>
<td>1</td>
<td>0.4549</td>
</tr>
<tr>
<td>_Iitn_use_2</td>
<td>-0.12949</td>
<td>1.79</td>
<td>1</td>
<td>0.1811</td>
</tr>
<tr>
<td>c.\text{ei}#hbf</td>
<td>-0.10322</td>
<td>1.14</td>
<td>1</td>
<td>0.2859</td>
</tr>
</tbody>
</table>

* global test | 8.70     | 10  | 0.5610    |

. local grafreg "graphregion(fcolor(white) lcolor(white) ifcolor(white) ilcolor(white))"
. local plotreg "plotregion(fcolor(white) lcolor(white) ifcolor(white) ilcolor(white))"
. estat phtest,plot(logmsp3) yline(0) 'grafreg' 'plotreg' title(D)
. graph save 4,replace
(file 4.gph saved)
. estat phtest,plot(logr0) yline(0) 'grafreg' 'plotreg' title(E)
. graph save 5,replace
(file 5.gph saved)
. estat phtest,plot(logr2) yline(0) 'grafreg' 'plotreg' title(F)
. graph save 6,replace
(file 6.gph saved)
. estat phtest, plot(ei) yline(0) `grafreg' `plotreg' title(G)
. graph save 7, replace
(file 7.gph saved)
. estat phtest, plot(hbf) yline(0) `grafreg' `plotreg' title(H)
. graph save 8, replace
(file 8.gph saved)
. graph combine 4.gph 5.gph 6.gph 7.gph 8.gph, `grafreg' `plotreg'
. graph save phtest, replace
(file phtest.gph saved)
. graph export phtest.tif, width(2049) replace
(file phtest.tif written in TIFF format)
.
. log close
name:  <unnamed>
log:  C:\Users\dkangoye\Documents\chap2_coxreg.log
log type:  text
closed on:  13 Sep 2016, 18:35:48
----------------------------------------------------------

7.2.3 Negative binomial regression Stata code / Chapter 2

clear all
macro drop _all
capture log close
set more off
log using chap2_nbreg,replace text
*chap2_nbreg.do: construct predictive model for number of episodes
*david kangoye,PhD student,Open University/KEMRI-WTRP
version 11.2
set linesize 80
*=================================================================
cd "C:\Users\dkangoye\Dropbox\projects\phdprogram_ou\work\data\data_bics\elisa_bics"
use bicsV0, clear
*combine morbidity data with exposure data
*---------------------------------------------------------------
joinby code using dist15ei, unmatch(both)
tab_merge,m
list code if _merge==2
drop if _merge==2
*gen data set with an id that uniquely identify each couple of (code+period)
*---------------------------------------------------------------
sort code datevisit
egen id=concat(code period)
order id, before(code)
*generate variable for number of episodes for each child for each period
*---------------------------------------------------------------
egen numep=total(malar10), by(id)
order numep, after(malar4)
*keep only one observ per child for each period
*---------------------------------------------------------------
bysort id (datevisit):keep if _n==1  
sort code datevisit  
*gen variable for number of episodes occurred in the previous period  
gen numpep=numep[_n-1] if code==code[_n-1] & period != period[_n-1]  
replace numpep=0 if period=="0-3"  
order numep numpep,after(malar4)  
*gen variable for age  
drop age  
genage=(datevisit-dob)/28  
order age,after(numpep)  
order period,after(malar4)  
*define local macros for potential explanatory variables  
llocal envfac season ei mob zone  
llocal socufac ethn  
llocal matfac agem primgrav del_n sg_birth itn_use iptp_n educ  
llocal infac1 age sex hbt hbf muac height_scr weight_scr epi_stat neo_inf  
llocal infac2 logmsp3 logr0 logr2  
llocal infac3 msp3 r0 r2  
*keep/order variables of interest and sort observations  
keep id code dob datevisit period numep numpep `envfac' `socufac' `matfac' `infac1' `infac2' `infac3' monthn months  
order id code datevisit age period season ei numep numpep msp3 r0 r2 logmsp3 logr0 logr2  
sort code datevisit  
save bicsVx,replace  
*select between poisson/nbreg for non negative count data regression:  
*compare mean and variance of outcome variable  
use bicsVx,clear  
hist numep,freq  
tabstat numep,s(mean v)  

codebook mob  
recode mob (10=1) (11=2) (12=3) (1=4)  
codebook mob  
order mob,after(dob)  
lab def cald 1"October" 2"November" 3"December" 4"January"  
lab val mob cald  
tab mob  
tab mob,nolabel  
foreach i in logmsp3 logr0 logr2{  
gen b’i’=’i’ if months=="M00"  
sort code datevisit  
replace b’i’=b’i’[_n-1] if code==code[_n-1]  
lab var b’i’ "baseline anti-’i’ titer"  
order b’i’,after(’i’)  
replace b’i’=round(b’i’,.01)  
sum b’i’,d  
}  
*mfp of age for nbreg model  
mfp nbreg numep age  
corr age lage__1 lage__2  
*define local macros for predictor var to be used in regression analysis  
llocal envfac i.season ei i.mob i.zone  
llocal socufac i.educ  
llocal matfac a i.primgrav i.itn_use i.iptp_n  
llocal infac1 i.sex  muac_scr height_scr weight_scr i.epi_stat
local infac2 i.hb_type hbf logmsp3 logr0 logr2 blogmsp3 blogr0 blogr2
local age Iage__1 Iage__2

*run univariate nbreg adjusting for clusters
*==============================================================================
foreach i in age `age' `infac2' `infac1' `matfac' `envfac' `socufac' {
    xi:nbreg numep `i', cluster(code) irr
}

*perform collinearity diagnostics
*==============================================================================
corr numep age logmsp3 logr0 logr2 hbf i.hb_type muac_scr mob epi_stat ///
    agem itn_use educ season ei

xi:collin numep age logmsp3 logr0 logr2 hbf i.hb_type muac_scr i.mob i.epi_stat ///
    agem i.itn_use i.educ i.season ei

*multivariate regression analysis using changing antibody titres adjusting
for clusters
*===================================================================================

*model0: include if p<0.2 or high importance var
xi:nbreg numep age logmsp3 logr0 logr2 hbf i.hb_type muac_scr i.mob i.epi_stat ///
    agem i.itn_use i.educ i.season ei, cluster(code) irr
xi:glm numep age logmsp3 logr0 logr2 hbf i.hb_type muac_scr i.mob i.epi_stat ///
    agem i.itn_use i.educ i.season ei, family(nb) cluster(code)

*run wald test to select covariates for the final model
xi:testparm i.epi_stat
    test agem
    xi:testparm i.mob
    xi:testparm i.educ
    test muac_scr

xi:nbreg numep age logmsp3 logr0 logr2 hbf i.hb_type muac_scr ///
    i.itn_use i.educ i.season ei, cluster(code) irr

log close
exit

7.2.4 Negative binomial regression output log / Chapter 2
*combine morbidity data with exposure data
*==========================================
joinby code using dist15ei, unn(both)
*tab _merge,m

<table>
<thead>
<tr>
<th>_merge</th>
<th>Freq.</th>
<th>Percent</th>
<th>Cum.</th>
</tr>
</thead>
<tbody>
<tr>
<td>both in master and using data</td>
<td>14,148</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>14,148</td>
<td>100.00</td>
</tr>
</tbody>
</table>

list code if _merge==2
drop if _merge==2
(0 observations deleted)
drop _merge

*gen data set with an id that uniquely identify each couple of (code+period)
*===========================================================================
sort code datevisit
egen id=concat(code period)
order id,before(code)

*generate variable for number of episodes for each child for each period
*===========================================================================
egen numep=total(malar10), by (id)
order numep, after(malar4)

*keep only one observ per child for each period
*===========================================================================
bysort id (datevisit): keep if _n==1
(13300 observations deleted)
sort code datevisit

*gen variable for number of episodes occured in the previous period
*===========================================================================
gen numpep=numep[_n-1] if code==code[_n-1] & period != period[_n-1]
(125 missing values generated)
replace numpep=0 if period=="0-3"
(123 real changes made)
order numep numpep, after(malar4)

*gen variable for age
*===========================================================================
drop age
generate age=(datevisit-dob)/28
order age, after(numpep)
order period, after(malar4)

*define local macros for potential explanatory variables
*===========================================================================
local envfac season ei mob zone
local socufac ethn
local matfac agem primgrav del_n sg_birth itn_use iptp_n educ
. local infac1 age sex hb_type hbf muac height_scr weight_scr epi_stat neo_inf
>  
. local infac2 logmsp3 logr0 logr2
. local infac3 msp3 r0 r2
>
. *keep/order variables of interest and sort observations
. *======================================================
. keep id code dob datevisit period numep numpep `envfac' `socufac' `matfac' ///
>     'infac1' 'infac2' 'infac3' monthn months
. order id code datevisit age period season ei numep numpep msp3 r0 r2 logr0 logr2
>
. sort code datevisit
>
. save bicsVx,replace
file bicsVx.dta saved
>
. *select between poisson/nbreg for non negative count data regression:
. *compare mean and variance of outcome variable
. *=======================================================
. use bicsVx,clear
(combination of all data sets {morb+geo+sero})
>
. hist numep,freq
(bin=29, start=0, width=.17241379)
>
. tabstat numep,s(mean v)
variable |      mean  variance
-------------+----------------------------------
umep |  .3419811   .631435

. codebook mob

--------------------------------------------------------------------------------
mob                                                                 month of birth
--------------------------------------------------------------------------------
type:  numeric (float)

range:  [1,12]                       units:  1
unique values:  4                        missing .:  0/848

  tabulation: Freq.  Value
             177   1
             74   10
             267  11
            330  12

. recode mob (10=1) (11=2) (12=3) (1=4)
(mob: 848 changes made)

. codebook mob

--------------------------------------------------------------------------------
mob                                                                 month of birth
--------------------------------------------------------------------------------
type:  numeric (float)

range:  [1,4]                       units:  1
unique values:  4                        missing .:  0/848

  tabulation: Freq.  Value
             74   1

216
. order mob,after(dob)

. lab def cald 1"October" 2"November" 3"December" 4"January"

. lab val mob cald

. tab mob

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>October</td>
<td>74</td>
<td>8.73</td>
<td>8.73</td>
</tr>
<tr>
<td>November</td>
<td>267</td>
<td>31.49</td>
<td>40.21</td>
</tr>
<tr>
<td>December</td>
<td>330</td>
<td>38.92</td>
<td>79.13</td>
</tr>
<tr>
<td>January</td>
<td>177</td>
<td>20.87</td>
<td>100.00</td>
</tr>
<tr>
<td>Total</td>
<td>848</td>
<td>100.00</td>
<td></td>
</tr>
</tbody>
</table>

. tab mob, nolabel

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>74</td>
<td>8.73</td>
<td>8.73</td>
</tr>
<tr>
<td>2</td>
<td>267</td>
<td>31.49</td>
<td>40.21</td>
</tr>
<tr>
<td>3</td>
<td>330</td>
<td>38.92</td>
<td>79.13</td>
</tr>
<tr>
<td>4</td>
<td>177</td>
<td>20.87</td>
<td>100.00</td>
</tr>
<tr>
<td>Total</td>
<td>848</td>
<td>100.00</td>
<td></td>
</tr>
</tbody>
</table>

. foreach i in logmsp3 logr0 logr2{
  2.         gen b`i'=`i' if months=="M00"
  3.         sort code datevisit
  4.         replace b`i'=b`i'[_n-1] if code==code[_n-1]
  5.         lab var b`i' "baseline anti-`i' titer"
  6.         order b`i',after(`i')
  7.         replace b`i'='round(b`i',.01)
  8.         sum b`i',d
  9. }

(725 missing values generated)
(713 real changes made)
(836 real changes made)

baseline anti-logmsp3 titer

<table>
<thead>
<tr>
<th>Percentiles</th>
<th>Smallest</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>.29</td>
</tr>
<tr>
<td>5%</td>
<td>.8</td>
</tr>
<tr>
<td>10%</td>
<td>2.05</td>
</tr>
<tr>
<td>25%</td>
<td>2.84</td>
</tr>
<tr>
<td>50%</td>
<td>4.33</td>
</tr>
<tr>
<td>75%</td>
<td>5.37</td>
</tr>
<tr>
<td>90%</td>
<td>6.5</td>
</tr>
<tr>
<td>95%</td>
<td>6.83</td>
</tr>
<tr>
<td>99%</td>
<td>7.58</td>
</tr>
</tbody>
</table>

Mean: 4.160275  Std. Dev.: 1.758796  Variance: 3.093364  Skewness: -.1311114  Kurtosis: 2.483507

(725 missing values generated)
(713 real changes made)
(836 real changes made)

baseline anti-logr0 titer

<table>
<thead>
<tr>
<th>Percentiles</th>
<th>Smallest</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>.52</td>
</tr>
<tr>
<td>5%</td>
<td>1.7</td>
</tr>
<tr>
<td>10%</td>
<td>2.13</td>
</tr>
<tr>
<td>25%</td>
<td>2.96</td>
</tr>
</tbody>
</table>

Mean: 3.230275  Std. Dev.: 1.158796  Variance: 1.348364  Skewness: -1.103114  Kurtosis: 2.243507

(725 missing values generated)
(713 real changes made)
(836 real changes made)
### Percentiles and Statistics

<table>
<thead>
<tr>
<th>Percentile</th>
<th>Small</th>
<th>Large</th>
<th>Mean</th>
<th>Std. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>.97</td>
<td>.52</td>
<td>3.076944</td>
<td>1.689534</td>
</tr>
<tr>
<td>5%</td>
<td>2.71</td>
<td>.52</td>
<td>3.259305</td>
<td>1.689534</td>
</tr>
<tr>
<td>10%</td>
<td>2.92</td>
<td>.52</td>
<td>3.259305</td>
<td>1.689534</td>
</tr>
<tr>
<td>25%</td>
<td>4.315</td>
<td>.52</td>
<td>3.526607</td>
<td>1.689534</td>
</tr>
<tr>
<td>50%</td>
<td>5.34</td>
<td></td>
<td>3.908612</td>
<td>1.4613</td>
</tr>
<tr>
<td>75%</td>
<td>6.45</td>
<td>9</td>
<td>4.912082</td>
<td>2.673533</td>
</tr>
<tr>
<td>90%</td>
<td>7.22</td>
<td>9</td>
<td>5.843594</td>
<td>2.673533</td>
</tr>
<tr>
<td>95%</td>
<td>7.7</td>
<td>9</td>
<td>6.515108</td>
<td>2.673533</td>
</tr>
</tbody>
</table>

### Variability Measures

- Variance: 2.135399
- Skewness: .1160334
- Kurtosis: 2.914036

(725 missing values generated)
(713 real changes made)
(836 real changes made)

### Anti-logr2 Titer

#### Percentiles

<table>
<thead>
<tr>
<th>Percentile</th>
<th>Small</th>
<th>Large</th>
<th>Mean</th>
<th>Std. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>.97</td>
<td>.52</td>
<td>3.076944</td>
<td>1.689534</td>
</tr>
<tr>
<td>5%</td>
<td>2.71</td>
<td>.52</td>
<td>3.259305</td>
<td>1.689534</td>
</tr>
<tr>
<td>10%</td>
<td>2.92</td>
<td>.52</td>
<td>3.259305</td>
<td>1.689534</td>
</tr>
<tr>
<td>25%</td>
<td>4.315</td>
<td>.52</td>
<td>3.526607</td>
<td>1.689534</td>
</tr>
<tr>
<td>50%</td>
<td>5.34</td>
<td></td>
<td>3.908612</td>
<td>1.4613</td>
</tr>
<tr>
<td>75%</td>
<td>6.45</td>
<td>9</td>
<td>4.912082</td>
<td>2.673533</td>
</tr>
<tr>
<td>90%</td>
<td>7.22</td>
<td>9</td>
<td>5.843594</td>
<td>2.673533</td>
</tr>
<tr>
<td>95%</td>
<td>7.7</td>
<td>9</td>
<td>6.515108</td>
<td>2.673533</td>
</tr>
</tbody>
</table>

### Deviance for Model with All Terms Untransformed

Deviance: 1164.137, 848 observations

#### Transformations of Covariates

- `gen double Iage__1 = X^3 - 1.440072283 if e(sample)`
- `gen double Iage__2 = X^3*ln(X) - .1750615751 if e(sample)`

(Where: X = age/10)

### Final Multivariable Fractional Polynomial Model for numep

#### Coefficients

| Term      | Coef.  | Std. Err. | z     | P>|z|  | 95% Conf. Interval |
|-----------|--------|-----------|-------|------|-------------------|
| `Iage__1` | 1.231002 | .0910916  | 13.51 | 0.000| 1.052466 - 1.409538 |
| `Iage__2` | -1.218107 | .0944402  | -12.90 | 0.000| -1.403206 - 1.033007 |
| _cons    | -1.324822 | .0967993  | -13.69 | 0.000| -1.514545 - 1.135098 |

#### Log Likelihood

- Number of obs = 848
- LR chi2(2) = 180.66
- Prob > chi2 = 0.0000
- Log likelihood = -537.81752
- Pseudo R2 = 0.1438

#### Likelihood-Ratio Test of alpha=0

- chibar2(01) = 17.48
- Prob > chibar2 = 0.000
- Deviance: 1075.635

### Correlation

- `corr age Iage__1 Iage__2`
*define local macros for predictor var to be used in regression analysis
local envfac i.season ei.i.mob i.zone
local socufac i.educ
local matfac agem i.primgrav i.itn_use i.iptp_n
local infac1 i.sex muac_scr height_scr weight_scr i.epi_stat
local infac2 i.hb_type hbf logmsp3 logr0 logr2 blogmsp3 blogr0 blogr2
local age Iage__1 Iage__2

*run univariate nbreg adjusting for clusters
foreach i in age `age' `infac2' `infac1' `matfac' `envfac' `socufac'{
   xi:nbreg numep `i', cluster(code) irr
}

Fitting Poisson model:
Iteration 0: log pseudolikelihood = -633.52912
Iteration 1: log pseudolikelihood = -633.52897
Iteration 2: log pseudolikelihood = -633.52897

Fitting constant-only model:
Iteration 0: log pseudolikelihood = -645.90918
Iteration 1: log pseudolikelihood = -628.39289
Iteration 2: log pseudolikelihood = -628.14696
Iteration 3: log pseudolikelihood = -628.14648
Iteration 4: log pseudolikelihood = -628.14648

Fitting full model:
Iteration 0: log pseudolikelihood = -591.75095
Iteration 1: log pseudolikelihood = -582.30352
Iteration 2: log pseudolikelihood = -582.06864
Iteration 3: log pseudolikelihood = -582.06863

Negative binomial regression
Number of obs = 848
Wald chi2(1) = 245.35
Prob > chi2 = 0.0000
Log pseudolikelihood = -582.06863
Pseudo R2 = 0.0734

(Std. Err. adjusted for 125 clusters in code)

| numep | IRR  | Std. Err. | z     | P>|z| | [95% Conf. Interval] |
|-------|------|-----------|-------|------|----------------------|
| age   | 1.115524 | .00776858 | 15.66 | 0.000 | 1.100368 1.130888 |
| /lnalpha | .6900406 | .1458838 | 4.78 | 0.000 | .5041136 .9759676 |
| alpha | 1.993797 | .2908626 | 3.41 | 0.001 | 1.497974 2.653734 |

Fitting Poisson model:
Iteration 0: log pseudolikelihood = -676.65514
Iteration 1: log pseudolikelihood = -676.64564
Iteration 2: log pseudolikelihood = -676.64564

Fitting constant-only model:

- Iteration 0: log pseudolikelihood = -645.90918
- Iteration 1: log pseudolikelihood = -628.39289
- Iteration 2: log pseudolikelihood = -628.14696
- Iteration 3: log pseudolikelihood = -628.14648

Fitting full model:

- Iteration 0: log pseudolikelihood = -613.16418
- Iteration 1: log pseudolikelihood = -610.26627
- Iteration 2: log pseudolikelihood = -610.19808
- Iteration 3: log pseudolikelihood = -610.19806

Negative binomial regression

<table>
<thead>
<tr>
<th>Number of obs</th>
<th>Wald ch2(1)</th>
<th>Dispersion</th>
<th>Log pseudolikelihood</th>
</tr>
</thead>
<tbody>
<tr>
<td>848</td>
<td>117.29</td>
<td>mean</td>
<td>-610.19806</td>
</tr>
</tbody>
</table>

Pseudo R2 = 0.0286

<table>
<thead>
<tr>
<th>(Std. Err. adjusted for 125 clusters in code)</th>
</tr>
</thead>
<tbody>
<tr>
<td>nump</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>Iage</td>
</tr>
<tr>
<td>cons</td>
</tr>
</tbody>
</table>

| /lnalpha | .9787 | .1358 | -7.86 | 0.000 | .7126296 | 1.244888 |

| alpha | 2.661 | .3613 | 2.0393 | 0.000 | 3.472546 |

<table>
<thead>
<tr>
<th>(Std. Err. adjusted for 125 clusters in code)</th>
</tr>
</thead>
<tbody>
<tr>
<td>nump</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>Iage</td>
</tr>
<tr>
<td>cons</td>
</tr>
</tbody>
</table>

| /lnalpha | 1.054 | .1340 | 7.9059 | 0.000 | 1.316522 |

| alpha | 2.86 | .3845 | 2.0551 | 0.000 | 3.730426 |

i.hb_type _Ihb_type_1 (naturally coded; _Ihb_type_1 omitted)
Fitting Poisson model:

Iteration 0:  log pseudolikelihood =  -697.05025
Iteration 1:  log pseudolikelihood =  -696.72745
Iteration 2:  log pseudolikelihood =  -696.72620
Iteration 3:  log pseudolikelihood =  -696.72609

Fitting constant-only model:

Iteration 0:  log pseudolikelihood =  -644.13887
Iteration 1:  log pseudolikelihood =  -642.54052
Iteration 2:  log pseudolikelihood =  -626.7411
Iteration 3:  log pseudolikelihood =  -626.73975
Iteration 4:  log pseudolikelihood =  -626.73975

Fitting full model:

Iteration 0:  log pseudolikelihood =  -625.50456
Iteration 1:  log pseudolikelihood =  -624.51526
Iteration 2:  log pseudolikelihood =  -624.51071
Iteration 3:  log pseudolikelihood =  -624.51071

Negative binomial regression

Number of obs   =        842
Dispersion           = mean                       Prob > chi2     =     0.0000
Log pseudolikelihood =  -624.51071

(Std. Err. adjusted for 124 clusters in code)

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Fitting Poisson model:

Iteration 0:  log pseudolikelihood =  -698.36129
Iteration 1:  log pseudolikelihood =  -698.36129

Fitting constant-only model:

Iteration 0:  log pseudolikelihood =  -644.13887
Iteration 1:  log pseudolikelihood =  -642.54052
Iteration 2:  log pseudolikelihood =  -626.7411
Iteration 3:  log pseudolikelihood =  -626.73975
Iteration 4:  log pseudolikelihood =  -626.73975

Fitting full model:

Iteration 0:  log pseudolikelihood =  -625.50456
Iteration 1:  log pseudolikelihood =  -624.51526
Iteration 2:  log pseudolikelihood =  -624.51071
Iteration 3:  log pseudolikelihood =  -624.51071

Negative binomial regression

Number of obs   =        842
Dispersion           = mean                       Prob > chi2     =     0.1326
Log pseudolikelihood =  -625.74249

(Std. Err. adjusted for 124 clusters in code)

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221
Fitting Poisson model:
Iteration 0:  log pseudolikelihood = -699.2549
Iteration 1:  log pseudolikelihood = -699.2549

Fitting constant-only model:
Iteration 0:  log pseudolikelihood = -645.90918
Iteration 1:  log pseudolikelihood = -628.39289
Iteration 2:  log pseudolikelihood = -628.14696
Iteration 3:  log pseudolikelihood = -628.14648
Iteration 4:  log pseudolikelihood = -628.14648

Fitting full model:
Iteration 0:  log pseudolikelihood = -626.43682
Iteration 1:  log pseudolikelihood = -626.3765
Iteration 2:  log pseudolikelihood = -626.37649

Negative binomial regression
Number of obs   =        848
Wald chi2(1)    =       4.37
Dispersion      = mean          Prob > chi2     =     0.0365
Log pseudolikelihood = -626.37649          Pseudo R2       =     0.0028
(Std. Err. adjusted for 125 clusters in code)

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Fitting Poisson model:
Iteration 0:  log pseudolikelihood = -699.72369
Iteration 1:  log pseudolikelihood = -699.72369

Fitting constant-only model:
Iteration 0:  log pseudolikelihood = -645.90918
Iteration 1:  log pseudolikelihood = -628.39289
Iteration 2:  log pseudolikelihood = -628.14696
Iteration 3:  log pseudolikelihood = -628.14648
Iteration 4:  log pseudolikelihood = -628.14648

Fitting full model:
Iteration 0:  log pseudolikelihood = -626.74277
Iteration 1:  log pseudolikelihood = -626.70723
Iteration 2:  log pseudolikelihood = -626.70722

Negative binomial regression
Number of obs   =        848
Wald chi2(1)    =       5.34
Dispersion      = mean          Prob > chi2     =     0.0209
Log pseudolikelihood = -626.70722          Pseudo R2       =     0.0023
(Std. Err. adjusted for 125 clusters in code)

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Fitting Poisson model:
Iteration 0:  log pseudolikelihood = -702.32192
Iteration 1:  log pseudolikelihood = -702.32192

Fitting constant-only model:
Iteration 0:  log pseudolikelihood = -645.90918
Iteration 1:  log pseudolikelihood = -628.39289
Iteration 2:  log pseudolikelihood = -628.14696
Iteration 3:  log pseudolikelihood = -628.14648
Iteration 4:  log pseudolikelihood = -628.14648

Fitting full model:
Iteration 0:  log pseudolikelihood = -628.14133
Iteration 1:  log pseudolikelihood = -628.14131

Negative binomial regression
Number of obs = 848
Wald chi2(1) = 0.01
Dispersion = mean
Prob > chi2 = 0.9145
Log pseudolikelihood = -628.14131
Pseudo R2 = 0.0000

(Std. Err. adjusted for 125 clusters in code)

|            | Robust
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Fitting Poisson model:
Iteration 0:  log pseudolikelihood = -692.14524
Iteration 1:  log pseudolikelihood = -692.14524

Fitting constant-only model:
Iteration 0:  log pseudolikelihood = -636.91081
Iteration 1:  log pseudolikelihood = -635.75433
Iteration 2:  log pseudolikelihood = -619.75637
Iteration 3:  log pseudolikelihood = -619.75511
Iteration 4:  log pseudolikelihood = -619.75511

Fitting full model:
Iteration 0:  log pseudolikelihood = -619.75496
Iteration 1:  log pseudolikelihood = -619.75496

Negative binomial regression
Number of obs = 836
Wald chi2(1) = 0.00
Dispersion = mean
Prob > chi2 = 0.9856
Log pseudolikelihood = -619.75496
Pseudo R2 = 0.0000

(Std. Err. adjusted for 123 clusters in code)

|            | Robust
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### Fitting Poisson model:

Iteration 0:  log pseudolikelihood = -691.31192
Iteration 1:  log pseudolikelihood = -691.31192

### Fitting constant-only model:

Iteration 0:  log pseudolikelihood = -636.91081
Iteration 1:  log pseudolikelihood = -635.75433
Iteration 2:  log pseudolikelihood = -619.75637
Iteration 3:  log pseudolikelihood = -619.75511
Iteration 4:  log pseudolikelihood = -619.75511

### Fitting full model:

Iteration 0:  log pseudolikelihood = -619.35038
Iteration 1:  log pseudolikelihood = -619.34922
Iteration 2:  log pseudolikelihood = -619.34922

### Negative binomial regression

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### Fitting Poisson model:

Iteration 0:  log pseudolikelihood = -689.9109
Iteration 1:  log pseudolikelihood = -689.9109

### Fitting constant-only model:

Iteration 0:  log pseudolikelihood = -636.91081
Iteration 1:  log pseudolikelihood = -635.75433
Iteration 2:  log pseudolikelihood = -619.75637
Iteration 3:  log pseudolikelihood = -619.75511
Iteration 4:  log pseudolikelihood = -619.75511

### Fitting full model:

Iteration 0:  log pseudolikelihood = -618.66005
Iteration 1:  log pseudolikelihood = -618.65157
Iteration 2:  log pseudolikelihood = -618.65157

### Negative binomial regression

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alpha | 3.009321  .4007784  2.317962   3.906885  2.317962   3.906885

+---------------------------------------------------------------------+-
| i.sex |   _Isex_1-2         | (naturally coded; _Isex_1 omitted) |
+---------------------------------------------------------------------+-

Fitting Poisson model:
Iteration 0:   log pseudolikelihood = -701.67573
Iteration 1:   log pseudolikelihood = -701.67573

Fitting constant-only model:
Iteration 0:   log pseudolikelihood = -645.90918
Iteration 1:   log pseudolikelihood = -628.39289
Iteration 2:   log pseudolikelihood = -628.14648
Iteration 3:   log pseudolikelihood = -628.14648
Iteration 4:   log pseudolikelihood = -628.14648

Fitting full model:
Iteration 0:   log pseudolikelihood = -627.82688
Iteration 1:   log pseudolikelihood = -627.82617
Iteration 2:   log pseudolikelihood = -627.82617

Negative binomial regression                      Number of obs   =        848
Wald chi2(1)    =       0.66
Dispersion           = mean                       Prob > chi2     =     0.4183
Log pseudolikelihood = -627.82617                 Pseudo R2       =     0.0005
(Std. Err. adjusted for 125 clusters in code)

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/lnalpha | 1.121781  .129468  8.680288   1.375534

alpha | 3.070319  .3975081  2.38221   3.95719

Fitting Poisson model:
Iteration 0:   log pseudolikelihood = -695.95391
Iteration 1:   log pseudolikelihood = -695.95391

Fitting constant-only model:
Iteration 0:   log pseudolikelihood = -645.90918
Iteration 1:   log pseudolikelihood = -628.39289
Iteration 2:   log pseudolikelihood = -628.14648
Iteration 3:   log pseudolikelihood = -628.14648
Iteration 4:   log pseudolikelihood = -628.14648

Fitting full model:
Iteration 0:   log pseudolikelihood = -625.03772
Iteration 1:   log pseudolikelihood = -624.96878
Iteration 2:   log pseudolikelihood = -624.96878

Negative binomial regression                      Number of obs   =        848
Wald chi2(1)    =       6.35
Dispersion           = mean                       Prob > chi2     =     0.0118
Log pseudolikelihood = -624.96876                 Pseudo R2       =     0.0051
(Std. Err. adjusted for 125 clusters in code)

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225
### Fitting Poisson model:

Iteration 0:  log pseudolikelihood = -698.63185
Iteration 1:  log pseudolikelihood = -698.63185

### Fitting constant-only model:

Iteration 0:  log pseudolikelihood = -643.84275
Iteration 1:  log pseudolikelihood = -641.93474
Iteration 2:  log pseudolikelihood = -626.50538
Iteration 3:  log pseudolikelihood = -626.50419
Iteration 4:  log pseudolikelihood = -626.50419

### Fitting full model:

Iteration 0:  log pseudolikelihood = -625.92657
Iteration 1:  log pseudolikelihood = -625.91939
Iteration 2:  log pseudolikelihood = -625.91939
Iteration 3:  log pseudolikelihood = -625.91939
Iteration 4:  log pseudolikelihood = -625.91939

### Negative binomial regression

| numep | IRR   | Std. Err. | z    | P>|z| | 95% Conf. Interval |
|-------|-------|-----------|------|-----|---------------------|
| height_scr | 1.034575 | .0311853 | 1.13 | .259 | .9752235 - 1.097539 |
| _cons | .0541612 | .0892531 | -1.77 | .080 | .0021428 - 1.368976 |

| /lnalpha | 1.105535 | .1300929 | 0.8505576 - 1.360513 |

| alpha | 3.02084 | .39299 | 2.340952 - 3.898191 |

### Fitting Poisson model:

Iteration 0:  log pseudolikelihood = -698.42233
Iteration 1:  log pseudolikelihood = -698.42233

### Fitting constant-only model:

Iteration 0:  log pseudolikelihood = -642.48049
Iteration 1:  log pseudolikelihood = -642.96432
Iteration 2:  log pseudolikelihood = -624.71768
Iteration 3:  log pseudolikelihood = -624.71721
Iteration 4:  log pseudolikelihood = -624.71721

### Fitting full model:

Iteration 0:  log pseudolikelihood = -624.51178
Iteration 1:  log pseudolikelihood = -624.51122
Iteration 2:  log pseudolikelihood = -624.51122

### Negative binomial regression

| numep | IRR   | Std. Err. | z    | P>|z| | 95% Conf. Interval |
|-------|-------|-----------|------|-----|---------------------|
| weight_scr | 1.095997 | .1522895 | 0.66 | .509 | .8347072 - 1.439078 |
| _cons | .2347934 | .1366372 | -2.49 | .013 | .0750468 - .7345812 |

(Std. Err. adjusted for 124 clusters in code)
Fitting Poisson model:
Iteration 0:  log pseudolikelihood = -700.47843
Iteration 1:  log pseudolikelihood = -700.47843

Fitting constant-only model:
Iteration 0:  log pseudolikelihood = -645.90918
Iteration 1:  log pseudolikelihood = -628.39289
Iteration 2:  log pseudolikelihood = -628.14696
Iteration 3:  log pseudolikelihood = -628.14648
Iteration 4:  log pseudolikelihood = -628.14648

Fitting full model:
Iteration 0:  log pseudolikelihood = -627.28304
Iteration 1:  log pseudolikelihood = -627.27783
Iteration 2:  log pseudolikelihood = -627.27783
Iteration 3:  log pseudolikelihood = -627.27783
Iteration 4:  log pseudolikelihood = -627.27783

Negative binomial regression
Number of obs = 848
Wald chi2(1) = 2.90
Dispersion = mean
Log pseudolikelihood = -627.27783
Pseudo R2 = 0.0014

(Std. Err. adjusted for 125 clusters in code)

-----------------------------------------------------------------------------
<p>|              Robust              |              |              |              |
| numep  |  IRR   | Std. Err. |    z     | P&gt;|z|   | [95% Conf. Interval] |
|-------------|--------|-----------|--------|-------|---------------------|
| _Iepi_stat_2 | 1.378817| .2601219 | 1.70   | 0.089 | .9526262  1.99568  |</p>
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Fitting Poisson model:
Iteration 0:  log pseudolikelihood = -699.12461
Iteration 1:  log pseudolikelihood = -699.12461

Fitting constant-only model:
Iteration 0:  log pseudolikelihood = -645.90918
Iteration 1:  log pseudolikelihood = -628.39289
Iteration 2:  log pseudolikelihood = -628.14696
Iteration 3:  log pseudolikelihood = -628.14648
Iteration 4:  log pseudolikelihood = -628.14648

Fitting full model:
Iteration 0:  log pseudolikelihood = -626.65079
Iteration 1:  log pseudolikelihood = -626.63393
Iteration 2:  log pseudolikelihood = -626.63393

Negative binomial regression
Number of obs = 848
Wald chi2(1) = 3.67
Dispersion = mean
Log pseudolikelihood = -626.63393
Pseudo R2 = 0.0024

(Std. Err. adjusted for 125 clusters in code)

-----------------------------------------------------------------------------
|              Robust              |              |              |              |
| numep  |  IRR   | Std. Err. |    z     | P>|z|   | [95% Conf. Interval] |
|-------------|--------|-----------|--------|-------|---------------------|
| agem        | 1.022515| .0118834 | 1.92   | 0.055 | .9994871  1.046073  |
| _cons       | .1884063| .0626125 | -5.02  | 0.000 | .0982241  .3613872  |
-----------------------------------------------------------------------------
/lnalpha |  1.105245   .1341747                      .8422678    1.368223
alpha |   3.019965   .4052028                      2.321626    3.928363

i.primgrav
_Iprimgrav_1-2  (naturally coded; _Iprimgrav_1 omitted)

Fitting Poisson model:
Iteration 0:  log pseudolikelihood = -695.53486
Iteration 1:  log pseudolikelihood = -695.53486

Fitting constant-only model:
Iteration 0:  log pseudolikelihood = -639.74823
Iteration 1:  log pseudolikelihood = -622.03197
Iteration 2:  log pseudolikelihood = -621.84529
Iteration 3:  log pseudolikelihood = -621.84503
Iteration 4:  log pseudolikelihood = -621.84503

Fitting full model:
Iteration 0:  log pseudolikelihood = -621.60308
Iteration 1:  log pseudolikelihood = -621.60267
Iteration 2:  log pseudolikelihood = -621.60267

Negative binomial regression  Number of obs  =   841
Wald chi2(1)    =      0.52
Dispersion        = mean                      Prob > chi2     =     0.4697
Log pseudolikelihood = -621.60267
Pseudo R2        =     0.0004

(Std. Err. adjusted for 124 clusters in code)

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_Iprimgrav_2 | 1.160122   .2383361     0.72   0.470     .7755907    1.735302
_cons    | .3028571    .0553221   -6.54   0.000    .2117148    .4332359

/lnalpha |  1.131013   .1324086                      .8714971    1.390529
alpha |   3.098794   .401307                      2.390487    4.016975

i.itn_use
_Iitn_use_1-2  (naturally coded; _Iitn_use_1 omitted)

Fitting Poisson model:
Iteration 0:  log pseudolikelihood = -699.02896
Iteration 1:  log pseudolikelihood = -699.02896

Fitting constant-only model:
Iteration 0:  log pseudolikelihood = -642.77572
Iteration 1:  log pseudolikelihood = -625.1816
Iteration 2:  log pseudolikelihood = -624.9517
Iteration 3:  log pseudolikelihood = -624.9513
Iteration 4:  log pseudolikelihood = -624.9513

Fitting full model:
Iteration 0:  log pseudolikelihood = -624.86646
Iteration 1:  log pseudolikelihood = -624.86641

Negative binomial regression  Number of obs  =   842
Wald chi2(1)    =      0.14
Dispersion        = mean                      Prob > chi2     =     0.7047
Log pseudolikelihood = -624.86641
Pseudo R2        =     0.0001

(Std. Err. adjusted for 124 clusters in code)

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<tbody>
<tr>
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-------------------|
_Iitn_use_2 | 1.110725   .3077197     0.38   0.705     .6453369    1.911732
Negative binomial regression                      Number of obs   =        848
Wald chi2(3)    =      19.75
Dispersion           = mean                       Prob > chi2     =     0.0000
Log pseudolikelihood = -619.47886                 Pseudo R2       =     0.0138

(Std. Err. adjusted for 125 clusters in code)
-----------------------------------------------------------------------------
| numep | IRR  | Std. Err. | z    | P>|z| |  [95% Conf. Interval] |
-------------|------|-----------|------|------|-----------------------|
_ipvtp_n_1   | 2.042857 | .8610699 | 1.69 | 0.090 | .8942402 - 4.666828 |
_ipvtp_n_2   | 1.5   | .6046066 | 1.01 | 0.314 | .6807639 - 3.305111 |
_ipvtp_n_3   | .7051282 | .6054798 | -0.41 | 0.684 | .1310261 - 3.794707 |
     _cons   | .2181818 | .0852793 | -3.90 | 0.000 | .101418 - .4693772 |
-------------|------|-----------|------|------|-----------------------|
/inalpha    | 1.093923 | .1299048 | 8.39 | < 0.001 | .8391572 - 1.348688 |
-------------|------|-----------|------|------|-----------------------|
     alpha   | 2.985964 | .3881301 | 7.67 | < 0.001 | 2.314415 - 3.852369 |
-------------|------|-----------|------|------|-----------------------|
i.season    | 1.039923 | .1299048 | 8.39 | < 0.001 | .8391572 - 1.348688 |
-------------|------|-----------|------|------|-----------------------|

Fitting Poisson model:
Iteration 0:   log pseudolikelihood = -685.2339
Iteration 1:   log pseudolikelihood = -685.2339
Iteration 2:   log pseudolikelihood = -685.2339

Fitting constant-only model:
Iteration 0:   log pseudolikelihood = -645.90918
Iteration 1:   log pseudolikelihood = -628.39289
Iteration 2:   log pseudolikelihood = -628.14648
Iteration 4:   log pseudolikelihood = -628.14648

Fitting full model:
Iteration 0:   log pseudolikelihood = -625.70365
Iteration 1:   log pseudolikelihood = -625.66106
Iteration 2:   log pseudolikelihood = -625.66105

Negative binomial regression                      Number of obs   =        848
Wald chi2(3)    =      19.75
Dispersion           = mean                       Prob > chi2     =     0.0000
Log pseudolikelihood = -619.47886                 Pseudo R2       =     0.0138

(Std. Err. adjusted for 125 clusters in code)
-----------------------------------------------------------------------------
| numep | IRR  | Std. Err. | z    | P>|z| |  [95% Conf. Interval] |
-------------|------|-----------|------|------|-----------------------|
_ipvtp_n_1   | 2.042857 | .8610699 | 1.69 | 0.090 | .8942402 - 4.666828 |
_ipvtp_n_2   | 1.5   | .6046066 | 1.01 | 0.314 | .6807639 - 3.305111 |
_ipvtp_n_3   | .7051282 | .6054798 | -0.41 | 0.684 | .1310261 - 3.794707 |
     _cons   | .2181818 | .0852793 | -3.90 | 0.000 | .101418 - .4693772 |
-------------|------|-----------|------|------|-----------------------|
/inalpha    | 1.093923 | .1299048 | 8.39 | < 0.001 | .8391572 - 1.348688 |
-------------|------|-----------|------|------|-----------------------|
     alpha   | 2.985964 | .3881301 | 7.67 | < 0.001 | 2.314415 - 3.852369 |
-------------|------|-----------|------|------|-----------------------|
i.season    | 1.039923 | .1299048 | 8.39 | < 0.001 | .8391572 - 1.348688 |
-------------|------|-----------|------|------|-----------------------|

Fitting Poisson model:
Iteration 0:   log pseudolikelihood = -685.2339
Iteration 1:   log pseudolikelihood = -685.2339
Iteration 2:   log pseudolikelihood = -685.2339

Fitting constant-only model:
Iteration 0:   log pseudolikelihood = -645.90918
Iteration 1:   log pseudolikelihood = -628.39289
Iteration 2:   log pseudolikelihood = -628.14648
Iteration 4:   log pseudolikelihood = -628.14648

Fitting full model:
Iteration 0:   log pseudolikelihood = -625.70365
Iteration 1:   log pseudolikelihood = -625.66106
Iteration 2:   log pseudolikelihood = -625.66105

Negative binomial regression                      Number of obs   =        848
Wald chi2(3)    =      19.75
Dispersion           = mean                       Prob > chi2     =     0.0000
Log pseudolikelihood = -619.47886                 Pseudo R2       =     0.0138

(Std. Err. adjusted for 125 clusters in code)
-----------------------------------------------------------------------------
|               Robust numep |        IRR   Std. Err.      z    P>|z|     [95% Conf. Interval] |
|--------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| _Iseason_1               | 2.022495        | .320576         | 4.44            | 0.000           | 1.482408        | 2.759354 |
| _cons                    | .2281106        | .0284638        | -11.84          | 0.000           | .1786208        | .2913123 |
| /lnalpha                 | 1.012293        | .1443095        |                  |                 | .7294516        | 1.295134 |
| alpha                    | 2.751904        | .3971258        |                  |                 | 2.073943        | 3.651486 |

Fitting Poisson model:

Iteration 0: log pseudolikelihood = -679.68018
Iteration 1: log pseudolikelihood = -679.67988
Iteration 2: log pseudolikelihood = -679.67988

Fitting constant-only model:

Iteration 0: log pseudolikelihood = -645.90918
Iteration 1: log pseudolikelihood = -628.39289
Iteration 2: log pseudolikelihood = -628.14648
Iteration 3: log pseudolikelihood = -628.14648
Iteration 4: log pseudolikelihood = -628.14648

Fitting full model:

Iteration 0: log pseudolikelihood = -617.89562
Iteration 1: log pseudolikelihood = -616.91966
Iteration 2: log pseudolikelihood = -616.91966

Negative binomial regression

|               Robust numep |        IRR   Std. Err.      z    P>|z|     [95% Conf. Interval] |
|--------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| ei                       | 1.063371        | .0187659        | 3.48            | 0.000           | 1.027219        | 1.100795 |
| _cons                    | .8058415        | .1861696        | -0.93           | 0.350           | .5123901        | 1.267356 |
| /lnalpha                 | .9712251        | .1435151        |                  |                 | .6899407        | 1.25251 |
| alpha                    | 2.641178        | .3790489        |                  |                 | 1.993597        | 3.499113 |

i.mob

|               Robust numep |        IRR   Std. Err.      z    P>|z|     [95% Conf. Interval] |
|--------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| _Imob_1-4                | (naturally coded; _Imob_1 omitted) |

Fitting Poisson model:

Iteration 0: log pseudolikelihood = -694.38589
Iteration 1: log pseudolikelihood = -694.38581
Iteration 2: log pseudolikelihood = -694.38581

Fitting constant-only model:

Iteration 0: log pseudolikelihood = -645.90918
Iteration 1: log pseudolikelihood = -628.39289
Iteration 2: log pseudolikelihood = -628.14648
Iteration 3: log pseudolikelihood = -628.14648
Iteration 4: log pseudolikelihood = -628.14648

Fitting full model:

Iteration 0: log pseudolikelihood = -624.4042
Iteration 1: log pseudolikelihood = -624.30405
Iteration 2: log pseudolikelihood = -624.30401

Negative binomial regression

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<th>Number of obs =</th>
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<tr>
<td>Wald chi2(1) =</td>
<td>12.12</td>
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<tr>
<td>Prob &gt; chi2 =</td>
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<tr>
<td>Pseudo R2 =</td>
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</table>
Wald chi2(3) =   8.80
Dispersion     = mean
Log pseudolikelihood = -624.30401
Prob > chi2     =  0.0321
Pseudo R2 =  0.0061

(Std. Err. adjusted for 125 clusters in code)

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<td>(naturally coded; _Izone_1 omitted)</td>
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Fitting Poisson model:
Iteration 0:  log pseudolikelihood = -675.28941
Iteration 1:  log pseudolikelihood = -675.28552
Iteration 2:  log pseudolikelihood = -675.28552

Fitting constant-only model:
Iteration 0:  log pseudolikelihood = -645.90918
Iteration 1:  log pseudolikelihood = -628.39289
Iteration 2:  log pseudolikelihood = -628.14696
Iteration 3:  log pseudolikelihood = -628.14648
Iteration 4:  log pseudolikelihood = -628.14648

Fitting full model:
Iteration 0:  log pseudolikelihood = -614.86058
Iteration 1:  log pseudolikelihood = -613.47266
Iteration 2:  log pseudolikelihood = -613.4616
Iteration 3:  log pseudolikelihood = -613.4616

Negative binomial regression
Number of obs   =     848
Wald chi2(2)     =    27.12
Dispersion       = mean
Log pseudolikelihood = -613.4616
Pseudo R2 =  0.0234

(Std. Err. adjusted for 125 clusters in code)

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Fitting Poisson model:
Iteration 0:  log pseudolikelihood = -688.70062
Iteration 1:  log pseudolikelihood = -688.67148
Iteration 2:  log pseudolikelihood = -688.67145

Fitting constant-only model:
Iteration 0:  log pseudolikelihood = -645.90918
Iteration 1:  log pseudolikelihood = -628.39289
Iteration 2:  log pseudolikelihood = -628.14696
Iteration 3:  log pseudolikelihood = -628.14648
Iteration 4:  log pseudolikelihood = -628.14648

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Fitting full model:

Iteration 0:  log pseudolikelihood = -620.91549
Iteration 1:  log pseudolikelihood = -620.49811
Iteration 2:  log pseudolikelihood = -620.49732
Iteration 3:  log pseudolikelihood = -620.49732

Negative binomial regression  
Number of obs   =        848  
Wald chi2(2)    =      24.63  
Dispersion          = mean  
Log pseudolikelihood = -620.49732  
Pseudo R2          =     0.0122

(Std. Err. adjusted for 125 clusters in code)

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_\lnalpha_          | 1.038688  | 0.1338688 |

_alpha_              | 2.825507  | 0.3782474 |

.*perform colllinearity diagnostics
.*================================

corr numep age logmsp3 logr0 logr2 hbf hb_type muac_scr mob epi_stat ///
agem itn_use educ season ei
(obs=836)

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.xi:collin numep age logmsp3 logr0 logr2 hbf i.hb_type muac_scr i.mob i.epi_stat i.agem i.itn_use i.educ i.season i.ei

> t ///
> agem i.itn_use i.educ i.season ei
> i.hb_type _Ihb_type_1-4 (naturally coded; _Ihb_type_1 omitted)
i.mob _Imob_1-4   (naturally coded; _Imob_1 omitted)
i.epi_stat _Iepi_stat_1-2 (naturally coded; _Iepi_stat_1 omitted)
Collinearity Diagnostics

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<th>Tolerance</th>
<th>Squared</th>
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Mean VIF 1.61

Eigen values & Cond Index computed from scaled raw sscp (w/ intercept)

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Condition Number 60.0076

Det(correlation matrix) 0.0287

: *multivariate regression analysis using changing antibody titres adjusting for clusters

```
xi: nbreg numep age logmsp3 logr0 logr2 hbf i.hb_type muac_scr i.mob i.epi_stat agem i.itn_use i.educ i.season ei, cluster(code) irr
```

Condition Number 60.0076
Eigenvalues & Cond Index computed from scaled raw sscp (w/ intercept)
Det(correlation matrix) 0.0287

---

233
Fitting Poisson model:

Iteration 0:  log pseudolikelihood = -571.09016
Iteration 1:  log pseudolikelihood = -570.82309
Iteration 2:  log pseudolikelihood = -570.77072
Iteration 3:  log pseudolikelihood = -570.75883
Iteration 4:  log pseudolikelihood = -570.75621
Iteration 5:  log pseudolikelihood = -570.75556
Iteration 6:  log pseudolikelihood = -570.75541
Iteration 7:  log pseudolikelihood = -570.75541

Fitting constant-only model:

Iteration 0:  log pseudolikelihood = -640.99977
Iteration 1:  log pseudolikelihood = -639.67455
Iteration 2:  log pseudolikelihood = -623.54382
Iteration 3:  log pseudolikelihood = -623.54202
Iteration 4:  log pseudolikelihood = -623.54202

Fitting full model:

Iteration 0:  log pseudolikelihood = -574.98152
Iteration 1:  log pseudolikelihood = -543.99099
Iteration 2:  log pseudolikelihood = -542.31197
Iteration 3:  log pseudolikelihood = -542.30631
Iteration 4:  log pseudolikelihood = -542.30631

Negative binomial regression
Number of obs = 836
Dispersion = mean
Log pseudolikelihood = -542.30631

(Std. Err. adjusted for 123 clusters in code)

<table>
<thead>
<tr>
<th></th>
<th>Robust</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IRR</td>
<td>Std. Err.</td>
<td>z</td>
<td>P&gt;</td>
</tr>
<tr>
<td></td>
<td>[95% Conf. Interval]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>age</td>
<td>1.114544</td>
<td>.0092731</td>
<td>13.03</td>
<td>0.000</td>
</tr>
<tr>
<td>logmsp3</td>
<td>1.163622</td>
<td>.0657381</td>
<td>2.68</td>
<td>0.007</td>
</tr>
<tr>
<td>logr0</td>
<td>.981701</td>
<td>.0639584</td>
<td>-0.28</td>
<td>0.777</td>
</tr>
<tr>
<td>logr2</td>
<td>.922641</td>
<td>.0545965</td>
<td>-1.36</td>
<td>0.174</td>
</tr>
<tr>
<td>hbf</td>
<td>.986981</td>
<td>.0066038</td>
<td>-1.96</td>
<td>0.050</td>
</tr>
<tr>
<td>_Ihb_type_2</td>
<td>1.06e-07</td>
<td>1.09e-07</td>
<td>15.52</td>
<td>0.000</td>
</tr>
<tr>
<td>_Ihb_type_3</td>
<td>1.082621</td>
<td>.2497315</td>
<td>0.34</td>
<td>0.731</td>
</tr>
<tr>
<td>_Ihb_type_4</td>
<td>.3923899</td>
<td>.1462107</td>
<td>-2.51</td>
<td>0.012</td>
</tr>
<tr>
<td>muac_scr</td>
<td>1.273454</td>
<td>.0973677</td>
<td>3.16</td>
<td>0.002</td>
</tr>
<tr>
<td>_Imob_2</td>
<td>1.501543</td>
<td>.5402344</td>
<td>1.13</td>
<td>0.259</td>
</tr>
<tr>
<td>_Imob_3</td>
<td>1.423391</td>
<td>.478914</td>
<td>1.05</td>
<td>0.294</td>
</tr>
<tr>
<td>_Imob_4</td>
<td>1.87437</td>
<td>.6193199</td>
<td>1.90</td>
<td>0.057</td>
</tr>
<tr>
<td>_Iepi_stat_2</td>
<td>1.073276</td>
<td>.2087134</td>
<td>0.36</td>
<td>0.716</td>
</tr>
<tr>
<td>agem</td>
<td>1.010588</td>
<td>.0123516</td>
<td>0.86</td>
<td>0.389</td>
</tr>
<tr>
<td>_Itn_use_2</td>
<td>1.065135</td>
<td>.250555</td>
<td>0.27</td>
<td>0.789</td>
</tr>
<tr>
<td>_Ieduc_1</td>
<td>1.233071</td>
<td>.2177</td>
<td>1.19</td>
<td>0.235</td>
</tr>
<tr>
<td>_Ieduc_2</td>
<td>1.493988</td>
<td>.1554438</td>
<td>-2.24</td>
<td>0.025</td>
</tr>
<tr>
<td>_Iseason_1</td>
<td>1.382405</td>
<td>.2231467</td>
<td>2.01</td>
<td>0.045</td>
</tr>
<tr>
<td>ei</td>
<td>1.049381</td>
<td>.0148523</td>
<td>3.41</td>
<td>0.001</td>
</tr>
<tr>
<td>_cons</td>
<td>1.006454</td>
<td>.007756</td>
<td>-4.20</td>
<td>0.000</td>
</tr>
<tr>
<td>/lnalpha</td>
<td>.2450085</td>
<td>1.190958</td>
<td>0.1291404</td>
<td>0.619573</td>
</tr>
<tr>
<td>alpha</td>
<td>1.277632</td>
<td>.2438946</td>
<td>1.8788506</td>
<td>1.857362</td>
</tr>
</tbody>
</table>

```bash
.i: glm numep age logmsp3 logr0 logr2 hbf i.hb_type muac_scr i.mob i.epi_stat /
> // agem i.itn_use i.educ i.season ei, family(nb) cluster(code)
> i.hb_type _Ihb_type_1-4 (naturally coded; _Ihb_type_1 omitted)
> i.mob _Imob_1-4 (naturally coded; _Imob_1 omitted)
```
Iteration 0: log pseudolikelihood = -545.48237
Iteration 1: log pseudolikelihood = -543.08806
Iteration 2: log pseudolikelihood = -542.92962
Iteration 3: log pseudolikelihood = -542.91109
Iteration 4: log pseudolikelihood = -542.90861
Iteration 5: log pseudolikelihood = -542.90815
Iteration 6: log pseudolikelihood = -542.90805
Iteration 7: log pseudolikelihood = -542.90803

Generalized linear models
Optimization : ML
Residual df = 816
Scale parameter = 1
Deviance = 501.3042028
(1/df) Deviance = .6143434
Pearson = 707.0400259
(1/df) Pearson = .8664706
Variance function: V(u) = u+(1)u^2 [Neg. Binomial]
Link function : g(u) = ln(u) [Log]
AIC = 1.34667
Log pseudolikelihood = -542.908029
BIC = -4989.257
(Std. Err. adjusted for 123 clusters in code)

| Coef. | Std. Err. | z  | P>|z| | [95% Conf. Interval] |
|-------|-----------|----|------|------------------|
| age | .1053882 | .0077728 | 13.56 | 0.000 | .0901537 - .1206227 |
| logmsp3 | .1532178 | .0548552 | 2.79 | 0.005 | .0457036 - .260732 |
| logr0 | -.016589 | .0649053 | -0.26 | 0.798 | -.143801 - .110623 |
| logr2 | -.0804812 | .059533 | -1.35 | 0.176 | -.1971637 - .0362013 |
| hbf | -.0127331 | .0066385 | -1.92 | 0.055 | -.0257444 - .0002781 |
| _Ihb_type_2 | -13.26747 | 1.034017 | -12.83 | 0.000 | -15.2941 - 11.24083 |
| _Ihb_type_3 | .0775009 | .2254079 | 0.34 | 0.731 | -.3642905 - .5270757 |
| _Ihb_type_4 | -.8973213 | .3696066 | -2.43 | 0.015 | -1.621737 - .12989 |
| muac_scr | .2429429 | .075917 | 3.20 | 0.001 | .0941483 - .3917375 |
| _Imob_2 | .3995843 | .3588732 | 1.11 | 0.266 | -.3077424 - 1.102963 |
| _Imob_3 | .3519822 | .3370354 | 1.04 | 0.296 | -.3085951 - .101256 |
| _Imob_4 | .6264551 | .331514 | 1.89 | 0.059 | -.0229187 - 1.275892 |
| _Iepi_stat_2 | .0779836 | .1890837 | 0.41 | 0.680 | -.2926137 - .448581 |
| agem | .0106353 | .0119971 | 0.89 | 0.375 | -.0128787 - .0341992 |
| _Iitn_use_2 | .074523 | .2308985 | 0.32 | 0.747 | -.3780297 - .5270757 |
| _Ieduc_1 | .3130504 | .1663306 | 1.88 | 0.060 | -.0129516 - .6390524 |
| _Iseason_1 | .0476603 | .0139306 | 3.42 | 0.001 | .0203568 - .0749638 |
| _cons | -5.050858 | 1.199093 | -4.21 | 0.000 | -7.401038 - 2.700679 |

*run wald test to select covariates for the final model
.xttestparm i.epi_stat
i.epi_stat          _Iepi_stat_1-2 (naturally coded; _Iepi_stat_1 omitted)
( 1)  [numep]_Iepi_stat_2 = 0
  chi2(  1) =  0.17
  Prob > chi2 =  0.6800

test agem
( 1)  [numep]agem = 0
  chi2(  1) =  0.79
  Prob > chi2 =  0.3754

.xttestparm i.mob
i.mob          _Imob_1-4 (naturally coded; _Imob_1 omitted)
( 1)  [numep]_Imob_2 = 0
(2) \[\text{numep}_1 = 0\]
(3) \[\text{numep}_2 = 0\]

\[
\chi^2(3) = 4.65
\]
\[
\text{Prob} > \chi^2 = 0.1991
\]

```
.xi:testparm i.educ
  i.educ         _Ieduc_0-2
                  (naturally coded; _Ieduc_0 omitted)
(1) \[\text{numep}_1 = 0\]
(2) \[\text{numep}_2 = 0\]

\[
\chi^2(2) = 8.00
\]
\[
\text{Prob} > \chi^2 = 0.0183
\]
```

```
.test muac_scr
  (1) \[\text{numep}_1 = 0\]

\[
\chi^2(1) = 10.24
\]
\[
\text{Prob} > \chi^2 = 0.0014
\]
```

```
.xi:nbreg numep age logmsp3 logr0 logr2 hbf i.hb_type muac_scr ///
   i.itn_use i.educ i.season ei,cluster(code) irr
  i.hb_type       _Ihb_type_1-4
                     (naturally coded; _Ihb_type_1 omitted)
  i.itn_use       _Iitn_use_1-2
                     (naturally coded; _Iitn_use_1 omitted)
  i.educ         _Ieduc_0-2
                     (naturally coded; _Ieduc_0 omitted)
  i.season       _Iseason_0-1
                     (naturally coded; _Iseason_0 omitted)
```

Fitting Poisson model:

Iteration 0: log pseudolikelihood = -575.47803
Iteration 1: log pseudolikelihood = -575.20508
Iteration 2: log pseudolikelihood = -575.14994
Iteration 3: log pseudolikelihood = -575.13425
Iteration 4: log pseudolikelihood = -575.13412
Iteration 5: log pseudolikelihood = -575.13412
Iteration 6: log pseudolikelihood = -575.13412

Fitting constant-only model:

Iteration 0: log pseudolikelihood = -640.99977
Iteration 1: log pseudolikelihood = -639.67455
Iteration 2: log pseudolikelihood = -623.54382
Iteration 3: log pseudolikelihood = -623.54202
Iteration 4: log pseudolikelihood = -623.54202

Fitting full model:

Iteration 0: log pseudolikelihood = -575.61397
Iteration 1: log pseudolikelihood = -570.17976
Iteration 2: log pseudolikelihood = -544.97805
Iteration 3: log pseudolikelihood = -544.39906
Iteration 4: log pseudolikelihood = -544.39838
Iteration 5: log pseudolikelihood = -544.39838

Negative binomial regression

```
<table>
<thead>
<tr>
<th></th>
<th>Number of obs</th>
<th>Wald chi2(14)</th>
<th>Prob &gt; chi2</th>
<th>Pseudo R2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.1269</td>
</tr>
</tbody>
</table>
```

```
(Std. Err. adjusted for 123 clusters in code)

<p>| | Robust |
|---|---|---|---|---|
|Robust | IRR | Std. Err. | z | P&gt;|z| | [95% Conf. Interval] |</p>
<table>
<thead>
<tr>
<th>---</th>
<th>---</th>
<th>---</th>
<th>---</th>
<th>---</th>
</tr>
</thead>
<tbody>
<tr>
<td>numep</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>logmsp3</td>
<td>1.165051</td>
<td>.0660603</td>
<td>2.69</td>
<td>0.000</td>
</tr>
<tr>
<td>logr0</td>
<td>1.002688</td>
<td>.061288</td>
<td>0.04</td>
<td>0.968</td>
</tr>
<tr>
<td>logr2</td>
<td>.920025</td>
<td>.0531677</td>
<td>-1.44</td>
<td>0.149</td>
</tr>
<tr>
<td>hbf</td>
<td>.9849169</td>
<td>.0060504</td>
<td>-2.47</td>
<td>0.013</td>
</tr>
<tr>
<td>_Ihb_type_2</td>
<td>4.11e-09</td>
<td>4.22e-09</td>
<td>-10.79</td>
<td>0.000</td>
</tr>
</tbody>
</table>
```
### 7.2.5 Cox regression Stata code / Chapter 3

```stata
clear all
macro drop _all
capture log close
set more off
log using chap3_coxreg,replace text
*chap3_coxreg.do: construct predictive model for number of episodes
*david kangoye,PhD student,Open University/KEMRI-WTRP
version 11.2
set linesize 80
*================================================================
cd "C:\Users\dkangoye\Dropbox\projects\phdprogram_ou\work\data\data_bics\elisa_bics\chap2_nbreg"
use bics_kwtrpV0,clear
*combine morbidity data with exposure
data
*===============================
joinby code using "C:\Users\dkangoye\Dropbox\projects\phdprogram_ou\work\data\data_bics\elisa_bics\dist15ei",unm(both)
tab _merge,m
list code if _merge==2
drop if _merge==2
drop _merge
*define febrile malaria episodes
*===============================
gen byte malar1=1 if fever==1 & tf>0 & tf !=.
lab var malar1 "febrile malaria episode_tf>0"
replace malar1=0 if malar1==.
gen byte malar2=1 if fever==1 & tf>10000 & tf !=.
lab var malar2 "febrile malaria episode_tf>10000"
replace malar2=0 if malar2==.
log close
name:  <unnamed>
log:  C:\Users\dkangoye\Dropbox\projects\phdprogram_ou\work\data\data_bics\elisa_bics\chap2_nbreg.log
log type:  text
closed on:  13 Sep 2016, 18:57:05
*================================================================
```

The code above sets up the environment, loads the data, combines morbidity data with exposure data, and defines febrile malaria episodes. It then generates variables for febrile malaria episodes and closes the log file.
*order malaria variables
order malar1 malar2,after(tf)

*censor episodes occurring within 21 days following previous malaria episode
*======================================================
*check number of episodes by definition before censoring
local defs "malar1 malar2"
foreach def in `defs'{
   tab `def',m
}
*censor episodes
sort malar1 code datevisit
replace malar1=0 if malar1[_n-1]==1 & code==code[_n-1] & datevisit-datevisit[_n-1]<=21
*check number of episodes by definition after censoring
foreach def in malar1 malar2{
   tab `def',m
}

* generate additional variables and explore distribution
*=================================================================
*generate variable age
gen age=(datevisit - dob)/30
lab var age "age of infant at current visit"
sum age

*gen variable season
gen moy=month(datevisit)
lab var moy "calendar month"
tab moy,m
gen season=1 if moy >=6 & moy <=11
replace season=0 if moy==12 | moy>=1 & moy<=5
lab var season "malaria transmission season"
tab season,m

*generate season of birth
gen sob=1 if month(dob)>=6 & month(dob)<=11
replace sob=0 if month(dob)==12 | month(dob)>=1 & month(dob)<=5
lab var sob "season of birth"
tab sob,m

*generate month of birth
gen mob=month(dob)
lab var mob "month of birth"
tab mob,m

*gen variables for seropositivity
gen amal1_sp=(AMA1_AU>1.1401198)
gen msp1_sp=(MSP1_AU>1.2326468)
gen msp2_sp=(MSP2_AU>0.09898795)
gen msp3_sp=(MSP3_AU>0.81264652)
save bics_kwtrpV1,replace

**************************************************************************
use bics_kwtrpV1,clear
keep if monthn<22

*univariate cox regression
*=================================================================
use bics_kwtrpV1,clear
keep if monthn<21
stset datevisit, failure(malar2) id(code) origin(datscren) scale(28)
stde
stsum
recode iptp_n 3=2
*create local macros for explanatory variables
local envfac i.season i.mob i.zone ei
local socufac i.educ
local matfac age i.primgrav i.sg_birth i.itn_use i.iptp_n
local infac1 i.sex muac height_scr weight_scr i.del_way i.rea i.neo_inf
local infac2 i.hb_type hbf AMA1_AU MSP1_AU MSP2_AU MSP3_AU
local infac3 i.ama1_sp i.msp1_sp i.msp2_sp i.msp3_sp /*i.ama1_pl i.msp1_pl i.msp2_pl i.msp3_pl*/
foreach i in `infac1' `infac2' `infac3' `envfac' `socufac' `matfac' {
    stcox `i'
    stcox i.zone
testparm i.zone
    stcox i.educ
testparm i.educ
    stcox i.iptp_n
testparm i.iptp_n
    stcox i.mob
testparm i.mob
    stcox i.hb_type
testparm i.hb_type
}
*perform collinearity diagnostic
pwcorr AMA1_AU MSP1_AU MSP2_AU MSP3_AU hbf muac_scr ei,star(.05)
graph matrix AMA1_AU MSP1_AU MSP2_AU MSP3_AU hbf muac_scr ei
collin AMA1_AU MSP1_AU MSP2_AU MSP3_AU
*multivariate cox regression
**include in baseline multivariable model if p<0.2 or theoretically high importance variable
stcox hbf i.hb_type AMA1_AU MSP1_AU MSP2_AU MSP3_AU i.ama1_sp i.msp1_sp i.msp2_sp ///
i.mob i.itn_use i.educ i.season ei
est store model0
*model simplification: backward elimination
*important variables that should not be removed:
* antibody titres, itn use, season, exposure index, foetal haemoglobin (literature)
*drop msp1_sp
stcox hbf i.hb_type AMA1_AU MSP1_AU MSP2_AU MSP3_AU i.ama1_sp i.msp2_sp ///
i.mob i.itn_use i.educ i.season ei
est store model1
est table model0 model1,b(%5.3f) p(%4.3f) stats(N ll aic bic)
testparm i.mob
testparm i.educ
*drop ama1_sp
stcox hbf i.hb_type AMA1_AU MSP1_AU MSP2_AU MSP3_AU i.msp2_sp ///
i.mob i.itn_use i.educ i.season ei
est store model2
est table model0 model1 model2,b(%5.3f) p(%4.3f) stats(N ll aic bic)
testparm i.mob
testparm i.educ
*drop msp2_sp
stcox AMA1_AU MSP1_AU MSP2_AU MSP3_AU hbf i.hb_type ///
i.itn_use i.mob i.educ i.season ei
est store model3
est table model0 model1 model2 model3,b(%5.3f) p(%4.3f) stats(N ll aic bic)
testparm i.mob
testparm i.educ
*drop educ
stcox AMA1_AU MSP1_AU MSP2_AU MSP3_AU hbf i.hb_type ///
i.itn_use i.mob i.season ei
est store model4
est table model0 model1 model2 model3 model4, bc(5.3) p(4.3) stats(N ll aic bic)
testparm i.mob
testparm i.hb_type

*test of proportional hazards assumption
*=============================================================================
estat phtest, detail
estat phtest,plot(ei) yline(0) title(Exposure index)
graph save 1,replace
estat phtest,plot(hbf) yline(0) title(Foetal Haemoglobin rate)
graph save 2,replace
estat phtest,plot(AMA1_AU) yline(0) title(Antibodies to AMA1)
graph save 3,replace
estat phtest,plot(MSP1_AU) yline(0) title(Antibodies to MSP1-19)
graph save 4,replace
estat phtest,plot(MSP2_AU) yline(0) title(Antibodies to MSP2)
graph save 5,replace
estat phtest,plot(MSP3_AU) yline(0) title(Antibodies to MSP3)
graph save 6,replace
grc1leg 1.gph 2.gph 3.gph 4.gph 5.gph 6.gph, ///
xcom l1(Scaled Schoenfeld) b1(Time (months))
graph save schoenfeld,replace
graph export schoenfeld.tif,width(2049)
log close
exit

7.2.6 Cox regression output log / Chapter 3

---
name: <unnamed>
log: C:\Users\dkangoye\Dropbox\projects\phdprogram_ou\work\data\data_bics\chap3_coxreg.log
opened on: 13 Sep 2016, 18:59:38

*chap3_coxreg.do: construct predictive model for number of episodes
*david kangoye, PhD student, Open University/KEMRI-WTRP
version 11.2
set linesize 80
*=============================================================================
set linesize 80
*cd "C:\Users\dkangoye\Dropbox\projects\phdprogram_ou\work\data\data_bics\elisa_bics\labwork\davidk_bics2014"
C:\Users\dkangoye\Dropbox\projects\phdprogram_ou\work\data\data_bics\elisa_bics_\labwork\davidk_bics2014

*use bics_kwtrpV0,clear
(combination of all data sets {morb+geo+sero})
*combine morbidity data with exposure data

*======================================

join by code using "C:\Users\dkangoye\Dropbox\projects\phdprogram_ou\work\data\>data_bics\elisa_bics\dist15ei", unm(both)

tab _merge,m

<table>
<thead>
<tr>
<th>_merge</th>
<th>Freq.</th>
<th>Percent</th>
<th>Cum.</th>
</tr>
</thead>
<tbody>
<tr>
<td>both in master and using data</td>
<td>14,089</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Total</td>
<td>14,089</td>
<td>100.00</td>
<td></td>
</tr>
</tbody>
</table>

list code if _merge==2

drop if _merge==2

(drop observations deleted)

drop _merge

*define febrile malaria episodes

*=======================================

*generate var for fever

gen fever=1 if (temp>=37.5 & temp !=.) | hof==1

(12982 missing values generated)

lab var fever "presence of subjective and/or objective fever"

tab fever,m

<table>
<thead>
<tr>
<th>presence of</th>
<th>Freq.</th>
<th>Percent</th>
<th>Cum.</th>
</tr>
</thead>
<tbody>
<tr>
<td>subjective</td>
<td>1,107</td>
<td>7.86</td>
<td>7.86</td>
</tr>
<tr>
<td>and/or</td>
<td>12,982</td>
<td>92.14</td>
<td>100.00</td>
</tr>
<tr>
<td>objective</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fever</td>
<td>14,089</td>
<td>100.00</td>
<td></td>
</tr>
</tbody>
</table>

*generate new variables for malaria

gen byte malar1=1 if fever==1 & tf>0 & tf !=.

(1396 missing values generated)

lab var malar1 "febrile malaria episode_tf>0"

.replace malar1=0 if malar1==.

(1396 real changes made)

gen byte malar2=1 if fever==1 & tf>10000 & tf !=.

(13799 missing values generated)

lab var malar2 "febrile malaria episode_tf>10000"

.replace malar2=0 if malar2==.

(13799 real changes made)

*order malaria variables

.order malar1 malar2, after(tf)

*check number of episodes by definition before censoring

*==========================================================================

*check number of episodes by definition before censoring

.local defs "malar1 malar2"
. foreach def in `defs'{
    2. tab `def',m
    3. }

    febrile | malaria | episode_tf> |
    0 | Freq. Percent Cum.
    -----------------------------------
    0 | 13,696 97.21 97.21
    1 |   393  2.79 100.00
    -----------------------------------
    Total | 14,089 100.00

    febrile | malaria | episode_tf> |
    10000 | Freq. Percent Cum.
    -----------------------------------
    0 | 13,799 97.94 97.94
    1 |    290  2.06 100.00
    -----------------------------------
    Total | 14,089 100.00

. *censor episodes
. sort malar1 code datevisit
. replace malar1=0 if malar1[_n-1]==1 & code==code[_n-1] & datevisit-datevisit[_n-1]<=21
(50 real changes made)

. *check number of episodes by definition after censoring
. foreach def in malar1 malar2{
    2. tab `def',m
    3. }

    febrile | malaria | episode_tf> |
    0 | Freq. Percent Cum.
    -----------------------------------
    0 | 13,746 97.57 97.57
    1 |    343  2.43 100.00
    -----------------------------------
    Total | 14,089 100.00

    febrile | malaria | episode_tf> |
    10000 | Freq. Percent Cum.
    -----------------------------------
    0 | 13,799 97.94 97.94
    1 |    290  2.06 100.00
    -----------------------------------
    Total | 14,089 100.00

. *_3_generate additional variables and explore distribution
. *=========================================================
. *generate variable age
    gen age= (datevisit - dob)/30
    lab var age "age of infant at current visit"
. sum age

<table>
<thead>
<tr>
<th>Variable</th>
<th>Obs</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>age</td>
<td>14089</td>
<td>13.11088</td>
<td>7.012261</td>
<td>.9</td>
<td>25.93333</td>
</tr>
</tbody>
</table>
*gen variable season
.
. gen moy=month(datevisit)
.
. lab var moy "calendar month"
.
. tab moy,m

<table>
<thead>
<tr>
<th>calendar</th>
<th>Freq.</th>
<th>Percent</th>
<th>Cum.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,161</td>
<td>8.24</td>
<td>8.24</td>
</tr>
<tr>
<td>2</td>
<td>1,088</td>
<td>7.72</td>
<td>15.96</td>
</tr>
<tr>
<td>3</td>
<td>1,205</td>
<td>8.55</td>
<td>24.52</td>
</tr>
<tr>
<td>4</td>
<td>1,148</td>
<td>8.15</td>
<td>32.66</td>
</tr>
<tr>
<td>5</td>
<td>1,185</td>
<td>8.41</td>
<td>41.07</td>
</tr>
<tr>
<td>6</td>
<td>1,187</td>
<td>8.43</td>
<td>49.50</td>
</tr>
<tr>
<td>7</td>
<td>1,151</td>
<td>8.17</td>
<td>57.67</td>
</tr>
<tr>
<td>8</td>
<td>1,231</td>
<td>8.74</td>
<td>66.41</td>
</tr>
<tr>
<td>9</td>
<td>1,196</td>
<td>8.49</td>
<td>74.90</td>
</tr>
<tr>
<td>10</td>
<td>1,182</td>
<td>8.11</td>
<td>83.45</td>
</tr>
<tr>
<td>11</td>
<td>1,190</td>
<td>8.45</td>
<td>91.89</td>
</tr>
<tr>
<td>12</td>
<td>1,142</td>
<td>8.11</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Total | 14,089 | 100.00

. gen season=1 if moy >=6 & moy <=11
(6929 missing values generated)
.
. replace season=0 if moy==12 | moy>=1 & moy<=5
(6929 real changes made)
.
. lab var season "malaria transmission season"
.
. tab season,m

<table>
<thead>
<tr>
<th>malaria</th>
<th>transmissio</th>
<th>Freq.</th>
<th>Percent</th>
<th>Cum.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>6,929</td>
<td>49.18</td>
<td>49.18</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>7,160</td>
<td>50.82</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Total | 14,089 | 100.00
.

*generate season of birth
.
. gen sob=1 if month(dob)>=6 & month(dob)<=11
(8425 missing values generated)
.
. replace sob=0 if month(dob)==12 | month(dob)>=1 & month(dob)<=5
(8425 real changes made)
.
. lab var sob "season of birth"
.
. tab sob,m

<table>
<thead>
<tr>
<th>season of</th>
<th>birth</th>
<th>Freq.</th>
<th>Percent</th>
<th>Cum.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>8,425</td>
<td>59.80</td>
<td>59.80</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>5,664</td>
<td>40.20</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Total | 14,089 | 100.00
.

*generate month of birth
.
. gen mob=month(dob)
.
. lab var mob "month of birth"
.
. tab mob,m
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2,905</td>
<td>20.62</td>
<td>20.62</td>
</tr>
<tr>
<td>10</td>
<td>1,243</td>
<td>8.82</td>
<td>29.44</td>
</tr>
<tr>
<td>11</td>
<td>4,421</td>
<td>31.38</td>
<td>60.82</td>
</tr>
<tr>
<td>12</td>
<td>5,520</td>
<td>39.18</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Total | 14,089 | 100.00

.save bics_kwtrpV1,replace
file bics_kwtrpV1.dta saved

.use bics_kwtrpV1,clear
(combination of all data sets {morb+geo+sero})

keep if monthn<22
(1539 observations deleted)

*univariate cox regression

.use bics_kwtrpV1,clear
(combination of all data sets {morb+geo+sero})

keep if monthn<21
(2717 observations deleted)

.stset datevisit, failure(malar2) id(code) origin(datscren) scale(28)

failure _d:  malar2 != 0 & malar2 < .
obs. time interval:  (datevisit[_n-1], datevisit]
exit on or before:  failure
t for analysis:  (time-origin)/28
origin:  time datscren

11372 total observations
118 observations end on or before enter()
2856 observations begin on or after (first) failure

8398 observations remaining, representing
125 subjects
69 failures in single-failure-per-subject data
1907.821 total analysis time at risk and under observation
at risk from t = 0
earliest observed entry t = 0
last observed exit t = 22.39286

.stde

failure _d:  malar2
analysis time _t:  (datevisit-origin)/28
origin:  time datscren
id:  code

<table>
<thead>
<tr>
<th>Category</th>
<th>total</th>
<th>mean</th>
<th>min</th>
<th>median</th>
<th>max</th>
</tr>
</thead>
<tbody>
<tr>
<td>no. of subjects</td>
<td>125</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no. of records</td>
<td>8398</td>
<td>67.184</td>
<td>5</td>
<td>81</td>
<td>100</td>
</tr>
<tr>
<td>(first) entry time</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(final) exit time</td>
<td></td>
<td>15.26257</td>
<td>1.142857</td>
<td>19.07143</td>
<td>22.39286</td>
</tr>
<tr>
<td>subjects with gap</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>time on gap if gap</td>
<td>0</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>time at risk</td>
<td>1907.8214</td>
<td>15.26257</td>
<td>1.142857</td>
<td>19.07143</td>
<td>22.39286</td>
</tr>
</tbody>
</table>
. stsum

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

<table>
<thead>
<tr>
<th>incidence</th>
<th>no. of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>time at risk</td>
<td>25% 50% 75%</td>
</tr>
</tbody>
</table>

---

total | 1907.821429   .0361669           125   9.142857  19.71429  22.39286

. recode iptp_n 3=2
(iptp_n: 185 changes made)

. *create local macros for explanatory variables
. local envfac i.season i.mob i.zone ei
. local socufac i.educ
. local matfac agem i.primgrav i.sg_birth i.itn_use i.iptp_n
. local infac1 i.sex  muac height_scr weight_scr i.del_way i.rea i.epi_stat i.ne > o_inf
. local infac2 i.hb_type hbf AMA1_AU MSP1_AU MSP2_AU MSP3_AU
. local infac3 i.amal_sp i.msp1_sp i.msp2_sp i.msp3_sp /*i.amal_pl i.msp1_pl i.msp2_pl i.msp3_pl*/

. foreach i in `infac1' `infac2' `infac3' `envfac' `socufac' `matfac' {
    2.         stcox `i'
    3. }

Cox regression -- Breslow method for ties

No. of subjects =          125                     Number of obs   =      8398
No. of failures =           69
Time at risk    =  1907.821429
Log likelihood  = -303.78456
LR chi2(1)      =      0.52
Prob > chi2     =    0.4720

------------------------------------------------------------------------------
_t | Haz. Ratio   Std. Err.    z  P>|z|    [95% Conf. Interval]
--------------|--------------------------|---------|---------|--------------------------
2.sex |    .839564   .2043459 -0.72   0.472   .5210452    1.352796

------------------------------------------------------------------------------

245
Cox regression -- Breslow method for ties

No. of subjects = 125  Number of obs = 8398
No. of failures = 69  Time at risk = 1907.821429
Log likelihood = -303.70034  LR chi2(1) = 0.69
Prob > chi2 = 0.4076

------------------------------------------------------------------------------
|     _t  | Haz. Ratio   Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|--------|-------------|------|------|------------------|
| muac_scr  | 1.092771    | .1156848 | 0.84 | 0.402 | .8880101   | 1.344747 |
------------------------------------------------------------------------------

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscreen
id: code

Iteration 0:  log likelihood = -303.23335
Iteration 1:  log likelihood = -303.16663
Iteration 2:  log likelihood = -303.16663
Refining estimates:
Iteration 0:  log likelihood = -303.16663

Cox regression -- Breslow method for ties

No. of subjects = 124  Number of obs = 8307
No. of failures = 69  Time at risk = 1887.035714
Log likelihood = -303.16663  LR chi2(1) = 0.13
Prob > chi2 = 0.7149

------------------------------------------------------------------------------
|     _t  | Haz. Ratio   Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|--------|-------------|------|------|------------------|
| height_scr  | 1.018011    | .0498422 | 0.36 | 0.715 | .9248628   | 1.120541 |
------------------------------------------------------------------------------

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscreen
id: code

Iteration 0:  log likelihood = -303.23335
Iteration 1:  log likelihood = -303.2065
Iteration 2:  log likelihood = -303.2065
Refining estimates:
Iteration 0:  log likelihood = -303.2065

Cox regression -- Breslow method for ties

No. of subjects = 124  Number of obs = 8312
No. of failures = 69  Time at risk = 1887.071429
Log likelihood = -303.2065  LR chi2(1) = 0.05
Prob > chi2 = 0.8168

------------------------------------------------------------------------------
|     _t  | Haz. Ratio   Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|--------|-------------|------|------|------------------|
| weight_scr  | .9538994    | .1947292 | -0.23 | 0.817 | .6393493   | 1.423203 |
------------------------------------------------------------------------------

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscreen
id: code

Iteration 0:  log likelihood = -302.41294
Iteration 1:  log likelihood = -302.21728
Iteration 2:  log likelihood = -302.21195
Iteration 3:  log likelihood = -302.21195
Refining estimates:
Iteration 0: log likelihood = -302.21195

Cox regression -- Breslow method for ties

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>123</td>
<td>Number of obs</td>
<td>8220</td>
<td></td>
</tr>
<tr>
<td>No. of failures</td>
<td>69</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time at risk</td>
<td>1866.321429</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log likelihood</td>
<td>-302.21195</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LR chi2(1)</td>
<td>0.40</td>
<td>Prob &gt; chi2</td>
<td>0.5261</td>
<td></td>
</tr>
</tbody>
</table>

| t | Haz. Ratio   | Std. Err. | z   | P>|z|   | [95% Conf. Interval] |
|---|--------------|-----------|-----|-------|----------------------|
| del_way | cesarean | .6537055 | .4692959 | -0.59 | 0.554     | .1600676    | 2.669689 |

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0: log likelihood = -304.04321
Iteration 1: log likelihood = -304.04319
Iteration 2: log likelihood = -304.04319
Refining estimates:
Iteration 0: log likelihood = -304.04319

Cox regression -- Breslow method for ties

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>125</td>
<td>Number of obs</td>
<td>8398</td>
<td></td>
</tr>
<tr>
<td>No. of failures</td>
<td>69</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time at risk</td>
<td>1907.821429</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log likelihood</td>
<td>-304.04319</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LR chi2(1)</td>
<td>0.00</td>
<td>Prob &gt; chi2</td>
<td>0.9939</td>
<td></td>
</tr>
</tbody>
</table>

| t | Haz. Ratio   | Std. Err. | z   | P>|z|   | [95% Conf. Interval] |
|---|--------------|-----------|-----|-------|----------------------|
| rea | no | .9967242 | .4268286 | -0.01 | 0.994     | .4305898    | 2.307205 |

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0: log likelihood = -304.04321
Iteration 1: log likelihood = -304.02786
Iteration 2: log likelihood = -304.02785
Refining estimates:
Iteration 0: log likelihood = -304.02785

Cox regression -- Breslow method for ties

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>125</td>
<td>Number of obs</td>
<td>8398</td>
<td></td>
</tr>
<tr>
<td>No. of failures</td>
<td>69</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time at risk</td>
<td>1907.821429</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log likelihood</td>
<td>-304.02785</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LR chi2(1)</td>
<td>0.03</td>
<td>Prob &gt; chi2</td>
<td>0.8609</td>
<td></td>
</tr>
</tbody>
</table>

| t | Haz. Ratio   | Std. Err. | z   | P>|z|   | [95% Conf. Interval] |
|---|--------------|-----------|-----|-------|----------------------|
| epi_stat | no | .9366541 | .3526131 | -0.17 | 0.862     | .4478532    | 1.958948 |

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code
Iteration 0: log likelihood = -304.04321
Iteration 1: log likelihood = -302.52313
Iteration 2: log likelihood = -302.52291

Refining estimates:
Iteration 0: log likelihood = -302.52291

Cox regression -- Breslow method for ties

No. of subjects = 125                     Number of obs = 8398
No. of failures = 69
Time at risk = 1907.821429
LR chi2(1) = 3.04
Log likelihood = -302.52291                     Prob > chi2 = 0.0812

------------------------------------------------------------------------------
t | Haz. Ratio   Std. Err.   z    P>|z|     [95% Conf. Interval]
-------------
neo_inf no |   .0840198   .0881232  -2.36  0.018      .010755    .6563778
-------------

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0: log likelihood = -303.25634
Iteration 1: log likelihood = -300.58057
Iteration 2: log likelihood = -300.16018
Iteration 3: log likelihood = -300.09198
Iteration 4: log likelihood = -300.05533
Iteration 5: log likelihood = -300.05351
Iteration 6: log likelihood = -300.05345
Iteration 7: log likelihood = -300.05343
Iteration 8: log likelihood = -300.05342
Iteration 9: log likelihood = -300.05342
Iteration 10: log likelihood = -300.05341
Iteration 11: log likelihood = -300.05341
Iteration 12: log likelihood = -300.05341
Iteration 13: log likelihood = -300.05341
Iteration 14: log likelihood = -300.05341
Iteration 15: log likelihood = -300.05341
Iteration 16: log likelihood = -300.05341
Iteration 17: log likelihood = -300.05341
Iteration 18: log likelihood = -300.05341
Iteration 19: log likelihood = -300.05341
Iteration 20: log likelihood = -300.05341
Iteration 21: log likelihood = -300.05341
Iteration 22: log likelihood = -300.05341
Iteration 23: log likelihood = -300.05341
Iteration 24: log likelihood = -300.05341
Iteration 25: log likelihood = -300.05341
Iteration 26: log likelihood = -300.05341
Iteration 27: log likelihood = -300.05341
Iteration 28: log likelihood = -300.05341
Iteration 29: log likelihood = -300.05341
Iteration 30: log likelihood = -300.05341
Iteration 31: log likelihood = -300.05341
Iteration 32: log likelihood = -300.05341
Iteration 33: log likelihood = -300.05341
Iteration 34: log likelihood = -300.05341
Iteration 35: log likelihood = -300.05341
Iteration 36: log likelihood = -300.05341

Refining estimates:
Iteration 0: log likelihood = -300.05341
Iteration 1: log likelihood = -300.05341

Cox regression -- Breslow method for ties

No. of subjects = 124                     Number of obs = 8321
No. of failures = 69
Time at risk = 1887.142857
LR chi2(3) = 6.41
Log likelihood = -300.05341  Prob > chi2 =  0.0935

------------------------------------------------------------------------------
   _t | Haz. Ratio   Std. Err.    z  P>|z|     [95% Conf. Interval]
-------------
   hb_type
   AS |  8.42e-17  1.04e-08  -0.00  1.000       0       .
   AC |  1.80663   .5776198  1.85  0.064       .9654311   3.380783
   CC | 36.13151   .3646519 -1.01  0.313       .0499831   2.611853
------------------------------------------------------------------------------
failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0:  log likelihood = -303.25634
Iteration 1:  log likelihood = -301.69767
Iteration 2:  log likelihood = -301.69451
Refining estimates:
Iteration 0:  log likelihood = -301.69451

Cox regression -- Breslow method for ties

No. of subjects =  124                     Number of obs   =      8321
No. of failures =    69                     Time at risk    = 1887.142857
Log likelihood = -301.69451  LR chi2(1)      =      3.12
                       Prob > chi2     =    0.0772

------------------------------------------------------------------------------
   _t | Haz. Ratio   Std. Err.    z  P>|z|     [95% Conf. Interval]
-------------
   hbf |  .9823996   .0097131  1.80  0.072       .9635456   1.001622
------------------------------------------------------------------------------
failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0:  log likelihood = -304.04321
Iteration 1:  log likelihood = -299.33605
Iteration 2:  log likelihood = -298.27137
Refining estimates:
Iteration 0:  log likelihood = -298.27137

Cox regression -- Breslow method for ties

No. of subjects =  125                     Number of obs   =      8398
No. of failures =    69                     Time at risk    = 1907.821429
Log likelihood = -299.22366  LR chi2(1)      =      9.64
                       Prob > chi2     =    0.0019

------------------------------------------------------------------------------
   _t | Haz. Ratio   Std. Err.    z  P>|z|     [95% Conf. Interval]
-------------
   AMA1_AU |  1.343157   .1279268  3.10  0.002       1.114437   1.618818
------------------------------------------------------------------------------
failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0:  log likelihood = -304.04321
Iteration 1:  log likelihood = -299.33605
Iteration 2:  log likelihood = -298.27137
Iteration 3:  log likelihood = -298.2656
Iteration 4:  log likelihood = -298.2656
Refining estimates:
Iteration 0:  log likelihood = -298.2656
Cox regression -- Breslow method for ties

| Variable   | Haz. Ratio | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|------------|------------|-----------|------|-------|----------------------|
| MSP1_AU    | 1.439863   | 0.1411553 | 3.72 | 0.000 | 1.188159 - 1.744888 |

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscreen
id: code

Iteration 0: log likelihood = -302.95718

Cox regression -- Breslow method for ties

| Variable   | Haz. Ratio | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|------------|------------|-----------|------|-------|----------------------|
| MSP2_AU    | 1.272589   | 0.1879093 | 1.63 | 0.103 | .9527977 - 1.699714 |

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscreen
id: code

Iteration 0: log likelihood = -303.73744

Cox regression -- Breslow method for ties

| Variable   | Haz. Ratio | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|------------|------------|-----------|------|-------|----------------------|
| MSP3_AU    | 1.277101   | 0.3939102 | 0.79 | 0.428 | .6977178 - 2.337602 |

variable ama1_sp not found
r(111);

end of do-file

r(111);

do "C:\Users\dkangoye\AppData\Local\Temp\STD0f000000.tmp"
clear all
macro drop _all
capture log close
set more off
log using chap3_nbreg,replace
text
chap3_nbreg.do: construct predictive model for number of episodes
david kangoye,PhD student,Open University/KEMRI-WTRP
version 11.2
set linesize 80
*=================================================================
cd "C:\Users\dkangoye\Dropbox\projects\phdprogram_ou\work\data\data_bics\elisa_bics_labwork\davidk_bics2014"
use bics_kwtrpV0,clear
*combine morbidity data with exposure data
*=================================================================
joinby code using "C:\Users\dkangoye\Dropbox\projects\phdprogram_ou\work\data\data_bics\elisa_bics\dist15ei",unm(both)
tab _merge,m
list code if _merge==2
drop if _merge==2
drop _merge
br
gen period=""
lab var period "period of observation"
replace period="0-3" if monthn==0 | monthn==1 | monthn==2
replace period="3-6" if monthn==3 | monthn==4 | monthn==5
replace period="6-9" if monthn==6
replace period="9-12" if monthn==9 | monthn==10 | monthn==11
replace period="12-15" if monthn==12 | monthn==13 | monthn==14
replace period="18-21" if monthn==18 | monthn==19 | monthn==20
order period,after(datevisit)
drop if period=""
codebook period
sort code datevisit
* _2_define clinical malaria episodes
*=================================================================
*generate var for fever
gen fever=1 if (temp>=37.5 & temp !=.) | hof==1
lab var fever "presence of subjective and/or objective fever"
tab fever,m
*generate new variables for malaria
gen byte malar1=1 if fever==1 & tf>0 & tf !=.
lab var malar1 "febrile malaria episode_tf>0"
replace malar1=0 if malar1==.
gen byte malar2=1 if fever==1 & tf>10000 & tf !=.
lab var malar2 "febrile malaria episode_tf>10000"
replace malar2=0 if malar2==.
*order malaria variables
order malar1 malar2,after(tf)
* _3_censor episodes occurring within 21 days following previous malaria episode

7.2.7 Negative binomial regression Stata code / Chapter 3
*check number of episodes by definition before censoring
local defs "malar1 malar2"
foreach def in `defs' {
    tab `def',m
}
*censor episodes
sort malar1 code datevisit
replace malar1=0 if malar1[_n-1]==1 & code==code[_n-1] & datevisit-datevisit[_n-1]<=21
*check number of episodes by definition after censoring
foreach def in malar1 malar2 {
    tab `def',m
}
* generate additional variables and explore distribution
*=========================================
generate variable age
    gen age= (datevisit - dob)/30
    lab var age "age of infant at current visit"
    sum age

*gen varible season
    gen moy=month(datevisit)
    lab var moy "calendar month"
    tab moy,m
    gen season=1 if moy >=6 & moy <=11
    replace season=0 if moy==12 | moy==1 & moy<=5
    lab var season "malaria transmission season"
    tab season,m

*generate season of birth
    gen sob=1 if month(dob)>=6 & month(dob)<=11
    replace sob=0 if month(dob)==12 | month(dob)>1 & month(dob)<=5
    lab var sob "season of birth"
    tab sob,m

*generate month of birth
    gen mob=month(dob)
    lab var mob "month of birth"
    tab mob,m

*gen variables for seropositivity
    gen ama1_sp=(AMA1_AU>1.1401198)
    gen msp1_sp=(MSP1_AU>1.2326468)
    gen msp2_sp=(MSP2_AU>0.09898795)
    gen msp3_sp=(MSP3_AU>0.81264652)

*_5_generate data set with an id that uniquely identify each couple of (code+period)
*==========================================================================
sort code datevisit
egen id=concat(code period)
    order id,before(code)
br
*_6_generate variable for number of episodes for each child for each period
*==========================================================================
egen numep=total(malar2),by (id)
    order numep,after(malar2)
*_7_keep only one observ per child for each period
*==========================================================================
bysort id (datevisit):keep if _n==1
    sort code datevisit
*_8_define local macros for potential explanatory variables
*==========================================================================
*create local macros for explanatory variables
local envfac season mob zone ei
local socufac educ
local matfac agem sex muac height_scr weight_scr del_way rea epi_stat neo_inf
local infa1 hh_type hbf AMA1_AU MSP1_AU MSP2_AU MSP3_AU
local infa3 ama1_sp msp1_sp msp2_sp msp3_sp /*ama1_pl msp1_pl msp2_pl msp3_pl*/

* _9_keep/order variables of interest and sort observations
*========================================================================================================
keep id code dob datevisit period numep `envfac' `socufac' `matfac' ///
`infa1' `infac2' `infac3' monthn month
sort code datevisit
drop if code=="B010" | code=="B016" | code=="B022" | code=="B034" | code=="F003" | ///
code=="F019" | code=="F022" | code=="F031" | code=="F036" | code=="K008" | ///
code=="N004" | code=="N010"
br
save bics_kwtrpV2,replace
* _10_select between pos/nbreg for non negative count data regression:
*compare mean and variance of outcome variable
*========================================================================================================
use bics_kwtrpV2,clear
hist numep,freq
tabstat numep,s(mean v)
* _11_compute mfp of age for nbreg model
*========================================================================================================
mfp nbreg numep age
corr age lage__1 lage__2
pwcorr age lage__1 lage__2
pcorr age lage__1 lage__2
* _12_define local macros for predictor var to be used in regression analysis
*========================================================================================================
local envfac i.season ei i.mob i.zone
local socufac i.educ
local matfac i.dob i.primgrav i.sg_birth i.iten_use i.iptp_n
local infa1 i.dob i.primgrav i.age i.sex muac height_scr weight_scr del_way i.rea i.epi_stat i.neo_inf
local infa2 hh_type hbf AMA1_AU MSP1_AU MSP2_AU MSP3_AU
local infa3 i.amap i.msp1 i.msp2 i.msp3
foreach i in `infa3' `infa2' `infa1' `matfac' `envfac' `socufac'{
    nbreg numep i.$i.cluster(code) irr }
breg numep i.zone.cluster(code) irr
testparm i.zone
breg numep i.educ,cluster(code) irr
testparm i.educ
recode iptp_n 3=2
breg numep i.iptp_n.cluster(code) irr
testparm i.iptp_n
breg numep i.mob,cluster(code) irr
testparm i.mob
breg numep i.hb_type.cluster(code) irr
testparm i.hb_type

* _14_perform collinearity diagnostics
*========================================================================================================
pwcorr age AMA1_AU MSP1_AU MSP2_AU MSP3_AU hbf muac_scr ei star(.05)
* _15_ multivariate regression analysis using changing antibody titres adjusting for clusters

*include in baseline multivariable model if p<0.2 or high importance variable

*==================================================================*
include in baseline multivariable model if p<0.2 or high importance variable
*==================================================================*

**nbreg numep age AMA1\_AU MSP1\_AU MSP2\_AU MSP3\_AU hbf i.hb\_type i.itn\_use i.mob i.season ei // i.ama1\_sp i.msp1\_sp i.msp3\_sp agem i.iptp\_n i.educ.cluster(code) irr**
est store model0

*model simplification: backward elimination
*==================================================================*
important variables that should not be removed: antibody titres, itn use, season, exposure index, foetal haemoglobin (literature)

drop msp3\_sp
**nbreg numep age AMA1\_AU MSP1\_AU MSP2\_AU MSP3\_AU hbf i.hb\_type i.itn\_use i.mob i.season ei // i.ama1\_sp i.msp1\_sp agem i.iptp\_n i.educ.cluster(code) irr**
est store model1

est table model0 model1,b(%5.3f) p(%4.3f) stats(N ll aic bic)

*drop msp1\_sp
**nbreg numep age AMA1\_AU MSP1\_AU MSP2\_AU MSP3\_AU hbf i.hb\_type i.itn\_use i.mob i.season ei // i.ama1\_sp agem i.iptp\_n i.educ.cluster(code) irr**
est store model2

est table model0 model1 model2,b(%5.3f) p(%4.3f) stats(N ll aic bic) testparm i.iptp\_n

*drop iptp\_n
**nbreg numep age AMA1\_AU MSP1\_AU MSP2\_AU MSP3\_AU hbf i.hb\_type i.itn\_use i.mob i.season ei // i.ama1\_sp agem i.iptp\_n i.educ.cluster(code) irr**
est store model3

est table model0 model1 model2 model3,b(%5.3f) p(%4.3f) stats(N ll aic bic) testparm i.mob testparm i.educ

*drop ama1\_sp
**nbreg numep age AMA1\_AU MSP1\_AU MSP2\_AU MSP3\_AU hbf i.hb\_type i.itn\_use i.mob i.season ei // agem i.iptp\_n i.educ.cluster(code) irr**
est store model4

est table model0 model1 model2 model3 model4,b(%5.3f) p(%4.3f) stats(N ll aic bic) testparm i.mob testparm i.hb\_type testparm i.educ

*drop agem
**nbreg numep age AMA1\_AU MSP1\_AU MSP2\_AU MSP3\_AU hbf i.hb\_type i.itn\_use i.mob i.season ei // agem i.iptp\_n i.educ.cluster(code) irr**
est store model5

est table model0 model1 model2 model3 model4 model5,b(%5.3f) p(%4.3f) stats(N ll aic bic) testparm i.mob testparm i.hb\_type testparm i.educ

*drop educ
**nbreg numep age AMA1\_AU MSP1\_AU MSP2\_AU MSP3\_AU hbf i.hb\_type i.itn\_use i.mob i.season ei // educ.cluster(code) irr**
est store model6

est table model0 model1 model2 model3 model4 model5 model6,b(%5.3f) p(%4.3f) stats(N ll aic bic) testparm i.mob testparm i.hb\_type

log close
exit
# 7.2.8 Negative binomial regression output log / Chapter 3

---

name: <unnamed>

log: C:\Users\dkangoye\Dropbox\projects\phdprogram_ou\work\data\data_bic

-log: s\elisa_bics_labwork\davidk_bics2014\chap3_coxreg.log

log type: text

opened on: 13 Sep 2016, 19:31:02

*chap3_coxreg.do: construct predictive model for number of episodes

* david kangoye, PhD student, Open University/KEMRI-WTRP

version 11.2

set linesize 80

*=============================================================================>

> ============================

C: \Users\dkangoye\Dropbox\projects\phdprogram_ou\work\data\data_bics\elisa

> _bics_labwork\davidk_bics2014

C: \Users\dkangoye\Dropbox\projects\phdprogram_ou\work\data\data_bics\elisa_bics_

> labwork\davidk_bics2014

use bics_kwtrpV0, clear

(combination of all data sets {morb+geo+sero})

*combine morbidity data with exposure data

*=============================================================================>

> cd "C:\Users\dkangoye\Dropbox\projects\phdprogram_ou\work\data\data_bics\elisa

> _bics_labwork\davidk_bics2014"

C: \Users\dkangoye\Dropbox\projects\phdprogram_ou\work\data\data_bics\elisa_bics_

> labwork\davidk_bics2014

*define febrile malaria episodes

*=============================================================================>

merge | Freq.  Percent  Cum.
-------|--------|--------|--------
both in master and using data | 14,089  100.00  100.00
-------|--------|--------|--------
Total | 14,089  100.00

list code if _merge==2

drop if _merge==2

(0 observations deleted)

drop _merge

*define febrile malaria episodes

*=============================================================================>

gen fever=1 if (temp>=37.5 & temp !=.) | hof==1

(12982 missing values generated)

lab var fever "presence of subjective and/or objective fever"

tab fever,m

| presence of | Freq.  Percent  Cum. |
|------------|--------|--------|--------|
| subjective | 1,107  7.86  7.86  |
| and/or     | 12,982 92.14 100.00 |
| objective | fever  | 14,089 100.00 |


. *generate new variables for malaria
. gen byte malar1=1 if fever==1 & tf>0 & tf !=.
(13696 missing values generated)
. lab var malar1 "febrile malaria episode_tf>0"
. replace malar1=0 if malar1==.
(13696 real changes made)

. gen byte malar2=1 if fever==1 & tf>10000 & tf !=.
(13799 missing values generated)
. lab var malar2 "febrile malaria episode_tf>10000"
. replace malar2=0 if malar2==.
(13799 real changes made)

. *order malaria variables
. order malar1 malar2,after(tf)

. *censor episodes occurring within 21 days following previous malaria episode
. *==========================================================================
. *check number of episodes by definition before censoring
. local defs "malar1 malar2"
. foreach def in `defs' {
    2. tab `def', m
    3. }

    febrile | malaria | episode_tf> | Freq. | Percent | Cum.
    0 | --------------------------
       | 0 | 13,696 | 97.21 | 97.21
       | 1 |  393  |  2.79 | 100.00
    Total |-----------------------------------
           | 14,089 | 100.00

    febrile | malaria | episode_tf> | Freq. | Percent | Cum.
    10000 | ------------------------
       | 0 | 13,799 | 97.94 | 97.94
       | 1 |   290  |  2.06 | 100.00
    Total |-----------------------------------
           | 14,089 | 100.00

. *censor episodes
. sort malar1 code datevisit
. replace malar1=0 if malar1[_n-1]==1 & code==code[_n-1] & datevisit-datevisit[_n-1]<21
(50 real changes made)

. *check number of episodes by definition after censoring
. foreach def in malar1 malar2 {
    2. tab `def', m
    3. }

    febrile | malaria | episode_tf> | Freq. | Percent | Cum.
    0 | ----------------------
       | 0 | 13,746 | 97.57 | 97.57
       | 1 |   343  |  2.43 | 100.00
    Total |-----------------------------------
           | 14,089 | 100.00
<table>
<thead>
<tr>
<th>Total</th>
<th>14,089</th>
<th>100.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>febrile</td>
<td></td>
<td></td>
</tr>
<tr>
<td>malaria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>episode_tf&gt;</td>
<td>10000</td>
<td>Freq.</td>
</tr>
<tr>
<td>0</td>
<td>13,799</td>
<td>97.94</td>
</tr>
<tr>
<td>1</td>
<td>290</td>
<td>2.06</td>
</tr>
<tr>
<td>Total</td>
<td>14,089</td>
<td>100.00</td>
</tr>
</tbody>
</table>

*3_generate additional variables and explore distribution
*=========================================================

*generate variable age
. gen age= (datevisit - dob)/30
. lab var age "age of infant at current visit"
. sum age

<table>
<thead>
<tr>
<th>Variable</th>
<th>Obs</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>age</td>
<td>14089</td>
<td>13.11088</td>
<td>7.012261</td>
<td>.9</td>
<td>25.93333</td>
</tr>
</tbody>
</table>

*gen variable season
. gen moy=month(datevisit)
. lab var moy "calendar month"
. tab moy,m

<table>
<thead>
<tr>
<th>calendar</th>
<th>Freq.</th>
<th>Percent</th>
<th>Cum.</th>
</tr>
</thead>
<tbody>
<tr>
<td>month</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1,161</td>
<td>8.24</td>
<td>8.24</td>
</tr>
<tr>
<td>2</td>
<td>1,088</td>
<td>7.72</td>
<td>15.96</td>
</tr>
<tr>
<td>3</td>
<td>1,205</td>
<td>8.55</td>
<td>24.52</td>
</tr>
<tr>
<td>4</td>
<td>1,148</td>
<td>8.15</td>
<td>32.66</td>
</tr>
<tr>
<td>5</td>
<td>1,185</td>
<td>8.41</td>
<td>41.07</td>
</tr>
<tr>
<td>6</td>
<td>1,187</td>
<td>8.43</td>
<td>49.50</td>
</tr>
<tr>
<td>7</td>
<td>1,151</td>
<td>8.17</td>
<td>57.67</td>
</tr>
<tr>
<td>8</td>
<td>1,231</td>
<td>8.74</td>
<td>66.41</td>
</tr>
<tr>
<td>9</td>
<td>1,196</td>
<td>8.49</td>
<td>74.90</td>
</tr>
<tr>
<td>10</td>
<td>1,205</td>
<td>8.55</td>
<td>83.45</td>
</tr>
<tr>
<td>11</td>
<td>1,190</td>
<td>8.45</td>
<td>91.89</td>
</tr>
<tr>
<td>12</td>
<td>1,142</td>
<td>8.11</td>
<td>100.00</td>
</tr>
<tr>
<td>Total</td>
<td>14,089</td>
<td>100.00</td>
<td></td>
</tr>
</tbody>
</table>

* gen season=1 if moy >=6 & moy <=11
(6929 missing values generated)

. replace season=0 if moy==12 | moy>=1 & moy<=5
(6929 real changes made)

. lab var season "malaria transmission season"
. tab season,m

<table>
<thead>
<tr>
<th>malaria</th>
<th>transmissio</th>
<th>Freq.</th>
<th>Percent</th>
<th>Cum.</th>
</tr>
</thead>
<tbody>
<tr>
<td>n season</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6,929</td>
<td>49.18</td>
<td>49.18</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7,160</td>
<td>50.82</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14,089</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
*generate season of birth*

```
. gen sob=1 if month(dob)>=6 & month(dob)<=11
(8425 missing values generated)

. replace sob=0 if month(dob)==12 | month(dob)>=1 & month(dob)<=5
(8425 real changes made)

. lab var sob "season of birth"

. tab sob,m

<table>
<thead>
<tr>
<th>season of birth</th>
<th>Freq.</th>
<th>Percent</th>
<th>Cum.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8425</td>
<td>59.80</td>
<td>59.80</td>
</tr>
<tr>
<td>1</td>
<td>5664</td>
<td>40.20</td>
<td>100.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14089</td>
<td>100.00</td>
<td></td>
</tr>
</tbody>
</table>
```

*generate month of birth*

```
. gen mob=month(dob)

. lab var mob "month of birth"

. tab mob,m

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2905</td>
<td>20.62</td>
<td>20.62</td>
</tr>
<tr>
<td>10</td>
<td>1243</td>
<td>8.82</td>
<td>29.44</td>
</tr>
<tr>
<td>11</td>
<td>4421</td>
<td>31.38</td>
<td>60.82</td>
</tr>
<tr>
<td>12</td>
<td>5520</td>
<td>39.18</td>
<td>100.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14089</td>
<td>100.00</td>
<td></td>
</tr>
</tbody>
</table>
```

*gen variables for seropositivity*

```
. gen ama1_sp=(AMA1_AU>1.1401198)

. gen msp1_sp=(MSP1_AU>1.2326468)

. gen msp2_sp=(MSP2_AU>0.09898795)

. gen msp3_sp=(MSP3_AU>0.81264652)
```

* save bics_kwtrpV1,replace
file bics_kwtrpV1.dta saved

> ************************************************************>
> ************
> use bics_kwtrpV1,clear
> (combination of all data sets {morb+geo+sero})

```
. keep if monthn<22
(1539 observations deleted)

. *univariate cox regression
 **-------------------------**
> use bics_kwtrpV1,clear
> (combination of all data sets {morb+geo+sero})

. keep if monthn<21
(2717 observations deleted)

. stset datevisit, failure(malar2) id(code) origin(datscren) scale(28)

  id:  code
  failure event: malar2 != 0 & malar2 < .

. 258
obs. time interval: (datevisit[_n-1], datevisit)
exit on or before: failure
t for analysis: (time-origin)/28
origin: time datscreen

11372 total observations
118 observations end on or before enter()
2856 observations begin on or after (first) failure

8398 observations remaining, representing
125 subjects
69 failures in single-failure-per-subject data
1907.821 total analysis time at risk and under observation
earliest observed entry t = 0
last observed exit t = 22.39286

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscreen
id: code

<table>
<thead>
<tr>
<th>Category</th>
<th>total</th>
<th>mean</th>
<th>min</th>
<th>median</th>
<th>max</th>
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</thead>
<tbody>
<tr>
<td>no. of subjects</td>
<td>125</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no. of records</td>
<td>8398</td>
<td>67.184</td>
<td>5</td>
<td>81</td>
<td>100</td>
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<tr>
<td>(first) entry time</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>(final) exit time</td>
<td>15.26257</td>
<td>1.142857</td>
<td>19.07143</td>
<td>22.39286</td>
<td></td>
</tr>
<tr>
<td>subjects with gap</td>
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<td></td>
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<tr>
<td>time on gap if gap</td>
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<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>time at risk</td>
<td>1907.8214</td>
<td>15.26257</td>
<td>1.142857</td>
<td>19.07143</td>
<td>22.39286</td>
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<tr>
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<td>.552</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

stsum

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscreen
id: code

<table>
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<tr>
<th>time at risk</th>
<th>rate</th>
<th>no. of subjects</th>
<th>25%</th>
<th>50%</th>
<th>75%</th>
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</thead>
<tbody>
<tr>
<td>total</td>
<td>1907.821429</td>
<td>.0361669</td>
<td>125</td>
<td>9.142857</td>
<td>19.71429</td>
</tr>
</tbody>
</table>

*recode iptp_n 3=2
(iptp_n: 185 changes made)

*create local macros for explanatory variables
.local envfac i.season i.mob i.zone ei
.local socufac i.educ
.local matfac agem i.primgrav i.sg_birth i.itn_use i.iptp_n
.local infac1 i.sex muac height_scr weight_scr i.del_way i.rea i.epi_stat i.ne > o_inf
.local infac2 i.hb_type hbf AMA1_AU MSP1_AU MSP2_AU MSP3_AU
.local infac3 i.ama1_sp i.msp1_sp i.msp2_sp i.msp3_sp /* i.ama1_pl i.msp1_pl i.msp2_pl i.msp3_pl */
. foreach i in `infac1' `infac2' `infac3' `envfac' `socufac' `matfac' 
  2.   stcox `i'
  3. }

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0:   log likelihood = -304.04321
Iteration 1:   log likelihood = -303.78456
Refining estimates:
Iteration 0:   log likelihood = -303.78456

Cox regression -- Breslow method for ties

No. of subjects =          125                     Number of obs   =      8398
No. of failures =           69
Time at risk    =  1907.821429                         LR chi2(1)      =      0.52
Log likelihood  = -303.78456                     Prob > chi2     =    0.4720

------------------------------------------------------------------------------
    _t  |  Haz. Ratio   Std. Err.     z    P>|z|     [95% Conf. Interval]
-------------+-------------------------------------------------------------
      sex  |   0.839564   .2043459  -0.72   0.472     .5210452    1.352796
------------------------------------------------------------------------------

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0:   log likelihood = -303.23335
Iteration 1:   log likelihood = -303.70034
Iteration 2:   log likelihood = -303.70034
Refining estimates:
Iteration 0:   log likelihood = -303.70034

Cox regression -- Breslow method for ties

No. of subjects =          124                     Number of obs   =      8307
No. of failures =           69
Time at risk    =  1887.035714                         LR chi2(1)      =    0.13
Log likelihood  = -303.16663                     Prob > chi2     =    0.7149

------------------------------------------------------------------------------
    _t  |  Haz. Ratio   Std. Err.     z    P>|z|     [95% Conf. Interval]
-------------+-------------------------------------------------------------
   muac_scr  |   1.092771   .1156848   0.84    0.402     .8880101    1.344747
------------------------------------------------------------------------------

260
height_scr | 1.018011   .0498422     0.36   0.715     .9248628    1.120541

failure_d: malar2
analysis time_t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0: log likelihood = -303.23335
Iteration 1: log likelihood = -303.2065
Iteration 2: log likelihood = -303.2065
Refining estimates:
Iteration 0: log likelihood = -303.2065

Cox regression -- Breslow method for ties
No. of subjects = 124                     Number of obs   = 8312
No. of failures = 69
Time at risk    = 1887.071429
Log likelihood  = -303.2065                     Prob > chi2     = 0.8168

_t | Haz. Ratio   Std. Err.    z    P>|z|     [95% Conf. Interval]
-------------+--------------------------------------------------
weight_scr  |   .9538994   .1947292   -0.23   0.817     .6393493    1.423203
-------------

failure_d: malar2
analysis time_t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0: log likelihood = -302.41294
Iteration 1: log likelihood = -302.21728
Iteration 2: log likelihood = -302.21195
Iteration 3: log likelihood = -302.21195
Refining estimates:
Iteration 0: log likelihood = -302.21195

Cox regression -- Breslow method for ties
No. of subjects = 123                     Number of obs   = 8220
No. of failures = 69
Time at risk    = 1866.321429
Log likelihood  = -302.21195                     Prob > chi2     = 0.5261

_t | Haz. Ratio   Std. Err.    z    P>|z|     [95% Conf. Interval]
-------------+--------------------------------------------------
del_way | cesarean  |   .6537055   .4692959   -0.59   0.554     .1600676    2.669689
-------------

failure_d: malar2
analysis time_t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0: log likelihood = -304.04321
Iteration 1: log likelihood = -304.04319
Iteration 2: log likelihood = -304.04319
Refining estimates:
Iteration 0: log likelihood = -304.04319

Cox regression -- Breslow method for ties
No. of subjects = 125                     Number of obs   = 8398
No. of failures = 69
Time at risk    = 1907.821429
Log likelihood  = -304.04319                     Prob > chi2     = 0.9939
| _t | Haz. Ratio | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|----|------------|-----------|------|------|-----------------------|
| rea | no         | 0.9967242 | 0.4268286 | -0.01 | 0.994 | 0.4305898 | 2.307205 |

Cox regression -- Breslow method for ties

No. of subjects = 125                     Number of obs = 8398
No. of failures = 69
Time at risk = 1907.821429
Log likelihood = -304.02785
LR chi2(1) = 3.04
Log likelihood = -302.52291
Prob > chi2 = 0.0812

| _t | Haz. Ratio | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|----|------------|-----------|------|------|-----------------------|
| epi_stat | no | 0.9366541 | 0.3526131 | -0.17 | 0.862 | 0.4478532 | 1.958948 |

Cox regression -- Breslow method for ties

No. of subjects = 125                     Number of obs = 8398
No. of failures = 69
Time at risk = 1907.821429
Log likelihood = -302.52291
LR chi2(1) = 0.03
Log likelihood = -300.52291
Prob > chi2 = 0.8609

| _t | Haz. Ratio | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|----|------------|-----------|------|------|-----------------------|
| neo_inf | no | 0.0840198 | 0.0881232 | -2.36 | 0.018 | 0.010755 | 0.6563778 |

Cox regression -- Breslow method for ties

No. of subjects = 125                     Number of obs = 8398
No. of failures = 69
Time at risk = 1907.821429
Log likelihood = -300.52291
LR chi2(1) = 0.03
Log likelihood = -300.0676
Prob > chi2 = 0.8609

262
<table>
<thead>
<tr>
<th>Iteration</th>
<th>Log Likelihood</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>-300.05351</td>
</tr>
<tr>
<td>10</td>
<td>-300.05345</td>
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<td>11</td>
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<td>-300.05341</td>
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<td>36</td>
<td>-300.05341</td>
</tr>
</tbody>
</table>

Cox regression -- Breslow method for ties

|                        | Haz Ratio | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|------------------------|-----------|-----------|------|-----|----------------------|
| _t | Haz Ratio | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|-------------------------------|-----------|-----------|------|-----|----------------------|
| hb_type |          |           |      |     |                      |
| AS  | 8.42e-17 | 1.04e-08  | -0.00| 1.000| 0                    |
| AC  | 1.80663  | .5776198  | 1.85 | 0.064|.9654311 3.380783   |
| CC  | .3613151 | .3646519  | -1.01| 0.313|.0499831 2.611853   |

failure d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datascreen
id: code

Iteration 0: log likelihood = -303.25634
Iteration 1: log likelihood = -301.69767
Iteration 2: log likelihood = -301.69451
Refining estimates:
Iteration 0: log likelihood = -301.69451

Cox regression -- Breslow method for ties

|                        | Haz Ratio | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|------------------------|-----------|-----------|------|-----|----------------------|
| _t | Haz Ratio | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|-------------------------------|-----------|-----------|------|-----|----------------------|
| hbf | .9823996 | .0097131  | -1.80| 0.072|.9635456 1.001622 |
Cox regression -- Breslow method for ties

No. of subjects =          125                     Number of obs   =      8398
No. of failures =           69                     Time at risk    =  1907.821429
LR chi2(1)      =     11.56                     Log likelihood  = -298.2656                     Prob > chi2     =    0.0007
------------------------------------------------------------------------------
     _t | Haz. Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
   MSP1_AU |   1.439863   .1411553     3.72   0.000     1.188159    1.744888
------------------------------------------------------------------------------
| _t | Haz. Ratio | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|----|------------|-----------|------|-----|----------------------|
| MSP2_AU | 1.272589 | .1879093 | 1.63 | 0.103 | .9527977 - 1.699714 |

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0: log likelihood = -304.04321
Iteration 1: log likelihood = -303.73807
Iteration 2: log likelihood = -303.73744
Refining estimates:
Iteration 0: log likelihood = -303.73744

Cox regression -- Breslow method for ties

No. of subjects = 125
Number of obs = 8398
No. of failures = 69
Time at risk = 1907.821429
LR chi2(1) = 0.61
Log likelihood = -303.73744
Prob > chi2 = 0.4342

| _t | Haz. Ratio | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|----|------------|-----------|------|-----|----------------------|
| MSP3_AU | 1.277101 | .3939102 | 0.79 | 0.428 | .6977178 - 2.337602 |

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0: log likelihood = -304.04321
Iteration 1: log likelihood = -302.71067
Iteration 2: log likelihood = -302.7041
Iteration 3: log likelihood = -302.7041
Refining estimates:
Iteration 0: log likelihood = -302.7041

Cox regression -- Breslow method for ties

No. of subjects = 125
Number of obs = 8398
No. of failures = 69
Time at risk = 1907.821429
LR chi2(1) = 2.68
Log likelihood = -302.7041
Prob > chi2 = 0.1017

| _t | Haz. Ratio | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|----|------------|-----------|------|-----|----------------------|
| 1.ama1_sp | 1.62308 | .4724089 | 1.66 | 0.096 | .9173807 - 2.871641 |

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0: log likelihood = -304.04321
Iteration 1: log likelihood = -301.14735
Iteration 2: log likelihood = -299.70465
Iteration 3: log likelihood = -299.69025
Iteration 4: log likelihood = -299.69025
Refining estimates:
Iteration 0: log likelihood = -299.69025

Cox regression -- Breslow method for ties

No. of subjects = 125
Number of obs = 8398
No. of failures = 69
Time at risk = 1907.821429
Cox regression -- Breslow method for ties

failure _d:  malar2
analysis time _t:  (datevisit-origin)/28
origin:  time dat scren
id:  code

Iteration 0:  log likelihood = -304.04321
Iteration 1:  log likelihood = -303.51434
Iteration 2:  log likelihood = -303.51033
Iteration 3:  log likelihood = -303.51033
Refining estimates:
Iteration 0:  log likelihood = -303.51033

Cox regression -- Breslow method for ties

failure _d:  malar2
analysis time _t:  (datevisit-origin)/28
origin:  time dat scren
id:  code

Iteration 0:  log likelihood = -304.04321
Iteration 1:  log likelihood = -298.21816
Iteration 2:  log likelihood = -297.86159
Iteration 3:  log likelihood = -297.85889
Iteration 4:  log likelihood = -297.85889
Refining estimates:
Iteration 0:  log likelihood = -297.85889

failure _d:  malar2
analysis time _t:  (datevisit-origin)/28
origin:  time dat scren
id:  code

Iteration 0:  log likelihood = -304.04321
Iteration 1:  log likelihood = -298.21816
Iteration 2:  log likelihood = -297.86159
Iteration 3:  log likelihood = -297.85889
Iteration 4:  log likelihood = -297.85889
Refining estimates:
Iteration 0:  log likelihood = -297.85889

Log likelihood = -299.69025  LR chi2(1) = 8.71  Prob > chi2 = 0.0032
----------------------------------------------------------
| _t | Haz. Ratio | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|----|------------|-----------|-----|-----|----------------------|
| 1.msp1_sp | 2.988049 1.001652 3.27 0.001 1.548989 5.764041 |
----------------------------------------------------------

No. of subjects = 125  Number of obs = 8398
No. of failures = 69  Time at risk = 1907.821429
Log likelihood = -301.86087  LR chi2(1) = 4.36  Prob > chi2 = 0.0367
----------------------------------------------------------
| _t | Haz. Ratio | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|----|------------|-----------|-----|-----|----------------------|
| 1.msp2_sp | 2.003354 .6227979 3.24 0.025 1.089277 3.684488 |
----------------------------------------------------------

No. of subjects = 125  Number of obs = 8398
No. of failures = 69  Time at risk = 1907.821429
Log likelihood = -303.51033  LR chi2(1) = 1.07  Prob > chi2 = 0.3019
----------------------------------------------------------
| _t | Haz. Ratio | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|----|------------|-----------|-----|-----|----------------------|
| 1.msp3_sp | 1.389899 .4327121 1.06 0.290 .7550607 2.558496 |
----------------------------------------------------------

No. of subjects = 125  Number of obs = 8398
No. of failures = 69  Time at risk = 1907.821429
Log likelihood = -303.51033  LR chi2(1) = 1.07  Prob > chi2 = 0.3019
----------------------------------------------------------
| _t | Haz. Ratio | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|----|------------|-----------|-----|-----|----------------------|
| 1.msp3_sp | 1.389899 .4327121 1.06 0.290 .7550607 2.558496 |
----------------------------------------------------------

No. of subjects = 125  Number of obs = 8398
No. of failures = 69  Time at risk = 1907.821429
Log likelihood = -303.51033  LR chi2(1) = 1.07  Prob > chi2 = 0.3019
----------------------------------------------------------
| _t | Haz. Ratio | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|----|------------|-----------|-----|-----|----------------------|
| 1.msp3_sp | 1.389899 .4327121 1.06 0.290 .7550607 2.558496 |
----------------------------------------------------------
Cox regression -- Breslow method for ties

No. of subjects = 125
No. of failures = 69
Time at risk = 1907.821429
Log likelihood = -297.85889

| _t | Haz. Ratio | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|----|------------|-----------|------|------|----------------------|
| 1.  | 8.275027   | 5.635893  | 3.10 | 0.002 | 2.177908 - 31.44122 |

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0: log likelihood = -304.04321
Iteration 1: log likelihood = -298.83732
Iteration 2: log likelihood = -298.28853
Iteration 3: log likelihood = -298.28696
Iteration 4: log likelihood = -298.28696
Refining estimates:
Iteration 0: log likelihood = -298.28696

Cox regression -- Breslow method for ties

No. of subjects = 125
No. of failures = 69
Time at risk = 1907.821429
Log likelihood = -298.28696

| _t | Haz. Ratio | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|----|------------|-----------|------|------|----------------------|
| 10 | .1891056   | .1177809  | -2.67| 0.007| .0557891 - .6410018 |
| 11 | .4389449   | .1428056  | -2.53| 0.011| .2319965 - .830498  |
| 12 | .531245    | .1594509  | -2.11| 0.035| .2949921 - .9567077 |

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0: log likelihood = -304.04321
Iteration 1: log likelihood = -293.26189
Iteration 2: log likelihood = -292.92575
Iteration 3: log likelihood = -292.92499
Refining estimates:
Iteration 0: log likelihood = -292.92499

Cox regression -- Breslow method for ties

No. of subjects = 125
No. of failures = 69
Time at risk = 1907.821429
Log likelihood = -292.92499

| _t | Haz. Ratio | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|----|------------|-----------|------|------|----------------------|
| urban | .3093051   | .1075754  | -3.37| 0.001| .1564378 - .615508  |
| mixed | 1.317749   | .3532078  | 1.03 | 0.303| .7791591 - 2.228638 |

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0:  log likelihood = -304.04321
Iteration 1:  log likelihood = -293.57271
Iteration 2:  log likelihood = -293.49031
Iteration 3:  log likelihood = -293.49028
Refining estimates:
Iteration 0:  log likelihood = -293.49028

Cox regression -- Breslow method for ties
No. of subjects =          125                     Number of obs   =      8398
No. of failures =           69
Time at risk    =  1907.821429
Log likelihood  = -293.49028
LR chi2(2)      =      8.01                     Prob > chi2     =    0.0183
-------------------------------------------------------------------------------
   _t | Haz. Ratio   Std. Err.   z    P>|z|     [95% Conf. Interval]
 -------------
   ei |  1.096877    .0234741  4.32  0.000      1.05182    1.143864
-------------------------------------------------------------------------------

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0:  log likelihood = -304.04321
Iteration 1:  log likelihood = -302.09653
Iteration 2:  log likelihood = -302.07665
Iteration 3:  log likelihood = -302.07665
Iteration 4:  log likelihood = -302.07665
Refining estimates:
Iteration 0:  log likelihood = -302.07665

Cox regression -- Breslow method for ties
No. of subjects =          125                     Number of obs   =      8398
No. of failures =           69
Time at risk    =  1907.821429
LR chi2(1)      =     3.93                     Prob > chi2     =    0.0473
-------------------------------------------------------------------------------
   _t | Haz. Ratio   Std. Err.   z    P>|z|     [95% Conf. Interval]
 -------------
educ |   primary sc..  | 1.51729    .3885309  1.63  0.103     .9185491    2.506311
   secondary ..  |   .4512767    .2152045 -1.67  0.095     .1772244    1.149155
-------------------------------------------------------------------------------

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0:  log likelihood = -304.04321
Iteration 1:  log likelihood = -302.09653
Iteration 2:  log likelihood = -302.07665
Iteration 3:  log likelihood = -302.07665
Iteration 4:  log likelihood = -302.07665
Refining estimates:
Iteration 0:  log likelihood = -302.07665

Cox regression -- Breslow method for ties
No. of subjects =          125                     Number of obs   =      8398
No. of failures =           69
Time at risk    =  1907.821429
LR chi2(1)      =     3.93                     Prob > chi2     =    0.0473
-------------------------------------------------------------------------------
| t  | Haz. Ratio | Std. Err. | z    | P>|z|   | [95% Conf. Interval] |
|----|-----------|-----------|------|--------|---------------------|
| age | 1.038145  | .0190669  | 2.04 | 0.042  | 1.001439 1.076196  |

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0: log likelihood = -299.21545
Iteration 1: log likelihood = -299.14403
Iteration 2: log likelihood = -299.14395
Refining estimates:
Iteration 0: log likelihood = -299.14395

Cox regression -- Breslow method for ties

No. of subjects = 124
No. of failures = 68
Time at risk = 1898.857143
Log likelihood = -299.14395
LR chi2(1) = 0.14
Prob > chi2 = 0.7053

| t  | Haz. Ratio | Std. Err. | z    | P>|z|   | [95% Conf. Interval] |
|----|-----------|-----------|------|--------|---------------------|
| primgrav | no | 1.122431  | .3468379 | 0.37  | 0.709   | .6125381 2.056771 |

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0: log likelihood = -304.04321
Iteration 1: log likelihood = -301.91106
Iteration 2: log likelihood = -301.16487
Iteration 3: log likelihood = -300.89514
Iteration 4: log likelihood = -300.79655
Iteration 5: log likelihood = -300.76036
Iteration 6: log likelihood = -300.74706
Iteration 7: log likelihood = -300.74217
Iteration 8: log likelihood = -300.74037
Iteration 9: log likelihood = -300.73977
Iteration 10: log likelihood = -300.73946
Iteration 11: log likelihood = -300.73937
Iteration 12: log likelihood = -300.73934
Iteration 13: log likelihood = -300.73933
Iteration 14: log likelihood = -300.73932
Iteration 15: log likelihood = -300.73932
Iteration 16: log likelihood = -300.73932
Iteration 17: log likelihood = -300.73932
Iteration 18: log likelihood = -300.73932
Iteration 19: log likelihood = -300.73932
Iteration 20: log likelihood = -300.73932
Iteration 21: log likelihood = -300.73932
Iteration 22: log likelihood = -300.73932
Iteration 23: log likelihood = -300.73932
Iteration 24: log likelihood = -300.73932
Iteration 25: log likelihood = -300.73932
Iteration 26: log likelihood = -300.73932
Iteration 27: log likelihood = -300.73932
Iteration 28: log likelihood = -300.73932
Iteration 29: log likelihood = -300.73932
Iteration 30: log likelihood = -300.73932
Iteration 31: log likelihood = -300.73932
Iteration 32: log likelihood = -300.73932
Iteration 33: log likelihood = -300.73932
Iteration 34: log likelihood = -300.73932
Iteration 35: log likelihood = -300.73932
Refining estimates:
Iteration 0: log likelihood = -300.73932
Cox regression -- Breslow method for ties

|                | hazard (95% CI) | P>|z|  
|----------------|-----------------|------|
| `sg_birth`     |                 |      |
| no             | 2.13e-16 (1.000, 4.18e-09) | 1.00 |

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Cox regression -- Breslow method for ties

|                | hazard (95% CI) | P>|z|  
|----------------|-----------------|------|
| `itn_use`      |                 |      |
| no             | 1.239 (0.945, 1.631) | 0.49 |

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Cox regression -- Breslow method for ties

|                | hazard (95% CI) | P>|z|  
|----------------|-----------------|------|
| `iptp_n`       |                 |      |
| 1              | 1.47 (0.90, 2.32) | 0.14 |
| 2              | 1.14 (0.76, 1.71) | 0.53 |

stcox i.zone
failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0:  log likelihood = -304.04321
Iteration 1:  log likelihood = -293.26189
Iteration 2:  log likelihood = -292.92575
Iteration 3:  log likelihood = -292.92499
Refining estimates:
Iteration 0:  log likelihood = -292.92499

Cox regression -- Breslow method for ties

|                | Haz. Ratio   | Std. Err. | z    | P>|z|     | [95% Conf. Interval] |
|----------------|--------------|-----------|------|---------|----------------------|
| zone           |              |           |      |         |                      |
| urban          | .3093051     | .1075754  | -.37 | 0.001   | .1564378    .6115508 |
| mixed          | 1.317749     | .3532878  | 1.03 | 0.303   | .7791591    2.228638 |

.testparm i.zone
( 1)  2.zone = 0
( 2)  3.zone = 0

ch2(  2) = 17.97
Prob > chi2 = 0.0001

.stcox i.educ

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscren
id: code

Iteration 0:  log likelihood = -304.04321
Iteration 1:  log likelihood = -300.22379
Iteration 2:  log likelihood = -300.04145
Iteration 3:  log likelihood = -300.04046
Iteration 4:  log likelihood = -300.04046
Refining estimates:
Iteration 0:  log likelihood = -300.04046

Cox regression -- Breslow method for ties

|                | Haz. Ratio   | Std. Err. | z    | P>|z|     | [95% Conf. Interval] |
|----------------|--------------|-----------|------|---------|----------------------|
| educ           |              |           |      |         |                      |
| primary sc.    | 1.51729      | .3885309  | 1.63 | 0.103   | .9185491    2.506311 |
| secondary ..   | .4512767     | .2152045  | -1.67| 0.095   | .177224     1.149115 |

.testparm i.educ
( 1)  1.educ = 0
( 2)  2.educ = 0
chi2( 2) = 6.97
Prob > chi2 = 0.0306

. stcox i.iptp_n

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscreen
id: code

Iteration 0: log likelihood = -304.04321
Iteration 1: log likelihood = -303.61417
Iteration 2: log likelihood = -303.61123
Iteration 3: log likelihood = -303.61123
Refining estimates:
Iteration 0: log likelihood = -303.61123

Cox regression -- Breslow method for ties
No. of subjects = 125                     Number of obs   = 8398
No. of failures = 69
Time at risk    = 1907.821429
Log likelihood  = -303.61123
LR chi2(2)      = 0.86
Prob > chi2     = 0.6492

------------------------------------------------------------------
   _t  | Haz. Ratio   Std. Err.     z    P>|z|     [95% Conf. Interval]
------------------------------------------------------------------
   1  |    1.474334   .824449     0.69   0.488     .4927236    4.411524
   2  |    1.149532   .5983478    0.27   0.789     .4144369    3.188479
------------------------------------------------------------------

. testparm i.iptp_n
( 1)  1.iptp_n = 0
( 2)  2.iptp_n = 0

chi2( 2) = 0.89
Prob > chi2 = 0.6393

. stcox i.mob

failure _d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscreen
id: code

Iteration 0: log likelihood = -304.04321
Iteration 1: log likelihood = -298.83732
Iteration 2: log likelihood = -298.28853
Iteration 3: log likelihood = -298.28696
Iteration 4: log likelihood = -298.28696
Refining estimates:
Iteration 0: log likelihood = -298.28696

Cox regression -- Breslow method for ties
No. of subjects = 125                     Number of obs   = 8398
No. of failures = 69
Time at risk    = 1907.821429
Log likelihood  = -298.28696
LR chi2(3)      = 11.51
Prob > chi2     = 0.0093

------------------------------------------------------------------
   _t  | Haz. Ratio   Std. Err.     z    P>|z|     [95% Conf. Interval]
------------------------------------------------------------------
   mob |    1.17 | .824449  | 0.27 | 0.789 | .4144369 | 3.188479
------------------------------------------------------------------
. testparm i.mob
( 1) 10.mob = 0
( 2) 11.mob = 0
( 3) 12.mob = 0
    chi2(  3) =   10.99
    Prob > chi2 =    0.0118

. stcox i.hb_type
failure _d:  malar2  
analysis time _t:  (datevisit-origin)/28
origin:  time datscren
id:  code

Iteration 0:   log likelihood = -303.25634
Iteration 1:   log likelihood = -300.58057
Iteration 2:   log likelihood = -300.16018
Iteration 3:   log likelihood = -300.09198
Iteration 4:   log likelihood = -300.05863
Iteration 5:   log likelihood = -300.05333
Iteration 7:   log likelihood = -300.04512
Iteration 8:   log likelihood = -300.05367
Iteration 9:   log likelihood = -300.05351
Iteration 10:  log likelihood = -300.05345
Iteration 11:  log likelihood = -300.05343
Iteration 12:  log likelihood = -300.05342
Iteration 13:  log likelihood = -300.05342
Iteration 14:  log likelihood = -300.05341
Iteration 15:  log likelihood = -300.05341
Iteration 16:  log likelihood = -300.05341
Iteration 17:  log likelihood = -300.05341
Iteration 18:  log likelihood = -300.05341
Iteration 19:  log likelihood = -300.05341
Iteration 20:  log likelihood = -300.05341
Iteration 21:  log likelihood = -300.05341
Iteration 22:  log likelihood = -300.05341
Iteration 23:  log likelihood = -300.05341
Iteration 24:  log likelihood = -300.05341
Iteration 25:  log likelihood = -300.05341
Iteration 26:  log likelihood = -300.05341
Iteration 27:  log likelihood = -300.05341
Iteration 28:  log likelihood = -300.05341
Iteration 29:  log likelihood = -300.05341
Iteration 30:  log likelihood = -300.05341
Iteration 31:  log likelihood = -300.05341
Iteration 32:  log likelihood = -300.05341
Iteration 33:  log likelihood = -300.05341
Iteration 34:  log likelihood = -300.05341
Iteration 35:  log likelihood = -300.05341
Iteration 36:  log likelihood = -300.05341
Refining estimates:
Iteration 0:   log likelihood = -300.05341
Iteration 1:   log likelihood = -300.05341

Cox regression -- Breslow method for ties

        No. of subjects =          124                      Number of obs =      8321
        No. of failures =           69
        Time at risk    =  1887.142857                      LR chi2(3) =      6.41
        Log likelihood  = -300.05341                      Prob > chi2 =    0.0935

------------------------------------------------------------------------------
  _t | Haz. Ratio   Std. Err.    z    P>|z|     [95% Conf. Interval]
-------------+---------------------------------------------------------------
  hb_type  |
  AS  |   8.42e-17  1.04e-08 -0.00  1.000            0           0 .
  AC  |    1.80663   .5776198     1.85  0.064     .9654311    3.380783
  CC  |   .3613151   .3646519   -1.01  0.313     .0499831    2.611853
------------------------------------------------------------------------------

. testparm i.hb_type
( 1) 2.hb_type = 0
( 2) 3.hb_type = 0
( 3) 4.hb_type = 0

chi2(  3) =    4.67
Prob > chi2 =    0.1979

. *perform collinearity diagnostic
. **============================================================================
. pwcorr AMA1_AU MSP1_AU MSP2_AU MSP3_AU hbf muac_scr ei, star(.05)

<table>
<thead>
<tr>
<th>AMA1_AU    MSP1_AU    MSP2_AU    MSP3_AU    hbf    muac_scr    ei</th>
</tr>
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<tbody>
<tr>
<td>AMA1_AU</td>
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<tr>
<td>MSP1_AU</td>
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<tr>
<td>hbf</td>
</tr>
<tr>
<td>muac_scr</td>
</tr>
<tr>
<td>ei</td>
</tr>
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</table>

. graph matrix AMA1_AU MSP1_AU MSP2_AU MSP3_AU
. collin AMA1_AU MSP1_AU MSP2_AU MSP3_AU
(obs=11372)

Collinearity Diagnostics

<table>
<thead>
<tr>
<th>Variable</th>
<th>VIF</th>
<th>VIF</th>
<th>Tolerance</th>
<th>R-Squared</th>
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<tr>
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<td>1.39</td>
<td>1.18</td>
<td>0.7215</td>
<td>0.2785</td>
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<tr>
<td>MSP1_AU</td>
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<td>1.15</td>
<td>0.7523</td>
<td>0.2477</td>
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<td>MSP2_AU</td>
<td>1.68</td>
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<td>0.5953</td>
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<tr>
<td>MSP3_AU</td>
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<td>1.14</td>
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Mean VIF 1.42

Eigenval Index

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<tr>
<td>5</td>
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</table>

Condition Number 2.8642
Eigenvalues & Cond Index computed from scaled raw sscp (w/ intercept)
Det(correlation matrix) 0.4495

. collin AMA1_AU MSP1_AU MSP2_AU MSP3_AU hbf muac_scr ei
(obs=11295)

Collinearity Diagnostics

<table>
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<tr>
<th>Variable</th>
<th>VIF</th>
<th>VIF</th>
<th>Tolerance</th>
<th>R-Squared</th>
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<tr>
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<td>muac_scr</td>
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Mean VIF      1.27

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<td>3.6075</td>
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<td>6</td>
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<td>5.9213</td>
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<tr>
<td>7</td>
<td>0.0249</td>
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<td>8</td>
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<td>36.0141</td>
</tr>
</tbody>
</table>

Condition Number        36.0141

Eigenvalues & Cond Index computed from scaled raw sscp (w/ intercept)
Det(correlation matrix)    0.4120.

*multivariate cox regression
*include in baseline multivariable model if p<0.2 or theoretically high import
> stcox hbf i.hb_type AMA1_AU MSP1_AU MSP2_AU MSP3_AU i.ama1_sp i.msp1_sp i.msp2
> _sp ///
>         i.mob i.itn_use i.educ i.season ei

failure _d:  malar2
analysis time _t:  (datevisit-origin)/28
origin:  time datSCREEN
id:  code

Iteration 0:  log likelihood = -298.44974
Iteration 1:  log likelihood = -266.375
Iteration 2:  log likelihood = -261.61083
Iteration 3:  log likelihood = -261.49205
Iteration 4:  log likelihood = -261.47772
Iteration 5:  log likelihood = -261.47248
Iteration 6:  log likelihood = -261.47055
Iteration 7:  log likelihood = -261.46984
Iteration 8:  log likelihood = -261.46958
Iteration 9:  log likelihood = -261.46948
Iteration 10: log likelihood = -261.46945
Iteration 11: log likelihood = -261.46944
Iteration 12: log likelihood = -261.46943
Iteration 13: log likelihood = -261.46943
Iteration 14: log likelihood = -261.46943
Iteration 15: log likelihood = -261.46943
Iteration 16: log likelihood = -261.46943
Iteration 17: log likelihood = -261.46943
Iteration 18: log likelihood = -261.46943
Iteration 19: log likelihood = -261.46943
Iteration 20: log likelihood = -261.46943
Iteration 21: log likelihood = -261.46943
Iteration 22: log likelihood = -261.46943
Iteration 23: log likelihood = -261.46943
Iteration 24: log likelihood = -261.46943
Iteration 25: log likelihood = -261.46943
Iteration 26: log likelihood = -261.46943
Iteration 27: log likelihood = -261.46943
Iteration 28: log likelihood = -261.46943
Iteration 29: log likelihood = -261.46943
Iteration 30: log likelihood = -261.46943
Iteration 31: log likelihood = -261.46943
Iteration 32: log likelihood = -261.46943
Iteration 33: log likelihood = -261.46943
Iteration 34: log likelihood = -261.46943
Iteration 35: log likelihood = -261.46943
Iteration 36: log likelihood = -261.46943
Iteration 37: log likelihood = -261.46943
Iteration 38: log likelihood = -261.46943
Iteration 39: log likelihood = -261.46943
Iteration 40: log likelihood = -261.46943

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Iteration 41: log likelihood = -261.46943
Iteration 42: log likelihood = -261.46943
Iteration 43: log likelihood = -261.46943

Refining estimates:
Iteration 0: log likelihood = -261.46943

Cox regression
Breslow method for ties

No. of subjects =          123                     Number of obs   =      8264
No. of failures =           68
Time at risk    =       1874.5
LR chi2(18)     =     73.96
Log likelihood  = -261.46943
Prob > chi2     =    0.0000

| t | Haz. Ratio   Std. Err. | z   | P>|z| | [95% Conf. Interval] |
|---|-------------------|-----|-------|---------------------|
|hbf| 0.9602224 .0123249 | -3.16 | 0.002 | 0.9363764 .9846852 |
|hb_type |       |       |       |       |       |       |       |
|     | AS | 2.42e-19 . . . . . . |
|     | AC | 1.623269 .5801645 1.36 0.175 .8056875 3.2705 |
|     | CC | .2685015 .2834482 -1.25 0.213 .0393126 2.125847 |
|     | AMA1_AU | 1.262122 .1863322 1.58 0.115 .9450069 1.685651 |
|     | MSP1_AU | 1.316635 .240752 1.50 0.132 .9200674 1.88413 |
|     | MSP2_AU | .6530574 .2248826 -1.24 0.216 .3325348 1.282524 |
|     | MSP3_AU | .282759 .4460321 0.72 0.474 .6488904 2.535824 |
|     | l.amal_sp | .6040203 .2567994 -1.19 0.236 .2625204 1.389761 |
|     | l.msp1_sp | 1.188667 .7086646 0.29 0.774 .3681268 3.825123 |
|     | l.msp2_sp | 2.575378 1.651516 1.48 0.140 .7328073 9.050908 |
|mob |       |       |       |       |       |       |       |
|     | 10 | .2017084 .1391441 -2.32 0.020 .0521845 .7796625 |
|     | 11 | .6807494 .2558631 -1.02 0.306 .3258809 1.422053 |
|     | 12 | .6460694 .2214892 -1.27 0.203 .3299631 1.265007 |
|itn_use |       |       |       |       |       |       |       |
|     | no | 1.072824 .4373913 0.17 0.863 .4824947 2.385416 |
|educ |       |       |       |       |       |       |       |
|     | primary sc.. | 1.870156 .5355262 2.19 0.029 1.066923 3.278103 |
|     | secondary .. | 1.07591 .6307863 0.12 0.901 .3409828 3.394843 |
|     | 1.season | 9.233465 6.726386 3.05 0.002 2.214533 38.49881 |
|ei |       |       |       |       |       |       |       |
|     | 1.094685 .0283509 3.49 0.000 1.040505 1.151686 |

est store model0

*model simplification: backward elimination
* important variables that should not be removed:
* antibody titres, itn use, season, exposure index, foetal haemoglobin (literature)
*drop map1_sp
stcox hbf i.hb_type AMA1_AU MSP1_AU MSP2_AU MSP3_AU i.amal_sp i.msp2_sp ///
i.mob i.itn_use i.educ i.season ei

failure_d: malar2
analysis time _t: (datevisit-origin)/28
origin: time datscreen
id: code

Iteration 0: log likelihood = -298.44974
Iteration 1: log likelihood = -264.81816
Iteration 2: log likelihood = -261.62226
Iteration 3: log likelihood = -261.53433
Iteration 4: log likelihood = -261.51928
Iteration 5: log likelihood = -261.51377
Iteration 6: log likelihood = -261.51174
Iteration 7: log likelihood = -261.511
Iteration 8: log likelihood = -261.51072
Iteration 9: log likelihood = -261.51062
Iteration 10: log likelihood = -261.51059
Iteration 11: log likelihood = -261.51057
Iteration 12: log likelihood = -261.51057
Iteration 13: log likelihood = -261.51057
Iteration 14: log likelihood = -261.51057
Iteration 15: log likelihood = -261.51057
Iteration 16: log likelihood = -261.51057
Iteration 17: log likelihood = -261.51057
Iteration 18: log likelihood = -261.51057
Iteration 19: log likelihood = -261.51057
Iteration 20: log likelihood = -261.51057
Iteration 21: log likelihood = -261.51057
Iteration 22: log likelihood = -261.51057
Iteration 23: log likelihood = -261.51057
Iteration 24: log likelihood = -261.51057
Iteration 25: log likelihood = -261.51057
Iteration 26: log likelihood = -261.51057

Refining estimates:
Iteration 0: log likelihood = -261.51056
Iteration 1: log likelihood = -261.51056
Iteration 2: log likelihood = -261.51056
Iteration 3: log likelihood = -261.51056
Iteration 4: log likelihood = -261.51056
Iteration 5: log likelihood = -261.51056
Iteration 6: log likelihood = -261.51056

Cox regression -- Breslow method for ties
No. of subjects = 123  Number of obs = 8264
No. of failures = 68  Time at risk = 1874.5
Log likelihood = -261.51056  LR chi2(18) = 73.88
Prob > chi2 = 0.0000

|                |    _t | Haz. Ratio | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|----------------|-------|------------|-----------|------|------|----------------------|
| hbf            | .9601089 | .0123754 | -3.16     | 0.002 | .9361573 | .9846732 |
|                | hb_type |           |           |      |      |                      |
| AS             | 1.52e+14  | 1.96e+07   | -0.00     | 1.000 | 0.000 |                      |
| AC             | 1.633136 | .5824928   | 1.38      | 0.169 | .8117515 | 3.285652 |
| CC             | .2717637 | .2869681   | -1.24     | 0.217 | .0340498 | 2.146555 |
|                | AMA1_AU |           |           |      |      |                      |
|                | 1.267528 | .1861146   | 1.61      | 0.106 | .9505467 | 1.690215 |
|                | MSP1_AU |           |           |      |      |                      |
|                | 1.365064 | .178841    | 2.38      | 0.018 | 1.055929 | 1.764702 |
|                | MSP2_AU |           |           |      |      |                      |
|                | .6453629 | .2207394   | -1.28     | 0.204 | .3301108 | 1.690215 |
|                | MSP3_AU |           |           |      |      |                      |
|                | 1.287881 | .4463949   | 0.73      | 0.465 | .6528889 | 2.54046   |
|                | l.amal_sp |         |           |      |      |                      |
|                | 0.6009246 | .2547343 | -1.20    | 0.230 | .2618136 | 1.379265 |
|                | l.msp2_sp |         |           |      |      |                      |
|                | 2.691987 | 1.672628   | 1.59      | 0.111 | .7965093 | 9.098189 |
|                | mob     |           |           |      |      |                      |
| 10             | .2004842 | .13833139 | -2.33     | 0.020 | .0518605 | .7750385 |
| 11             | .6790914 | .2550871  | -1.03     | 0.303 | .3252306 | 1.417964 |
| 12             | .6473024 | .2215481  | -1.27     | 0.204 | .3309572 | 1.266026 |
|                | itn_use |           |           |      |      |                      |
| no             | 1.063971 | .4327034  | 0.15      | 0.879 | .4794654 | 2.361036 |
|                | educ    |           |           |      |      |                      |
| primary sc.    | 1.859814 | .5307199  | 2.17      | 0.030 | 1.063088 | 3.253643 |
| secondary ..   | 1.070595 | .6270348  | 0.12      | 0.907 | .3396931 | 3.374146 |
|                | l.season |           |           |      |      |                      |
|                | 9.175112 | 6.64336   | 3.06      | 0.002 | 2.219666 | 37.92582 |
|                | ei      |           |           |      |      |                      |
|                | 1.094991 | .0282963  | 3.51      | 0.000 | 1.040912 | 1.151879 |

. est store modell
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Legend: b/p

.testparm i.mob

( 1) 10.mob = 0
( 2) 11.mob = 0
( 3) 12.mob = 0

chi2( 3) = 5.72
Prob > chi2 = 0.1262
. testparm i.educ
( 1)  1.educ = 0
( 2)  2.educ = 0

chi2(  2) =    4.85
Prob > chi2 =    0.0886

. *drop ama1_sp
. stcox hbf i hb_type AMA1_AU MSP1_AU MSP2_AU MSP3_AU i.msp2_sp ///
>                  i.mob
>                  i.itn_use i.educ i.season ei

failure _d:  malar2
analysis time _t:  (datevisit-origin)/28
origin:  time datscren
id:  code

Iteration 0:   log likelihood = -298.44974
Iteration 1:   log likelihood = -265.98151
Iteration 2:   log likelihood = -262.235205
Iteration 4:   log likelihood = -262.234008
Iteration 5:   log likelihood = -262.238955
Iteration 6:   log likelihood = -262.23652
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Iteration 42:  log likelihood = -262.23534
Iteration 43:  log likelihood = -262.23534
Iteration 44:  log likelihood = -262.23534

Refining estimates:
Iteration 0:   log likelihood = -262.23534

Cox regression -- Breslow method for ties

No. of subjects =          123                     Number of obs   =      8264
No. of failures =           68
Time at risk    =       1874.5
LR chi2(16)     =     72.43
Log likelihood  = -262.23534                     Prob > chi2     =    0.0000
| t | Haz. Ratio | Std. Err. | z   | P>|z| | [95% Conf. Interval] |
|---|------------|-----------|-----|------|-----------------|
|hbf | 0.9602003 | 0.0124723 | -3.13 | 0.002 | 0.936036  | 0.984933 |
|   |            |           |      |      |                 |           |
| hb_type |          |           |      |      |                 |           |
| AS  | 1.03e-19  | .   | .   | .   | .               |             |
| AC  | 1.667372  | .5950362 | 1.43 | 0.152 | .828451  | 3.355841  |
| CC  | .2949369  | .3094506 | -1.16 | 0.245 | .037726  | 2.30578   |
| AMA1_AU | 1.127067 | .12368 | 1.09 | 0.276 | .908955  | 1.397518  |
| MSP1_AU | 1.364107 | .1789437 | 2.37 | 0.018 | 1.054843 | 1.764043  |
| MSP2_AU | .6389871 | .2125111 | -1.35 | 0.178 | .3329689 | 1.226254  |
| MSP3_AU | 1.387617 | .4736641 | 0.96 | 0.337 | .7107408 | 2.709118  |
| l.msp2_sp | 2.555521 | 1.558997 | 1.54 | 0.124 | .7730426 | 8.448028  |
|   |            |           |      |      |                 |           |
| mob |          |           |      |      |                 |           |
| 10 | .2080973  | .1430321 | -2.28 | 0.022 | .0541013 | 0.804338  |
| 11 | .6814446  | .256321  | -1.02 | 0.308 | .3260293 | 1.423411  |
| 12 | .6475021  | .2231985 | -1.26 | 0.207 | .3294777 | 1.272496  |
| itn_use |          |           |      |      |                 |           |
| no | 1.058391  | .433304  | 0.14 | 0.890 | .4774221 | 2.361169  |
| educ |          |           |      |      |                 |           |
| primary sc. | 1.827461 | .5159321 | 2.14 | 0.033 | 1.050837 | 3.178051  |
| secondary .. | 1.043523 | .6076163 | 0.07 | 0.942 | .3333259 | 3.266891  |
| l.season | 9.250968  | 6.645039 | 3.10 | 0.002 | 2.263408 | 37.81042 |
| ei | 1.097981  | .0284054 | 3.61 | 0.000 | 1.043695 | 1.15509   |

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legend: b/p

. testparm i.mob
( 1) 10.mob = 0
( 2) 11.mob = 0
( 3) 12.mob = 0

chi2(  3) =  5.50
Prob > chi2 =   0.1385

. testparm i.educ
( 1) 1.educ = 0
( 2) 2.educ = 0

chi2(  2) =  4.71
Prob > chi2 =   0.0948

. *drop msp2_sp
. stcox AMA1_AU MSP1_AU MSP2_AU MSP3_AU hbf i hb_type ///
  > i.itn_use i.mob i.educ i.season ei

  failure _d: malar2
  analysis time _t: (datevisit-origin)/28
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  id: code

Iteration 0:  log likelihood = -298.44974
Iteration 1:  log likelihood = -266.55962
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Refining estimates:
Iteration 0: log likelihood = -263.40417

Cox regression -- Breslow method for ties

No. of subjects = 123                     Number of obs = 8264
No. of failures = 68
Time at risk   = 1874.5
LR chi2(15)     = 70.09
Log likelihood  = -263.40417                     Prob > chi2     = 0.0000
-------------------------------------------------------------------------------
  _t | Haz. Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
------------------------------------------------------------------------------
AMA1_AU |   1.137384      .1234     1.19   0.235     .9195097    1.406883
MSP1_AU |   1.393096   .1789241     2.58   0.010     1.083068    1.791869
MSP2_AU |   .9140762   .1664022    -0.49   0.622     .6397733    1.305986
MSP3_AU |   1.201256   .3939675     0.56   0.576      .631645    2.284537
hbf |   .9602566   .0123626     3.15   0.002     .9363295    .9847951

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   AC |   1.598977 .5736798    1.31   0.191     .7914962    3.230247
   CC |   .3081119 .3222807    -1.13   0.260     .039661    2.39361

itn_use |
   no |   1.10239   .4515609     0.24   0.812     .4939312    2.460393

mob |
   10 |   .2072943 .1420115    -2.30   0.022     .0541319    .7938191
   11 |   .7084804 .2649145    -0.62   0.537     .3404451    1.474377
   12 |   .6451689 .2224681    -1.27   0.204     .3282168    1.268195

educ |
primary sc.. |   1.778631 .4979711    2.06   0.040     1.027475    3.078934
secondary .. |   1.033114 .6015039     0.06   0.955     .3300339    3.233987

1.season |   9.215965 6.708862    3.05   0.002     2.212582    38.36803
   ei |   1.098306 .0284761    3.62   0.000     1.043888    1.15556

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N | 8264 | 8264 | 8264 | 8264 |
11 | -261.469 | -261.511 | -262.235 | -263.404 |
aic | 558.939 | 559.021 | 556.471 | 556.808 |
bic | 685.293 | 685.375 | 668.785 | 662.103 |

legend: b/p

.testparm i.mob
( 1) 10.mob = 0
( 2) 11.mob = 0
( 3) 12.mob = 0

chi2(  3) =  5.55
Prob > chi2 =  0.1354
. testparm i.educ
( 1) 1.educ = 0
( 2) 2.educ = 0

\[ \text{chi}^2(2) = 4.38, \quad \text{Prob} > \text{chi}^2 = 0.1117 \]

. *drop educ
. stcox AMA1_AU MSP1_AU MSP2_AU MSP3_AU hbf i.hb_type ///
>                  i.itn_use i.mob i.seas on ei

failure _d:  malar2
analysis time _t:  (datevisit-origin)/28
origin:  time datscreen
id:  code

Iteration 0:   log likelihood = -298.44974
Iteration 1:   log likelihood = -268.64842
Iteration 2:   log likelihood = -265.62348
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Iteration 4:   log likelihood = -265.51359
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Iteration 44:  log likelihood = -265.51237

Refining estimates:
Iteration 0:   log likelihood = -265.51237

Cox regression -- Breslow method for ties

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### Legend: b/p

- b: testparm i.mob
- p: testparm i.hb_type

#### Command

```
stcox AMA1_AU MSP1_AU MSP2_AU MSP3_AU hbf i.hb_type ///
>                 i.itn_use i.mob i.season##c.ei
```

---

### Stata Output

```
. testparm i.mob
( 1)  10.mob = 0
( 2)  11.mob = 0
( 3)  12.mob = 0

   chi2(  3) =    3.93
   Prob > chi2 =    0.2693

. testparm i.hb_type
( 1)  2.hb_type = 0
( 2)  3.hb_type = 0
( 3)  4.hb_type = 0
Constraint 1 dropped

   chi2(  2) =    4.23
   Prob > chi2 =    0.1205
```

---

```
. stcox AMA1_AU MSP1_AU MSP2_AU MSP3_AU hbf i.hb_type ///
>                 i.itn_use i.mob i.season##c.ei
```

### Failure Code: malar2

```
failure _d:  malar2
analysis time _t:  (datevisit-origin)/28
origin:  time datscren
id:  code
```

---

### Iteration Log

**Iteration 0:** log likelihood = -298.44974
**Iteration 1:** log likelihood = -268.44624
**Iteration 2:** log likelihood = -265.50167
**Iteration 3:** log likelihood = -265.51404
**Iteration 4:** log likelihood = -265.512
**Iteration 5:** log likelihood = -265.39039
**Iteration 6:** log likelihood = -265.39039
**Iteration 7:** log likelihood = -265.39039
**Iteration 8:** log likelihood = -265.3892
**Iteration 9:** log likelihood = -265.3892
**Iteration 10:** log likelihood = -265.3892
**Iteration 11:** log likelihood = -265.3892
**Iteration 12:** log likelihood = -265.3892
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**Iteration 14:** log likelihood = -265.3892
**Iteration 15:** log likelihood = -265.3892
**Iteration 16:** log likelihood = -265.3892
**Iteration 17:** log likelihood = -265.3892
**Iteration 18:** log likelihood = -265.3892
**Iteration 19:** log likelihood = -265.3892
**Iteration 20:** log likelihood = -265.3892
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Iteration 39: log likelihood = -265.3892
Iteration 40: log likelihood = -265.3892
Iteration 41: log likelihood = -265.3892
Iteration 42: log likelihood = -265.3892
Iteration 43: log likelihood = -265.3892
Iteration 44: log likelihood = -265.3892
Refining estimates:
Iteration 0: log likelihood = -265.3892

Cox regression -- Breslow method for ties

No. of subjects = 123
Time at risk = 1874.5

LR chi2(14) = 66.12
Prob > chi2 = 0.0000

Log likelihood = -265.3892

| t   | Haz. Ratio | Std. Err. | z     | P>|z| | 95% Conf. Interval |
|-----|------------|-----------|-------|-----|-------------------|
| AMA1_AU | 1.143541 | .124997   | 1.23  | 0.220       | .9230167 1.416753 |
| MSP1_AU | 1.404919 | .180271  | 2.65  | 0.008       | 1.092522 1.806644 |
| MSP2_AU | .8905581 | .1604702 | -0.64 | 0.520       | .6255813 1.267771 |
| MSP3_AU | 1.197047 | .3896018 | 0.55  | 0.581       | .6325162 2.265432 |
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*test of proportional hazards assumption
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. estat phtest, detail

Test of proportional-hazards assumption

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-----------------------------------------------
Assessing malaria morbidity during the first two years of life and age-specific sero-prevalence of adenovirus type Ad5, Ad35 and AdCh63, potential malaria vectored vaccine candidates in two settings of seasonal malaria transmission.
## CONDUCT OF THE STUDY IN BURKINA FASO

<table>
<thead>
<tr>
<th>Research Institution</th>
<th>Centre National de Recherche et de Formation sur le Paludisme (CNRFP), 01 BP 2208 Ouagadougou 01 Tel : +226 50 32 46 95/96 Fax : +226 50 30 52 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Research Team</td>
<td></td>
</tr>
<tr>
<td>Dr Sirima B. Sodiomon</td>
<td>Principal Investigator</td>
</tr>
<tr>
<td>Dr Ouédraogo Nébié Issa</td>
<td>Co-Principal investigator</td>
</tr>
<tr>
<td>Dr Yaro Jean-Baptiste</td>
<td>Clinical Coordinator (Clinical Team)</td>
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<tr>
<td>Dr Kangoye Tiga David</td>
<td>Sub-investigateur (Clinical Team)</td>
</tr>
<tr>
<td>Dr Coulibaly Espérance</td>
<td>Sub-investigator</td>
</tr>
<tr>
<td>Dr Kaboré Youssouf</td>
<td>Sub-investigator</td>
</tr>
<tr>
<td>Dr Kargougou Désiré</td>
<td>Sub-investigator</td>
</tr>
<tr>
<td>Dr Ouédraogo Alphonse</td>
<td>Sub-investigator</td>
</tr>
<tr>
<td>Dr Tiono Alfred</td>
<td>Sub-investigator</td>
</tr>
<tr>
<td>Dr Konaté Amadou</td>
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</tr>
<tr>
<td>Dr Soulama Issiaka</td>
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</tr>
<tr>
<td>Dr Ouédraogo André Lin</td>
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</tr>
<tr>
<td>Diarra Amidou</td>
<td>Lab Team</td>
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<td>Eye-Nose-Throat</td>
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<td>Expanded Program of Immunization</td>
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<tr>
<td>RDT</td>
<td>Rapid Diagnostic Test (for Malaria)</td>
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<td>Université Cheikh Anta Diop</td>
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<td>WBC</td>
<td>White Blood Cells</td>
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<tr>
<td>β-HCG</td>
<td>Beta-Human Chorionic Gonadotrophin</td>
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<td>To measure the time to the first malaria infection from birth to two years of age</td>
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<td>To establish a relationship between antibody level and time to the first malaria infection</td>
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<td>To assess the impact of maternal antibodies (and haemoglobin F) on morbidity in the first two years of life.</td>
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<td>To detect levels of antibodies which protect against Plasmodium falciparum infection</td>
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<td>To assess the prevalence of plasmodial infection in study volunteers from 6 months to 45 years</td>
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<td>To assess <em>P. falciparum</em> parasites load (asexual and sexual forms) in study volunteers from 6 months to 45 years.</td>
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<td><strong>Study Design</strong></td>
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<td>Assessment of malaria clinical incidence in infants: cohort study with and active longitudinal survey made of scheduled home visits, systematic monthly blood smear collection until a positive smear is obtained, scheduled blood sampling for immunological assessments once a month for the first six months and every 3 months for the remaining of the study duration, and a passive follow-up.</td>
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<td>Prematurity</td>
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<td>Anaemia (Hb ≤ 8 g/dL)</td>
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<td>Any congenital abnormality (cardio-vascular, hepatic and renal) suspected by the physician to cause any supplementary risk to the infants</td>
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<td>Any other circumstances and condition suspected by the physician to be a risk for the infant health</td>
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- Age between 4 to 6 weeks
- Written informed consent obtained from the parents/guardian prior to any study enrolment
- Available and willing to participate in the longitudinal follow up

2. Assessment of age-specific sero-prevalence of adenovirus type Ad5, Ad35, AdCh63:

- Males or females aged six months to 45 years.
- Resident of the study areas in Burkina and Senegal at the start of the study, and intend to remain a resident for the duration of the study.
- Willingness to participate in the study as evidenced by the completed informed consent document.
2. Assessment of age-specific sero-prevalence of adenovirus type Ad5, Ad35, AdCh63:

Clinical evidence or suspected acute or chronic disease (respiratory, cardiovascular, gastrointestinal, hepatic, genitourinary or lymphatic system), or any other findings that in the opinion of the examining physician may impact the safety of individual at the assessment of the laboratory parameters that are under study.

Acute allergy episode.

History of splenectomy.

All individuals on prescription drugs will be excluded.

History of the administration of any blood products within the three months preceding the study.

Pregnancy (either assessed clinically or by means of a positive urine β-hCG test) or breast-feeding.

Women with history of gynaecological disease (fibromyoma).

Recent (within 4 weeks) hospitalisation.

Recent (within 2 weeks) of blood donation.

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<th>Assessment of malaria clinical incidence in infants: 2 ans.</th>
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<td>Assessment of age-specific sero-prevalence of adenovirus type Ad5, Ad35, AdCh63: the day of the survey.</td>
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**Primary Endpoint**

Incidence of clinical malaria due to *P. falciparum*

Seroprevalence of adenovirus type Ad5, Ad35 and AdCh63.

**Secondary Endpoints**

First malaria infection in infants

First malaria episode in infants

Clearance of maternal antibodies in infants
| Assessment of neutralizing antibodies (Adenovirus) |
1. BACKGROUND

Malaria remains one of the major health problems in sub-Saharan Africa and *Plasmodium falciparum* causes much more severe and progressive illness than any of the other species of malaria parasites. Children living in endemic areas bear the major burden of the disease as well as the resultant mortality. A relative insusceptibility to malaria of young infants has been observed (Garnham, P.C.C.; 1949), and the most important factor in modifying the clinical symptoms has been ascribed to the passive transfer of maternal anti-malarial IgG antibodies (Bruce-Chwatt, L. J.; 1952; Sehgal, V.M., 1989). Previous studies have provided evidence for associations, at a population level, between decreasing levels of maternally derived malaria-specific IgG and increasing risk of clinical malaria (Hogh, Marbiah et al. 1995, Branch, Udhayakumar et al. 1998). These maternal antibodies against pathogen-specific antigens disappear during the first months of life while the neonate’s own immune system develops. The interval between the loss of protection once maternal antibodies have disappeared and possible protection by vaccination should be as narrow as possible for all vaccine-preventable diseases, because of the risk of early infection. Vaccination at too young age could waste too much vaccine on children with maternally derived protection that could potentially lower vaccine efficacy. Studies using mice have shown that the progeny of immune mothers respond poorly to active immunization, and it was concluded that maternal antibodies interfered with both priming and helper T-cell function (Harte PG et al., 1982), and neonatal T-cell tolerance was induced by peptides causing clonal inactivation in mice (Gammon G et al., 1986). In other studies, active immunization with radiation-attenuated of human and monkey malaria parasite have been shown to induce sterile immunity against live sporozoite challenge (Nussenzweig et al Ad. Imm. 1989). However contrasting observations have been made in rats i.e. enhanced responsiveness to vaccination in offspring of *P. berghei*-infected female rats was demonstrated (Desowitz RS et al, 1971). The immune responsiveness to malaria vaccination in infants in areas where malaria is endemic therefore needs careful consideration.

We propose a cohort study involving a longitudinal follow up of infants from birth to two years of age to characterize the dynamics of maternal antibodies, asymptomatic infection and clinical episodes in infants from 0 to 2 years in hyperendemic areas of Burkina Faso and Senegal during a longitudinal follow up of two years. Data from the study will help us understand the naturally acquired immunity to malaria, estimate sample size for the efficacy trial, in an evidence-based choice of the appropriate immunization schedule for the malaria vaccine candidate and capacity strengthening of these two institution in conducting cohort studies.
Adenovirus serotypes vector based vaccines have been proven to be immunogenic and are been used in clinical trials. This approach has some limitations because in malaria endemic countries the majority of people have some pre-existing immunity to the serotypes that have been used as vectors (Thorner, Vogels et al. 2006). However levels of antibodies to chimpanzee viruses are very low in humans in Africa and elsewhere and we have confirmed this recently for the AdCh63 strain in Kenyan children from Kilifi. As future clinical trials may need to use these vectors, we plan also to adequately assess Ad5, Ad35 (the vector for a Crucell candidate malaria vaccine) and AdCh63 serotypes age specific prevalence in our investigational sites.

2. OBJECTIVES

2.1. Primary objective

- To assess the incidence of clinical malaria in children from birth to two years of age
- To determine the age-specific seroprevalence of adenovirus types Ad5, Ad35 and AdCh63

2.2. Secondary objectives

- To measure the antibody responses (IgG, IgM and IgG1 to 4) to malaria specific antigens in infants from birth to two years over a period of two years
- To measure the time to the first malaria infection from birth to two years of age
- To measure the time to the first clinical malaria episode from birth to two years of age
- To establish a relationship between antibody level and time to the first malaria infection
- To establish a relationship between antibody level and time to first clinical malaria episode
- To assess the impact of maternal antibodies (and haemoglobin F) on morbidity in the first two years of life.
- To detect levels of antibodies which protect against \textit{Plasmodium falciparum} infection

2.3. Tertiary objectives

- To assess the prevalence of plasmodial infection in study volunteers from 6 months to 45 years
- To assess \textit{P. falciparum} parasites load (asexual and sexual forms) in study volunteers from 6 months to 45 years

3. METHODOLOGY

3.1. Study area
3.1.1. Burkina Faso

In Burkina Faso the study will be conducted in the southern part of the country in the Province of Comoé located 441 km from Ouagadougou, the capital of Burkina Faso. The surface area of the province is 15871 km² and the total population is 277 384 inhabitants and 17% represent children between 0 and 4 years. The entire province is covered by the health district of Banfora and has 24 community clinics named Centre de Santé et de Promotion Sociale (CSPS) and the Banfora district hospital is the first level of referral for the community clinics. These health centers provide basic health care services such as immunization of children, antenatal surveillance, delivery services and family planning.

The study area belongs to the Sudan-Guinea zone with more than 900mm of rain a year and cooler average temperatures. The malaria transmission is markedly seasonal; most transmission occurs during the rainy season from May to November and low during the dry season from December to April. *P. falciparum* is the predominant species accounting for more than 95% of infections in children under five years of age.

3.1.2. Senegal

In Senegal, the survey will be carried out in the health post of Lamarame, located in the health district of Ndoffane. Ndoffane is located 17 km to the south of Kaolack. The relief is generally flat with depressions in this zone. The climate is Sudano-Sahelian type and daytime temperatures between 24°C and 40°C;. Average rainfall is about 600 mm per year. There are approximately 200 villages in the district of Ndoffane with a total population of nearly 75,000 inhabitants of which about 7% are between 0- to 24 months .Malaria is the most important public health problem, accounting for 45% of general morbidity. The most recent entomological surveys showed an entomological inoculation rate of between 9 and 12 infective bites per night during the period of high transmission.

3.2. Study population

This study will involve infants for the assessment of clinical malaria incidence and participants above six months for the assessment of age-specific seroprevalence of adenovirus type Ad5, Ad35 and AdCh63.

3.2.1. For the assessment of clinical malaria incidence

The study will enrol infants from parents living in the study areas in Burkina and Senegal. Study staff will daily visit the maternity ward to identify potential participants. Appointment will be made
with parents interested to participate and they will be visited at home for the informed consent discussion. Infants from parents who have consented will be included in the study cohort.

Due to the routine registration in the study areas, numerous background factors will be covered for the children living in the study areas, including maternal factors (age, parity, spacing, survival of previous children, maternal education); health related factors (breastfeeding, mid-arm circumference, vaccination status, reported infections, previous hospitalizations) and cultural and socio economic conditions (ethnic group, family structure, family size, housing) (See in appendix A).

Existing breeding sites will be also reported and their distance to the households.

Eligibility and exclusion criteria

Inclusion criteria will be the following:

- Age between 4 to 6 weeks
- Written informed consent obtained from the parents/guardian prior to any study enrolment
- Available and willing to participate in the longitudinal follow up

The following exclusion criteria will be checked at the time of study entry; if any apply, the subject must not be included in the study.

- Documented history clinical malaria
- Malaria infection or documented history of malaria infection
- Prematurity
- Anemia (Hb ≤ 8 g/dL)
- Any confirmed or suspected condition of immunosuppressive diseases including HIV (no screening test will be done for this purpose) by the physician
- Any congenital abnormality (cardio-vascular, hepatic and renal) suspected by the physician to cause any supplementary risk to the infants
- Any other circumstances and condition suspected by the physician to be a risk for the infant health

3.2.2. For the assessment of the age-specific seroprevalence of adenovirus type Ad5, Ad35 and AdCh63

The study population will be drawn from the population above six months who are permanent residents of Banfora health district in Burkina Faso, the health district of Ndoffane in Senegal. People
are mostly subsistence farmers growing millet as well as domestic animals (poultry, cattle etc.). Houses are typically made of mud walls and grass or corrugated iron roofs.

Eligibility and exclusion criteria

Inclusion criteria will be the followings:

- Males or females aged six months to 45 years.
- Resident of the study areas in Burkina and Senegal at the start of the study, and intend to remain a resident for the duration of the study.
- Willingness to participate in the study as evidenced by the completed informed consent document.

The following exclusion criteria will be checked at the time of study entry; if any apply, the subject must not be included in the study.

- Clinical evidence or suspected acute or chronic disease (respiratory, cardiovascular, gastrointestinal, hepatic, genitourinary or lymphatic system), or any other findings that in the opinion of the examining physician may impact the safety of individual at the assessment of the laboratory parameters that are under study.
- Acute allergy episode.
- History of splenectomy.
- All individuals on prescription drugs will be excluded.
- History of the administration of any blood products within the three months preceding the study.
- Pregnancy (either assessed clinically or by means of a positive urine β-hCG test) or breastfeeding.
- Women with history of gynecological disease (fibromyoma).
- Recent (within 4 weeks) hospitalisation.
- Recent (within 2 weeks) of blood donation.

3.3. Study design

3.3.1. Malaria clinical incidence in infants

For the assessment of the clinical malaria incidence, we will carry out a longitudinal survey (See CRF in appendix B). Infants whose mothers consent to participate in the study will be actively followed-up throughout their first two years of life. This will involve bi weekly home visits to be
executed by a study nurse. During each home visit, the health status of the child will be checked. If history of fever within the last 24 hours or documented fever is observed ($T \geq 37.5^\circ C$) blood sample will be obtained through finger prick to prepare a blood smear. A malaria rapid diagnostic test will be done for febrile infants. The study nurses will be provided with the essential drugs for the management of mild disease. Any other serious illness will be referred to the nearest local health staff or to the District Hospital to receive adequate treatment and follow-up as clinically appropriate. The treatment and transportation costs will be met by the study. Between the two scheduled field-worker visits, parents of children will be encouraged to report to the nearest community clinic or hospital at any time should their child feels sick (See CRF in appendix C).

To assess the time to the first malaria infection, a blood smear will be systematically obtained every month from all the infants starting from the inclusion day (See appendix D). This systematic monthly blood smear collection will end for a child when a positive smear is obtained during any visit.

For immunological assessments 500µl of blood sample will be taken during the scheduled home visits. The sample collection schedule will be the following: once a month, during the first six (6) months of life and every three (3) months for the remaining study duration.

Any infant who develops malaria during the follow up period will receive appropriate treatment according to local standard. Treatment cost will be supported by the study.

3.3.2. Age specific seroprevalence of Ad5, Ad35 and AdCh63.

The volunteers will come from randomly selected villages of the study areas and the assessment of age specific seroprevalence will be done twice during the malaria high transmission season and during the malaria low transmission season. Participants from the first survey may be included in the second survey. All individuals in the sampled villages will be grouped into pre-specified age groupings and sex. All persons in a village will qualify to participate if they satisfy the inclusion criteria. As such, all healthy persons in a selected village will be invited to participate in the study. During each survey, each study participant will undergo clinical examination if he fulfils the study inclusion criteria, 5ml blood will be taken for thick/thin blood film preparation for malaria diagnosis purpose and the remaining blood will be centrifuged and the plasma collected will be used for the adenovirus serotyping. Malaria symptomatic participant will be treated following national guidelines and those presenting others symptoms will be referred to the nearest local health staff or to the District Hospital to receive adequate treatment and follow-up as clinically appropriate. CRP is appended in appendix E.
4. SAMPLE SIZE CONSIDERATION

4.1. Malaria clinical incidence in infants

Assuming an incidence of one or more episodes of febrile malaria of 20% during one year of follow up, with a sample size of 140 infants including 10% lost of follow up this incidence will be known within a 95% confidence interval of 13-27%.

4.2. Age specific seroprevalence of Ad5, Ad35 and AdCh63.

Compared to Ad5, Ad35 is reported to be less prevalent in sub-Saharan Africans (Thorner, Vogels et al. 2006). Our sample size will be then calculated using the lowest age specific seroprevalence. Assuming age specific Ad35 seroprevalence rates of 1% in the [six (6) months- three (3) years], 3% in children aged [3years-10 years] and 10% in individuals above 10 years, then with a sample size of 200 volunteers in each age group, the 95% confidence intervals will be 0.1-3.6%, 1.1-6.4% and 6.2-15.0% respectively.

5. LABORATORY PROCEDURES

Capillary blood will be obtained by finger prick and collected in microtubes and on slides. Thick and thin films will be stained with Giemsa stain. Parasite density will be scored as the number of parasites per 200 white blood cells (WBC) and converted to parasites per microliter based on an average WBC count of 8000/µl of normal whole blood. Slides will be classified negative only after one hundred fields (Approximately 2000 WBC) have been counted.

At the central laboratory, the plasma will be separated and stored in EDTA tubes at -20°C until analysis. The antibody measurement will be done by ELISA. The level of IgG, IgM and IgG subclasses will be determined using a standard curve.

Ad5, Ad35 and AdCh63 serological assays will be run at Oxford University.

6. STATISTICAL ANALYSES

The clinical malaria incidence will be calculated as the number of episodes divided by the number of children per time at risk. The times to the first infection and to the first clinical malaria episode will also be determined, and survival plots presented. The effect of maternal antibody will be determined by proportional hazard models. Time-dependent covariates will be used to account for age in months, and the effect of waning antibody titres. We will also consider multiple events, using
variations on the Cox-proportional hazard model to enter individuals more than once (adjusting p values and confidence intervals by the Robust Sandwich Estimator), and by Poisson regression where events are clustered by individual only. The former method has the advantage of allowing for time-dependent covariates, which is essential when the effects of waning maternal antibody are being considered.

Age specific seroprevalence rates for Ad5, AdCh63 and Ad35 will be determined as proportions. 95%CI will be computed using test based methods.

7. EVALUATION AND QUALITY CONTROL

Parasitaemia and anaemia at different time points: Haemoglobin measurements using a Hemocue machine, and finger prick sampling for thick smears for parasite density determination, will be done at pre-defined time points (once a month, during the first six (6) months of life and every three (3) months for the remaining study duration) and at the end of the longitudinal survey (at 24 months of age). A slide will be declared parasite negative only after 200 high power fields have been examined. A second independent laboratory technician will reread all collected slides. If parasite densities recorded by the two readers differ by more than 30%, a third senior laboratory technician will be asked to adjudicate. Anemia will be defined by haemoglobin level of less than 8 g/dL.

Clinical episodes: Impact on clinical malaria will be determined through active and passive surveillance, used to estimate the proportion of fever cases that have malaria, and by determining the prevalence of clinical malaria in cross-sectional surveys at different time points.

8. DATA MANAGEMENT

Data collection will done using CRF in hard copy. Collected data will be double entered, checked and validated. In stream data cleaning process will be implemented. A statistical analysis plan will be written before the data base is released to the statistician for analysis.

9. INFORMED CONSENT

The protocol, the informed consent form and any subsequent modifications will be reviewed and approved by the Institutional committee for bioethics of CNRFP and UCAD local Independent Ethical Committee responsible for oversight of the study. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject representative, and acceptance or refusal of the copy provided will be documented in the subject’s record.
Informed consent will be obtained via oral presentation in local language, accompanied by a short-form written consent document (stating the necessary elements and a written summary of what is presented orally) in French. An impartial witness (A relative, friend, or social worker not affiliated with the study) to the oral presentation will be required. The witness must be fluent in French and in the local language spoken by the subject. The subject will be offered copies of the short-form document and the summary. Acceptance or refusal of the copies provided will be documented. All subjects will be asked to answer a standardized set of questions to show that they fully understand the purpose and procedures of the study. If the subjects cannot answer the questions correctly the pertinent information will be reviewed again and if they fail to answer questions correctly the second time, they will not be enrolled. Information sheets for volunteers and informed consent forms are in Appendix F and G.

10. THE STUDY IMPORTANT MILESTONES

The following milestones will be tracked during the course of the study to inform all the partners involved about the study status and the progress made:

- The first infant enrolled
- The last infant enrolled
- The first infant first malaria infection
- The first infant first malaria episode
- The first infant last home visit
- The last infant last home visit
- The database release
- The study draft report release
- The study final report publication

11. INITIAL CENSUS AT BASELINE AND DEMOGRAPHIC SURVEILLANCE SYSTEM

Planning and delivering health interventions to specific population group is necessarily based on thorough knowledge of the target group. Human populations are not stable as there are births, deaths, out and in migrations. Therefore in our settings where there is no reliable system for the registration of vitals events, the Demographic Surveillance Systems (DSSs) are important to monitor all these events in order to provide the whole picture of the population under study at any point in time. A demographic surveillance system will be established in the different field sites where there is no DSS (in Burkina Faso and Senegal).
11.1. Burkinabe investigational site, Banfora

The setting up the Banfora DSS in South-Western Burkina Faso which covers the Banfora health district is ongoing.

During the first quarter of the year 2009, the entire Banfora Health District has been mapped by a specialist in GIS (See map below). The key points in each village (compounds community clinics, schools, village chief compound etc.) were geographically referenced.

Due to the resource constraints in May 2009, the general census was carried out in six (6) villages of four (4) community clinics catchments areas surrounding the Regional Hospital of Banfora where vaccines will be administrated and the laboratory analysis performed. During this general survey the following data on study population have been collected:

- The compounds were numbered.
- Demographic status (age, sex and matrimonial status).
- Composition of the household (household head, links between the head and the others members, etc.).

A total of 1046 children aged 0 to 59 months representing an average 19.5% of the total population, have been enumerated in these six (6) villages. Every quarter, the DSS team will update the database by visiting each village and compound in order to record the demographic and vital events (birth, death, marriage, in and out migrations etc.). To prepare for the phase IIb clinical trial of viral vectored candidate malaria vaccines we are planning to add 10 more villages as soon as resources are made available. To date this embryonic Banfora DSS is managed by a team composed by five (5) field workers in charge of the data collection and two (2) supervisors. The CNRFP GIS specialist, two (2) data manager and 10 Data clerks are also dedicating some time for the DSS data entry and management.

A database prepared on the Access software is planned to be updated quarterly. If an error is noticed at the community level or from the data base, the field workers will check and send the right information back to the computer centre (data base).
11.2. Senegalese investigational site, Keur Socé

Keur Socé is in central Senegal, with a Soudano-Saharan climate, characterized by a yearly average of 600 mm rainfall (mainly between July and September). The rural community of Keur Socé is populated by 20,415 inhabitants divided into 71 villages. The population is very young and under 15 yrs old group is estimated at 10,738 (52.6%). As it is at the national level, approximately 20% of this population are children under 5 years.

The vision of the Department of Parasitology Mycology is to upgrade the monitoring of the population through a DSS. It is also our intention to build suitable centers for carrying out clinical trials in the best conditions respecting GCP and GLP guidelines.

The Malaria Vectored Vaccine Consortium (MVVC) will give the site an opportunity to start this population surveillance.

The first step is the mapping of the site using experienced field technicians. This shouldn’t exceed 24 working days. We will follow this up with the baseline census enumeration using well trained
enumerators. The census is designed for completion within six (6) weeks. Finally a quarterly demographic surveillance will be performed (four (4) rounds per year).

12. COMPLIANCE WITH NATIONAL AND INTERNATIONAL STANDARDS OR RESEARCH

No potential ethical issue is anticipated and this study is considered as minimal risk to the participants. All the blood sampling will be performed after appropriate disinfection using sterile materials. For the FACS studies, consent for the sampling will be taken under the umbrella of ongoing immunologic studies in Kilifi. Ethical approval is provided as appendix 8a.

13. POTENTIAL IMPACT

Beyond the scientific achievements expected, the project will provide the opportunity for trial staff to prepare for future clinical trials.

In the three sites, the research staff is made up of young scientists, trained as MDs and PharmD’s with some staff receiving MSc training. Some of the staff has participated in previous baseline epidemiological studies. The investigational sites currently lack support staff (field workers, nurses). The clinical laboratory at the field station also needs to be set up.

The project will contribute to strengthening the capacity of the staff in cohort studies. These competencies will be needed when efficacy trials commence as this will require the longitudinal follow up of the vaccinated volunteers. The study will also allow recruiting and training of support staff (field workers, nurses) who will be familiarized with the SOPs on home visits. Basic laboratory equipment such as microscopes, Coulter counter, spectrophotometer will be acquired for the field laboratories. Beyond their use in the current proposal, these equipments will be used for the setup of laboratory normal values for future clinical trials in the target population.

Training the staff and equipping the field stations will significantly improve skills and quality of the work; direct consequence will be the ability of the teams to obtain more grants from other funding agencies; this will help in maintaining the sites when this epidemiological study ends.

Networking with other research institutions will also increase the ability of the team in resource mobilization.

14. PROJECT MANAGEMENT

CNRFP
Dr Sodiomon Sirima of CNRFP will function as the work package leader for the capacity building work package. As WP leader he will be responsible for formulating the detailed work plan of activity and proposing allocation of tasks and resources, identifying and discussing potential difficulties and barriers associated with achieving the objectives, highlighting and presenting any difficult issues that need the attention of the PC and PSC as well as technical and administrative reporting of the progress of the work plan to the PC. He will also be responsible for coordinating the development of a multi-site protocol for the conduct of the baseline study.

Université Cheikh Anta Diop

Dr Badara Cisse of the University of Dakar will participate in the development of the multi-site protocol for this baseline study. Prior to the conduct of the baseline study, the greatest challenge of the Senegalese site will be to build up a reliable DSS. In this regard, there will be close collaboration with an experienced demographer (Dr Momodou Jasseh) based at the site in The Gambia and the French Institut de Recherche pour le Développement (IRD). Discussions on the modalities for this collaboration are on-going with Dr Kalifa Bojang and Dr Cheikh Sokhna.

15. THE INVOLVEMENT OF THE PRIVATE SECTOR

Many of the collaborators in this proposal have considerable experience in capacity building. The Oxford and Kilifi partners are funded by the Wellcome Trust which has made major investments in African capacity building in several countries including Kenya, South Africa and Malawi. The KEMRI-Wellcome programme at Kilifi is well recognized for supporting the development of young African scientists and will continue to do so in the key area of malaria vaccine development though this proposal. Kilifi has the most recent experience of phase I and II trials of vectored vaccines for malaria in Africa and will play an active role in supporting optimized trial design and immunomonitoring. All northern partners in the proposal have strong records and competencies in capacity building and will collaborate in this goal with the southern partners.

REFERENCES


### ANNEXE A

**Etude épidémiologique de base MVVC : Morbidité palustre au cours des deux premières années de vie et cinétique des anticorps maternels, dans deux zones à transmission saisonnière.**

**FORMULAIRE DE SELECTION**

| Date de visite | __ | __ | __ | __ | __ | __ | __ | __ | __ |

#### 1. DEMOGRAPHIE

| Numéro de Screening | __ | __ | __ | __ |
| N°DSS du participant (si applicable) | __ | __ | __ | __ | __ | __ | __ | __ | __ | __ | __ | __ | __ | __ | __ |
| Nom de l’enfant | ______________________________________________________ |

| Date de naissance | __ | __ | __ | __ | __ | __ | __ | __ | __ | __ | __ | __ | __ | __ | __ | __ |
| Nom | Prénom(s) |

| Sexe | 1 = Masculin, 2 = Féminin |
| Nom de la mère | ______________________________________________________ |

| Age de la mère | __ | __ | __ | __ |
| Nombre de grossesse | __ | __ |
| Parité | __ |
| Nombre d’enfants vivants | __ |
| Niveau d’éducation de la mère | __ |
| Village: | __ | __ |
| N°DSS de la concession | __ | __ | __ | __ | __ |
| Nom du chef de ménage | ______________________________________________________ |

| Groupe ethnique | 1 = Gouin, 2 = Turka, 3 = Karaboro, 9 = Autres (Spécifier ___________ ) |
| Nom | Prénom(s) |

#### 2. ANTECEDANTS

| Accouchement à terme | 1 = Oui, 2 = Non, Si non Préciser : …………………. |
| Accouchement unique | 1 = Oui, 2 = Non, Si non Préciser : …………………. |
| Voie d’accouchement | 1 = Basse, 2 = Césarienne |
| Réanimation à la naissance | 1 = Oui, 2 = Non |
| Infection néonatale | 1 = Oui, 2 = Non |

| Statut vaccinal (est à jour selon son âge): | 1 = Oui, 2 = Non |
| Mode d’allaitement | 1 = lait maternel, 2 = lait artificiel |

L’enfant dort-il sous une moustiquaire depuis sa naissance? | 1 = Oui, 2 = Non |

Le nombre de TPI/SP reçu par la maman durant la grossesse: | __ |

Autres (préciser): ………………………………………………………………………………………………………………………………

#### 3. SIGNES VITAUX

---

7.4 Appendix 4: study case report forms
Température axillaire [____] [____]°C
L’enfant est-il fébrile (Température axillaire ≥ 37.5°C)? [____] 1=Oui 2=Non.
Poids [____] [____] kg
Taille [____] [____] cm
FC : [____] /mn
FR : [____] /mn
Périmètre brachial : [____] [____] cm

4. HISTOIRE MEDICALE ET EXAMEN PHYSIQUE

<table>
<thead>
<tr>
<th>HISTOIRE MEDICALE</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1=Oui 2=Non)</td>
</tr>
<tr>
<td>Traitement antipaludique depuis la naissance [<strong><strong>] ➜ si oui, prière spécifier : [</strong></strong>_________________________________]</td>
</tr>
<tr>
<td>Histoire de fièvre (corps chaud) dans les 24 heures précédentes [____]</td>
</tr>
<tr>
<td>Vomissement [<strong><strong>] ; si oui, fréquence [</strong></strong>] /24h</td>
</tr>
<tr>
<td>Diarrhée [<strong><strong>] ; si oui, fréquence [</strong></strong>] /24h</td>
</tr>
<tr>
<td>Constipation [____]</td>
</tr>
<tr>
<td>Toux [____]</td>
</tr>
<tr>
<td>Autres [____] si oui, préciser : ………….</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EXAMEN PHYSIQUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Examen des appareils et systèmes [____] 1=Normal / 2=Anormal</td>
</tr>
<tr>
<td>(Si 2, noter les anomalies)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Etat général</th>
</tr>
</thead>
<tbody>
<tr>
<td>[____] [__________________________]</td>
</tr>
<tr>
<td>Extrémité céphalique</td>
</tr>
<tr>
<td>[____] [__________________________]</td>
</tr>
<tr>
<td>Appareil respiratoire</td>
</tr>
<tr>
<td>[____] [__________________________]</td>
</tr>
<tr>
<td>Appareil cardio-vasculaire</td>
</tr>
<tr>
<td>[____] [__________________________]</td>
</tr>
<tr>
<td>Système nerveux</td>
</tr>
<tr>
<td>[____] [__________________________]</td>
</tr>
<tr>
<td>Appareil digestif</td>
</tr>
<tr>
<td>[____] [__________________________]</td>
</tr>
<tr>
<td>Peau et phanères</td>
</tr>
<tr>
<td>[____] [__________________________]</td>
</tr>
<tr>
<td>Appareil locomoteur</td>
</tr>
<tr>
<td>[____] [__________________________]</td>
</tr>
<tr>
<td>Autres anomalies:</td>
</tr>
<tr>
<td>[____] [__________________________]</td>
</tr>
</tbody>
</table>

Section complétée par: Initiales du clinicien [____] [____] [____] Date : [____] / [____] / [____] [____] [____]

5. EXAMENS BIOLOGIQUES DE BASE

| Le taux d’Hb a-t-il été mesuré? [____] 1=Oui 2=Non |
| Si Oui, noter le résultat: Hb: [____] [____] g/dl |
| *Un TDR a-t-il été réalisé: [____] 1=Oui, 2=Non, 3=Non disponible |
| Si Oui, noter le résultat: [____] 1=Positif, 2=Négatif, 3=Invalide, 4=Non disponible |
| *Le TDR sera fait uniquement si le l’enfant est fébrile (Température axillaire ≥ 37.5°C) ou présente une histoire de fièvre dans les 24 heures précédentes. Le TDR est toujours associé à une goutte épaisse. |

| Une goutte épaisse a-t-elle été réalisée [____] 1=Oui 2=Non |
| Si oui noter le résultat : |

<table>
<thead>
<tr>
<th>P.falciparum</th>
<th>P.malariae</th>
<th>P.ovale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trophozoïtes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schizontes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

316
Un échantillon de sang a-t-il été prélevé pour le dosage des AC maternels?
[ ] 1=Oui 2=Non
Un échantillon de sang a-t-il été prélevé pour le dosage de l’hémoglobine fœtale ?
[ ] 1=Oui 2=Non ;
Si oui, résultat : HbF: [ ____ ] %
Section complétée par: Initiales du technicien de laboratoire [ _____ ]
Date : [ _____ ] / [ _____ ] / [ _____ ]

6. ELIGIBILITE DU PARTICIPANT

Critères d’inclusion

Cocher tous les critères d’inclusion remplis par le participant
☐ âge entre 4 et 6 semaines
☐ consentement éclairé par écrit obtenu des parents/tuteurs avant tout enrôlement dans l’étude
☐ disponibilité et désir de participer au suivi longitudinal

Critères d’exclusion

Cocher tous les critères d’exclusion s’appliquant au participant
☐ Histoire d’épisode clinique de paludisme documentée
☐ Infécction paludique ou histoire d’infection paludique documentée
☐ Prématurité
☐ Anémie (Hb≤ 8g/dL)
☐ Toutes formes de maladies immunosuppressives dont le VIH (il n’y aura pas de test de dépistage à cet effet) confirmées ou suspectées par le médecin
☐ Toute anomalité congénitale (cardio-vasculaire, hépatique et rénale) qui, selon le médecin, pourrait créer un risque supplémentaire pour les enfants
☐ Toutes autres circonstances et conditions qui, selon le médecin, pourraient constituer un risque pour la santé de l’enfant en bas âge

Le participant remplit-il tous les critères d’inclusion et aucun critère d’exclusion?
[ ] 1=Oui 2=Non
Si Non, donner la(les) raison(s):
1. ___________________________ 2. ___________________________
3. ___________________________ 4. ___________________________
Si Oui attribuer un numéro d’étude : [ _____ ]
L’enfant a-t-il été référé [ ____ ] 1=Oui 2=Non

DECLARATION DE L’INVESTIGATEUR SUR LA VERIFICATION DES DONNEES DE L’ENQUETE

J’ai vérifié l’exactitude, la cohérence et la complétude des données de toutes les pages de ce formulaire d’enquête de base. Pour autant que je sache, ces données sont complètes et correctes.
Toutes les informations collectées dans ce formulaire l’ont été soit par moi ou par une personne sous ma supervision.

Date: ____________________________

Jour   Mois   Année   Initiales et Signature de l’investigateur
**ANNEXE B**

**Etude épidémiologique de base MVVC : Morbidité palustre au cours des deux premières années de vie et cinétique des anticorps maternels, dans deux zones à transmission saisonnière.**

**FORMULAIRE DE SUIVI ACTIF**

Date de visite | | | | / | | | |

Code du participant : | | | |

1. L’enfant a-t-il été vu ?

|   | 1=Oui 2=Non

2. **HISTOIRE MEDICALE**

L’enfant s’est-il rendu au CSPS depuis la dernière visite ? | | 1=Oui 2=Non
L’enfant a-t-il reçu un traitement depuis la dernière visite ? | | 1=Oui 2=Non
Si Oui indiquer les traitements reçus :
Traitements antipaludiques : ……………………………………………. Autres traitements : ……………………………………………………………

Histoire de fièvre dans les dernières 24 heures ? | | 1=Oui 2=Non

3. **SIGNES/SYMPTOMES**

Température axillaire | | | | °C
L’enfant est-il fébrile (Température axillaire > 37.5°C) ? | | 1=Oui 2=Non
Vomissement | | si oui, fréquence | | /24h
Diarrhée | | si oui, fréquence | | /24h
Constipation | |
Toux |
Autres | si oui, préciser : ……………………………………………………………

4. **EXAMENS BIOLOGIQUES**

*Un TDR a-t-il été réalisé? | | 1=Oui 2=Non 3=Non disponible.
Si Oui, noter le résultat du TDR | | 1=positif 2=négatif, 3=invalide, 4=Non disponible
*Le TDR sera fait uniquement si le l’enfant est fébrile (Température axillaire > 37.5°C) ou présente une histoire de fièvre dans les 24 heures précédentes. Le TDR est toujours associé à une goutte épaissie.

Une goutte épaissie a-t-elle été réalisée | | 1=Oui 2=Non 3=Non disponible
Si oui noter le résultat :

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
</table>
P.falciparum P.malariae P.ovale |
| Trophozoïtes | | |
| Schizontes | | |
| Gamètocytes | | |
5. TRAITEMENT

L’enfant a-t-il été traité par l’enquêteur ? [ ] 1=Oui 2=Non
Si oui, compléter le tableau ci-dessous :

<table>
<thead>
<tr>
<th>MEDICAMENT</th>
<th>1=Oui</th>
<th>Si oui, indiquer le traitement</th>
<th>Posologie</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antipaludique</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antipyrétique</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

L’enfant a-t-il été référé au CSPS ? [ ] 1=Oui 2=Non
Si oui préciser Le motif : ………………………………………………………………………
…………………………………………………………………………………………………..

Formulaire complété par: Initiales de l’enquêteur : [ ] [ ] [ ]
Date:[____]/[____]/[____]

DECLARATION DE L’INVESTIGATEUR SUR LA VERIFICATION DES DONNEES DE L’ENQUETE

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Date: [____]/[____]/[____] Initiales et Signature de l’investigateur
# ANNEXE C

**Etude épidémiologique de base MVVC** :
Morbidité palustre au cours des deux premières années de vie et cinétique des anticorps maternels, dans deux zones à transmission saisonnière.

## FORMULAIRE DE SUIVI PASSIF

<table>
<thead>
<tr>
<th>Date de visite</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Code du participant</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## 1. MOTIFS DE CONSULTATION

L’enfant a-t-il été référé au CSPS par l’enquêteur? 1=Oui 2=Non

<table>
<thead>
<tr>
<th>Symptôme</th>
<th>Présent</th>
<th>Durée / Fréquence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histoire de fièvre de 24H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhée</td>
<td></td>
<td>Fréquence dans les dernières</td>
</tr>
<tr>
<td>Vomissement</td>
<td></td>
<td>Fréquence dans les dernières</td>
</tr>
<tr>
<td>Toux</td>
<td></td>
<td>Durée en jours</td>
</tr>
<tr>
<td>Ecoulement nasal</td>
<td></td>
<td>Durée en jours</td>
</tr>
<tr>
<td>Difficultés respiratoires</td>
<td></td>
<td>Durée en jours</td>
</tr>
<tr>
<td>Convulsions</td>
<td></td>
<td>Fréquence dans les dernières</td>
</tr>
<tr>
<td>Perte de connaissance</td>
<td></td>
<td>Durée en jours</td>
</tr>
<tr>
<td>Insomnie inhabituelle</td>
<td></td>
<td>Durée en jours</td>
</tr>
<tr>
<td>Perte d’appétit</td>
<td></td>
<td>Durée en jours</td>
</tr>
<tr>
<td><strong>Autres (préciser)</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## 2. SIGNES VITAUX

Histoire de fièvre de 24 heures 1=Oui 2=Non.
Température axillaire |   | | °C
L’enfant est-il fébrile (Température axillaire ≥ 37.5°C)? 1=Oui 2=Non.
Fréquence cardiaque : |   | btm/ min
Fréquence respiratoire : |   | cycles/min
Poids : |   | kg
Taille : |   | cm

## 3. EXAMEN PHYSIQUE

**Examen des appareils et systèmes** 1=Normal / 2=Anormal
(Si 2, noter les anomalies)

<table>
<thead>
<tr>
<th>Etat général</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Extrémité céphalique</td>
<td></td>
</tr>
<tr>
<td>Appareil respiratoire</td>
<td></td>
</tr>
</tbody>
</table>
Appareil cardio-vasculaire
Système nerveux
Appareil digestif
Peau et phanères
Appareil locomoteur
Autres anomalies:

4. EXAMENS BIOLOGIQUES

*Un TDR a-t-il été réalisé? [ ] 1=Oui 2=Non 3=Non disponible.
Si Oui, noter le résultat du TDR [ ] 1=positif 2=négatif 3=invalide 4=Non disponible
*Le TDR sera fait uniquement si le l’enfant est fébrile (Température axillaire ≥ 37.5°C) ou présente une histoire de fièvre dans les 24 heures précédentes. Le TDR est toujours associé à une goutte épaisse.

Une goutte épaisse a-t-elle été réalisée [ ] 1=Oui 2=Non
Si oui noter le résultat :

<table>
<thead>
<tr>
<th></th>
<th>P.falciparum</th>
<th>P.malariae</th>
<th>P.ovale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trophozoïtes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schizontes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gamètocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Le taux d’Hb a-t-il été mesuré? [ ] 1=Oui 2=Non
Si Oui, noter le résultat : Hb: [ ] g/dl
Un prélèvement de sang capillaire a-t-il été prélevé pour la glycémie [ ] 1=Oui 2=Non
Si Oui, noter le résultat : Glycémie: [ ] g/L

Autres examens (préciser) :

1. ____________________________________________________________
3. ____________________________________________________________

5. DIAGNOSTIC

<table>
<thead>
<tr>
<th>Diagnostic</th>
<th>1=Oui 2=Non</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paludisme simple</td>
<td></td>
</tr>
<tr>
<td>Paludisme grave</td>
<td></td>
</tr>
<tr>
<td>Infection respiratoire aigue haute</td>
<td></td>
</tr>
<tr>
<td>Infection respiratoire aigue basse</td>
<td></td>
</tr>
<tr>
<td>Otite moyenne</td>
<td></td>
</tr>
<tr>
<td>Gastroentérite</td>
<td></td>
</tr>
<tr>
<td>Infection de la peau/tissus mous</td>
<td></td>
</tr>
<tr>
<td>Ménéngite</td>
<td></td>
</tr>
<tr>
<td>Septicémie</td>
<td></td>
</tr>
<tr>
<td>Malnutrition</td>
<td></td>
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<tr>
<td>Autre (préciser)</td>
<td></td>
</tr>
<tr>
<td>Autre (préciser)</td>
<td></td>
</tr>
</tbody>
</table>

Cet enfant devrait-il être hospitalisé? [ ] 1=Oui 2=Non

6. TRAITEMENT
**Médicament**

<table>
<thead>
<tr>
<th>Médicament</th>
<th>1=Oui</th>
<th>Si oui, indiquer traitement</th>
<th>Posologie</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antipaludique</td>
<td>☐</td>
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<tr>
<td>Antibiotique</td>
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<td>Antipyrétique</td>
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<tr>
<td>Antiparasitaire</td>
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<tr>
<td>Pansement</td>
<td>☐</td>
<td></td>
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<tr>
<td>Autres (préciser)</td>
<td>☐</td>
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</tr>
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**Formulaire complété par : initiales de l’infirmier et date:** ☐ ☐ ☐ Date ☐ ☐ ☐ / ☐ ☐ ☐ / ☐ ☐ ☐

**Déclaration de l’investigateur sur la vérification des données de l’enquête**

J’ai vérifié l’exactitude, la cohérence et la complétude des données de toutes les pages de ce formulaire d’enquête de base. Pour autant que sache, ces données sont complètes et correctes. Toutes les informations collectées dans ce formulaire l’ont été soit par moi ou par une personne sous ma supervision.

Date: ☐ ☐ ☐ / ☐ ☐ ☐ / ☐ ☐ ☐

__________________________
Jour ☐ ☐ ☐ Mois ☐ ☐ ☐ Année ☐ ☐ ☐ ☐

Initiales et Signature de l’investigateur

323
1. L’enfant a-t-il été vu ?

<p>| |</p>
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<tr>
<td>1=Oui 2=Non.</td>
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</table>

2. SIGNES VITAUX

Température axillaire [____] [____] [____] °C
L’enfant est-il fébrile (Température axillaire > 37.5°C)? [____] 1=Oui 2=Non.

3. HISTOIRE MEDICALE ET EXAMEN PHYSIQUE

**HISTOIRE MEDICALE**
(1=Oui 2=Non)

Histoire de fièvre de 24 heures [____]
Vomissement [____] si oui, fréquence [____]/24h
Diarrhée [____] si oui, fréquence [____]/24h
Ecoulement nasal [____]
Toux [____]
Autres [____] si oui, préciser : …………………………………………………………………………………

**EXAMEN PHYSIQUE**
(Si 2, noter les anomalies)

<table>
<thead>
<tr>
<th></th>
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</table>

Autres anomalies:

6. EXAMENS BIOLOGIQUES

Un échantillon de sang a-t-il été prélevé pour le dosage des AC maternels? [____] 1=Oui 2=Non

Une goutte épaisse a-t-elle été réalisée [____] 1=Oui 2=Non
Si oui noter le résultat :

| P.falciparum | P.malariae | P.ovale |
### Trophozoïtes
### Schizontes
### Gamètocytes

#### 7. TRAITEMENT

L’enfant a-t-il été traité par l’enquêteur ? [__] 1=Oui 2=Non  
Si oui, préciser le traitement reçu : …………………………………………………………
………………………………………………………………………………………………

L’enfant a-t-il été référé au CSPS ? [__] 1=Oui 2=Non  
Si oui préciser la raison : …………………………………………………………
………………………………………………………………………………………………

### DECLARATION DE L’INVESTIGATEUR SUR LA VERIFICATION DES DONNEES DE L’ENQUETE

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Date: [___/___/___]  
Initiales et Signature de l’investigateur