Risk factors for malaria infection and transmission in Burkina Faso, an area of high, persistent malaria transmission and high insecticide resistance

YARO, JEAN BAPTISTE

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Jean Baptiste Bibié YARO

Department of Biosciences

Durham University, UK

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Abstract

Malaria remains a public health problem despite the large decline in infection and disease between 2000 and 2015, due largely to the massive deployment of insecticide-treated nets (ITN). Recent years have been marked, however, by a stagnation in progress in high burden countries. In 2020, the World Health Organisation (WHO) reported that the key targets of WHO's global malaria strategy have been missed. The 11 high burden high impact countries (10 in sub-Saharan Africa including Burkina Faso, plus India) accounted for approximately 70% or more of the world's malaria case burden and 71% of global estimated deaths from malaria in 2020. About 253 million ITNs have been distributed in malaria endemic countries in 2019 with an increase of 56 million ITNs compared with 2018. Approximatively 84% of these ITNs were delivered to countries in sub-Saharan Africa. In parallel, the WHO global malaria 2020 report on insecticide resistance on malaria vectors highlights resistance to the four common insecticide classes (pyrethroids, organochlorines, carbamates, organophosphates) in 74 out the of the 82 endemic countries. The resistance to pyrethroids, the only insecticide class currently used in ITNs continues to be widespread in all major malaria vectors across malaria endemic countries.

Burkina Faso is one of the few countries not to have shown an association between ITN ownership and a reduction in child mortality. Artemisinin-based combination therapy (ACT) and Intermittent Preventive Treatment of Malaria in pregnant women using sulfadoxine-pyrimethamine (IPTp-SP) was introduced in 2006, ITNs have been distributed free of charge since 2010 every three years and seasonal malaria chemoprevention (SMC) in children under five years has been scaled up across the country in 2016. Following the mass distribution of more than 30 million ITNs in 2010, 2013, 2016 and 2019, more than 97% of households owned at least one ITN, although estimates of the proportion of children sleeping under an ITN range from <30% in the dry season to >70% in the peak transmission season. Despite the high coverage, cases of malaria remain high with 380 cases per 1000 in 2015, 536 cases per 1000 in 2017 and 528 cases per 1000 in 2018. Reasons for the lack of effectiveness of ITNs may include the high levels of insecticide resistance in malaria vectors, incomplete coverage of ITNs, poor condition of the nets and whether people use them correctly.

Here I explored the risk factors for malaria in Burkina Faso, mainly in children and pregnant women and for indoor densities of malaria mosquitoes. This thesis hypothesised that malaria in Burkina Faso was less common among children and pregnant women living in modern housing and with high socio-economic status.

Two epidemiological study models (cohort study and cross-sectional survey) were implemented to help determine major risk factors for *Plasmodium falciparum* infection in an area of persistent and intense malaria transmission in rural Burkina Faso, with high ITN coverage and high levels of insecticide resistance, throughout different population groups (children, pregnant women and all age population).

Incidence of *P. falciparum* infection remains overwhelmingly high in the study area with school-age children at greatest risk. Caregivers with high socio-economic status (odds ratio, OR =1.05, 95% CI 1.00 - 1.11, p=0.04), having travelled out of the study area (OR=1.52, 95% CI 1.45 - 1.52, p<0.001), or being literate (OR=1.71, 95% CI 1.26 - 2.32, p=0.001), were at increased risk. Conversely, sleeping in a metal-roofed house (OR=0.6, 95% CI 0.4 - 1.0, p=0.03) and having an electricity supply in the child's bedroom (OR=0.4, 95% CI 0.3 - 0.7, p=0.001), reduced the risk of house entry by *Anopheles gambiae*, the dominant malaria vectors in the study area.

During pregnancy there was a reduced risk of *P. falciparum* infection associated with the use of ITNs (Odds ratio, OR=0.31, 95% CI 0.12–0.79, p=0.02), while this association was not found in the general population or among children. In pregnant women, increasing the dose of IPTp-SP, was associated with the reducing the odds of infection by 40% (OR=0.59, 95% CI 0.43–0.81, p<0.001).

The study findings suggest that malaria control in Burkina Faso and other countries in sub-Saharan Africa experiencing persistently high malaria transmission need additional interventions to contribute to accelerate the disease burden reduce and to achieve the goals of WHO global strategies by 2030. My findings highlight the potential of improved housing to reduce malaria transmission as further opportunities for improving malaria control.

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Abbreviations

ACD	active case detection
ACT	Artemisinin-based combination therapy
AL	artemether-lumefantrin
ANC	antenatal clinic
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CNRFP	Centre National de Recherche et de Formation sur le Paludisme
COVID-19	Corona Virus Disease 2019
CSP	circumsporozoites protein
DfID	Department for International Development
EDCTP	European and Developing Countries Clinical Trial Partnership
EIR	Entomological inoculation rate
G6PD	glucose-6-phosphate dehydrogenase
HBHI	high burden to high impact
HbS	Sickle haemoglobin
HbSS	homozygous sickle cell
HDSS	Health and Demographic Surveillance System
HPF	high-power fields
INSP	Institut National de Santé Publique
IPTp-SP	Intermittent preventive treatment in pregnancy - sulfadoxine-pyrimethamine
IRR	incidence rate ratio
IRS	indoor residual spraying
ITN	Insecticide-treated nets
LLIN	long-lasting insecticide-treated net
MDG	Millennium Development Goal
MIRA	Malaria insecticide resistant Africa
MIS	Malaria Indicator Survey
MRC	Medical Research Council
NMCP	National Malaria Control Program
PBO	piperonyl butoxide
PCA	Principal component analysis
PCD	passive case detection
PCR	polymerase chain reaction

PDA	personal digital assistant
pHI	proportionate hole index
QGIS	Quantum Geographic Information System
RDT	malaria rapid diagnostic test
s.l.	*sensu lato*
s.s.	*sensus stricto*
SD	standard deviation
SES	socio-economic status
SMC	seasonal malaria chemoprevention
UK	United Kingdom
WHO	World Health Organization

Declaration

The work contained in this thesis has not been submitted elsewhere for any other degree or qualification and is the authors own work unless otherwise stated.

Statement of Copyright

Jean Baptiste Bibié YARO

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Contribution

Some of the work in this thesis is reproduced from published manuscripts in which Jean Baptiste Bibie YARO was first author.

Chapter 1 use material from literature review largely based on PubMed, Google Scholar, Institutional documentations and some person resources. Jean Baptiste B. Yaro searched the literature and identified relevant studies for inclusion in collaboration with Steve W. Lindsay and Anne L. Wilson.

Chapter 2 was published as Yaro et al., 2020 (Malaria Journal 19: 371). Anne L. Wilson (ALW), Steve W. Lindsay (SWL), Alfred B. Tiono (ABT) and MIRA research team member conceived the study. Jean Baptiste B. Yaro (JBY) conceived data collection materials and conducted fieldwork as study physician and study field coordinator in collaboration with demography fieldworkers, entomological fieldworkers, socio-economic fieldworkers and clinical fieldworkers including laboratory workers, collected data and managed data with Z. Amidou Ouedraogo (ZAO). Jean Baptiste B. Yaro searched the literature and identified relevant studies for inclusion in collaboration with SWL and ALW. Jean Baptiste B. Yaro conducted data analysis in collaboration with ALW, SWL, ABT, ZAO, Alphonse Ouedraogo, Efundem Agboraw, Eve Worrall, and Blami Koté. JBY drafted the manuscript and the list of all those who contributed to and approved the final manuscript is listed in the authors list of the chapter.

Chapter 3 is being prepared for submission to a scientific journal. ALW, SWL, ABT and MIRA research team member conceived the study. JBY conceived data collection materials and conducted fieldwork as study physician and study field coordinator in collaboration with demography fieldworkers, entomological fieldworkers, socio-economic fieldworkers and clinical fieldworkers including laboratory workers, collected data and managed data with ZAO. JBY searched the literature and identified relevant studies for inclusion in collaboration with SWL and ALW. JBY Conducted data analysis in collaboration with ALW, SWL, ABT, ZAO, AO, EA and EW. JBY drafted the manuscript and the list of all those who contributed to and approved the final manuscript is listed in the authors list of the chapter as followed: JBY, SWL, ALW, ABT, ZAO, AO, EA and EW.

Chapter 4 was submitted to the Malaria Journal for publication. ALW, SWL, ABT and MIRA research team member conceived the study. JBY conceived data collection materials and conducted fieldwork as study physician and study field coordinator in collaboration with demography fieldworkers, entomological fieldworkers, socio-economic fieldworkers and clinical fieldworkers including laboratory workers, collected data and managed data with ZAO. JBY searched the literature and identified relevant studies for inclusion in collaboration with SWL and ALW. JBY Conducted data analysis in collaboration with ALW, SWL, ABT, ZAO, Alphonse Ouedraogo, Efundem Agboraw and Eve Worrall. JBY drafted the manuscript and the list of all those who contributed to and approved the final manuscript for submission are followed: JBY, ALW, SWL, ABT, AO, N'Fale Sagnon, Hilary Ranson, K. Hyacinthe Toé, Antoine Sanou, W. Moussa Guelbeogo, Efundem Agboraw.

Chapter 5 was submitted to the Malaria Journal for publication. JBY, ABT, SWL and ALW conceived and designed the study. JBY searched the literature and identified relevant studies for inclusion in collaboration with SWL and ALW. JBY, ABT, AO, Sombie Salif, Amidou Diarra conducted field and laboratory work. Pinsaret Momo made the study map in collaboration with JBY and ALW. JBY, SWL, ALW, ABT, ZAO and AO did the data analysis. JBY drafted the manuscript and the list of all those who contributed to and approved the final manuscript for submission are followed: JBY, AO, AD, SS, ZAO, Issa Nebie Ouedraogo, Chris Drakeley, Sodiomon B. Sirima, ABT, SWL, ALW.

Chapter 6 was conceived and designed by JBY, SWL and ALW. JBY searched the literature and identified relevant studies and risk factors for inclusion in collaboration with SWL and ALW.

CHAPTER 1

Background and literature review

Introduction

Malaria is one of the oldest diseases in the world and has existed since the dawn of humanity [1]. Indeed the history of our planet has been shaped by the millions of deaths from this disease over the centuries [2, 3]. Malaria is an infectious disease cause by the *Plasmodium* parasite which is transmissible to humans by the bite of female *Anopheles* mosquitoes. Malaria continues to be a public health challenge in most developing tropical countries despite the identification of the causative parasites, and more than half a century after having effective drugs and insecticides at our disposal [4]. Recent reports show that today trends in the burden of malaria is not declining, but it's stable [5], and remains the fifth cause of death from infectious diseases worldwide (after respiratory infections, HIV/AIDS, diarrhoeal diseases, and tuberculosis) and the second in Africa, after HIV/AIDS [6].

Global malaria situation in the world and sub-Saharan Africa

Malaria is found in five of the six World Health Organization (WHO) regions, with only Europe being malaria free [7]. WHO estimate that at least 3.4 billion people in the world continue to be at risk of being infected and developing disease in 92 countries [5]; Among them, 1.1 billion are often at high risk with >1 in 1000 chance of getting malaria each year [5]. The WHO Africa Region is most affected by the disease, representing 94% of the total burden in 2019 with an estimated 219 million cases, followed by the WHO South-East Asia Region (3%) and the WHO Eastern Mediterranean Region (2%) [5]. Most of the disease burden in Africa occurs in sub-Saharan Africa and in children under five years old (estimated bearing more than two thirds of all deaths) and pregnant women [5]. The most widespread and actively harmful of malaria parasites is *Plasmodium falciparum* and is the dominant parasite in sub-Saharan Africa [8]. This is one reason why there are more infections and deaths from malaria in Africa than elsewhere and most burden in children [9, 10]. In fact, in 2018, *P. falciparum* accounted for 99.7% of estimated global malaria cases in the WHO African Region, while

involving in 50% of cases in the WHO South-East Asia Region, 71% of cases in the Eastern Mediterranean and 65% in the Western Pacific and 91% of malaria deaths [11, 12]. The good news was that the global distribution of malaria was shrinking [13]: *Plasmodium falciparum* incidence per 1000 individuals at risk and count in millions of cases reduced in global sub-Saharan Africa (figure 1.1, A-D), in different WHO regions (figure 1.2, A-B), *P. falciparum* parasites rate spatial distribution in children two-ten years old (figure 1.3) and in all age (figure 1.4) [14]. About 25 countries are on track to eliminate malaria by 2025 and over 60 aim to do this by 2030 [10, 15]. As COVID-19 continues to challenge the weak health systems in sub-Saharan Africa, it's urgent for malaria endemic countries to keep up the fight against malaria. This creates a double challenge for Africa. To achieve the ambitious, but attainable, goal of malaria eradication by 2040, malaria-endemic countries must work together to achieve the challenging targets set out by WHO [10, 15].

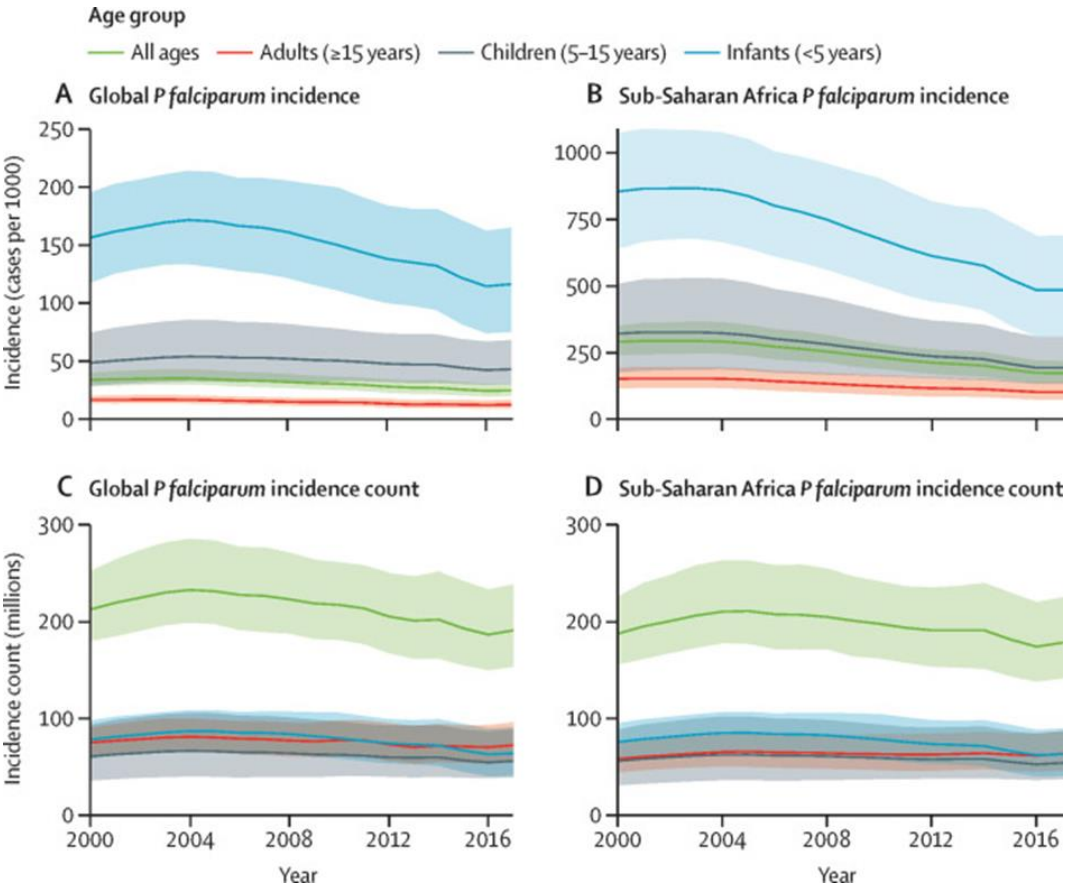

Figure 1.1: World global Malaria incidence rates from 2000–2017. *Plasmodium falciparum* incidence per 1000 individuals (A and B) and count in millions of cases (C and D) globally and for sub-Saharan Africa. Source: Weiss D.J. et *al.*, 2019

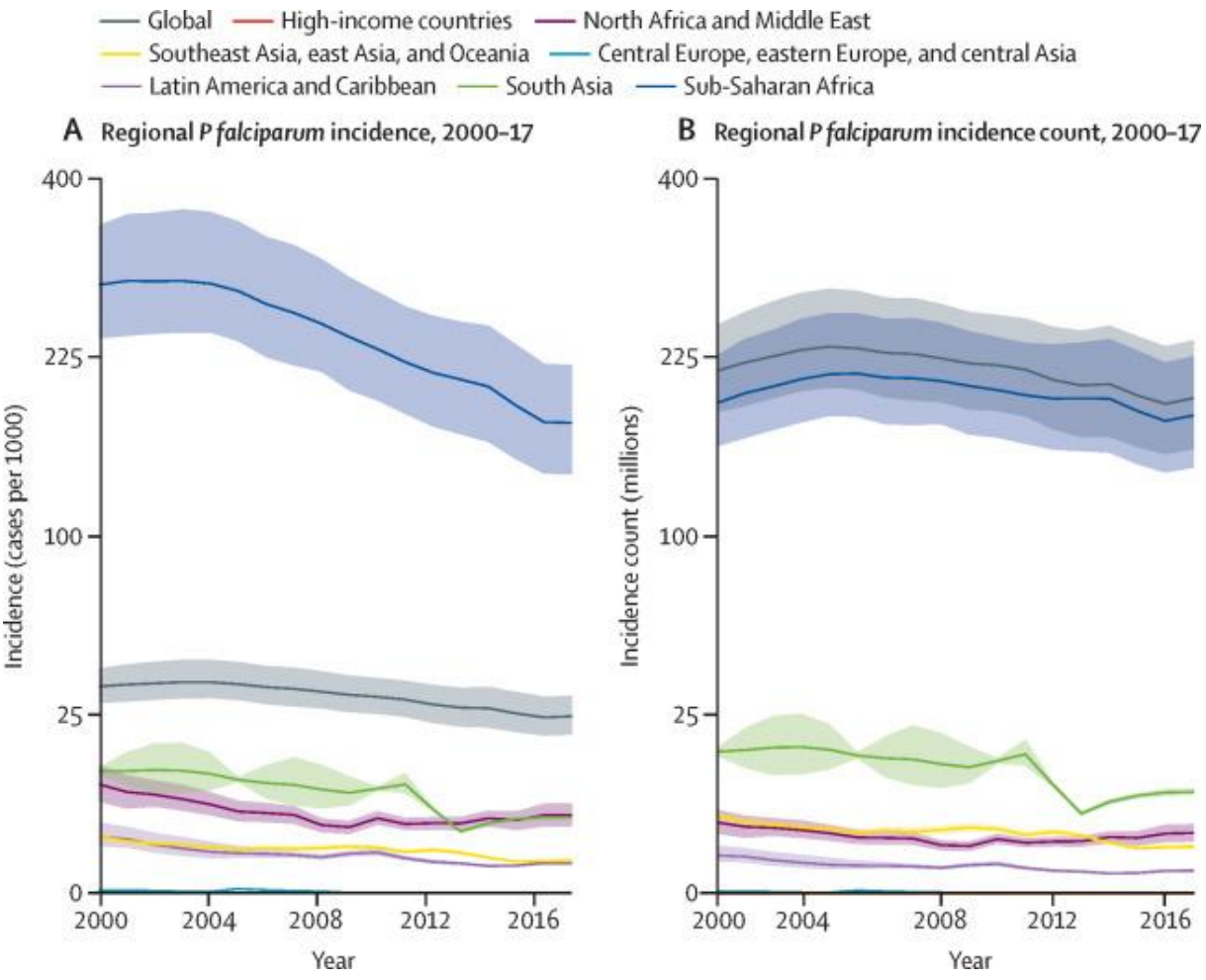

Figure 1.2: Regional distribution of *Plasmodium falciparum* incidence per 1000 individuals (A) and count in millions of cases (B). Source: Weiss D.J. et *al.*, 2019

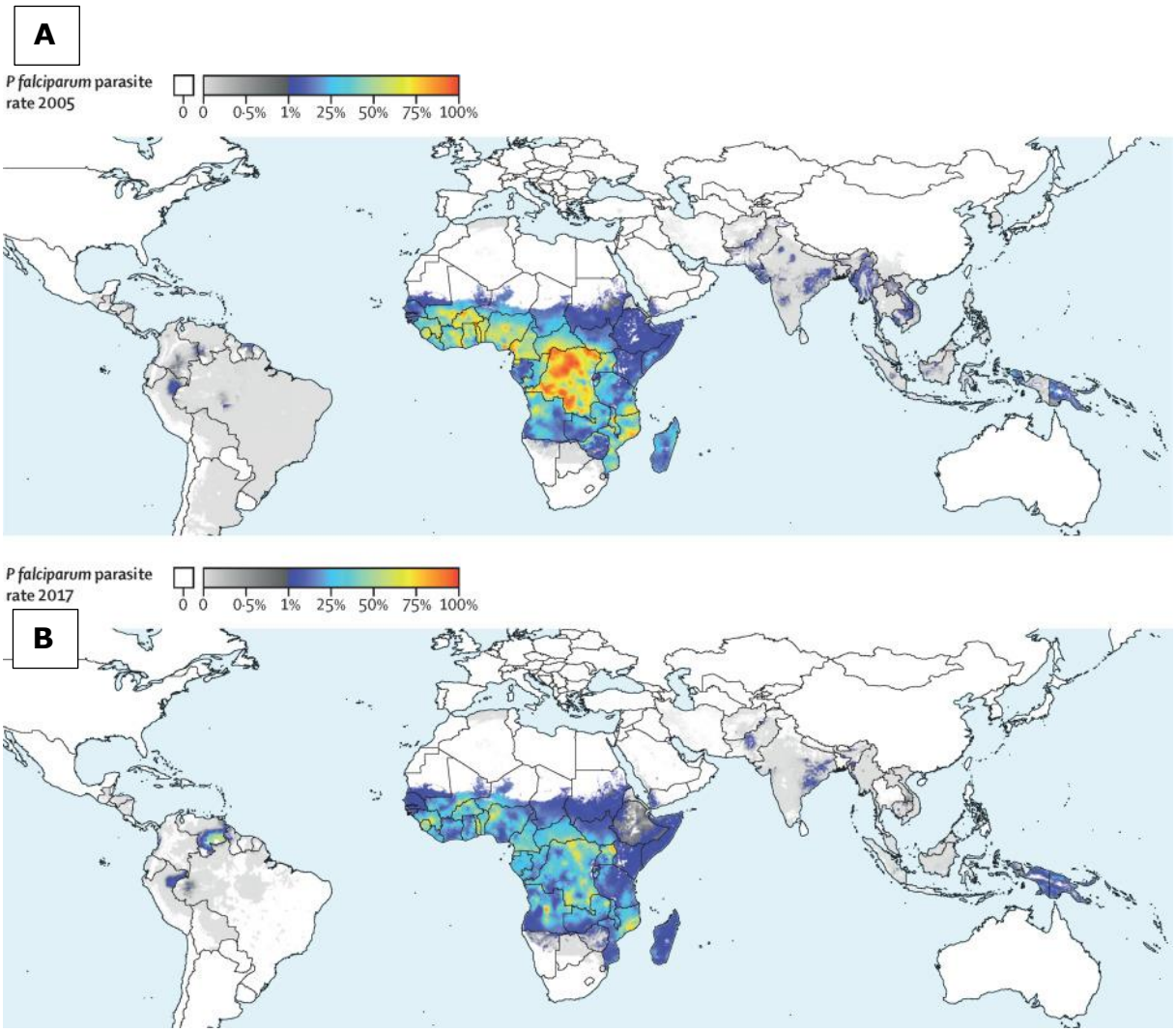

Figure 1.3: Spatial distribution of age-standardised *P. falciparum* parasite rate$_{2-10}$ in 2005 (**A**) and 2017 (**B**). Source: Weiss D.J. et *al.*, 2019.

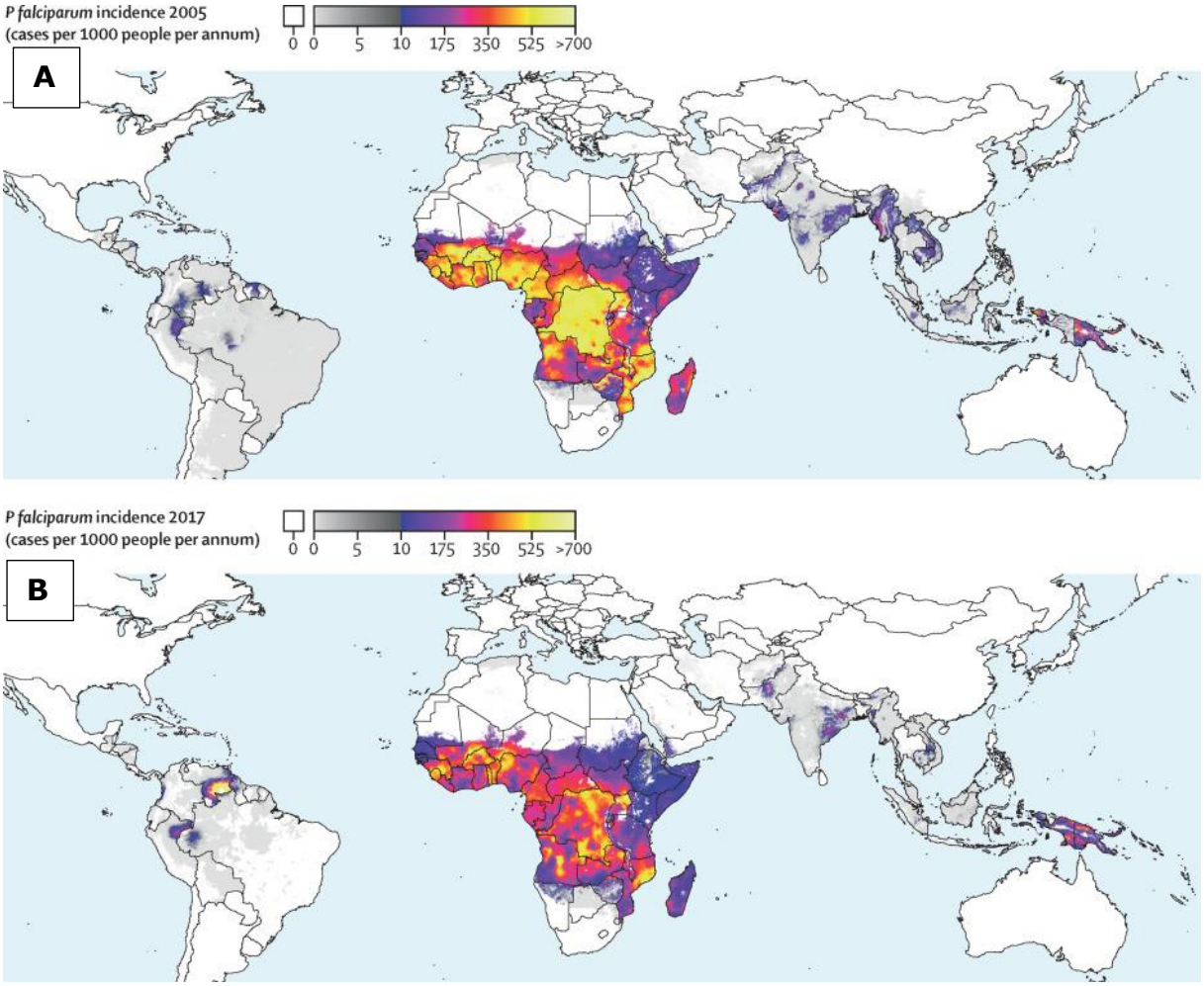

Figure 1.4: Spatial distribution of all-age *Plasmodium falciparum* incidence in 2005 (**A**) and 2017 (**B**). Source: Weiss D.J. et *al.*, 2019.

In sub-Saharan Africa, the *P. falciparum* prevalence rate halved in children two to 10 years old (Figure 1.3), with a 50% reduction in hyper-endemic areas and a 75% reduction in holo-endemic areas [4]. In spite of a growing population in endemic regions, *P. falciparum* all age cases declined between 2005 and 2017, from 232.3 million (95% uncertainty interval 198.8 – 277.7) to 193.9 million (156.6 – 240.2), (Figure 1.4). Three-quarters of this decline was achieved after 2005. This extraordinary achievement is attributed to the massive deployment of insecticide-treated nets (ITNs), accounting for 68% of the reduction, indoor residual spraying (IRS) for 13% reduction and artemisinin-based combination therapies (ACT) for 19% reduction [4] (Figure 1.5).

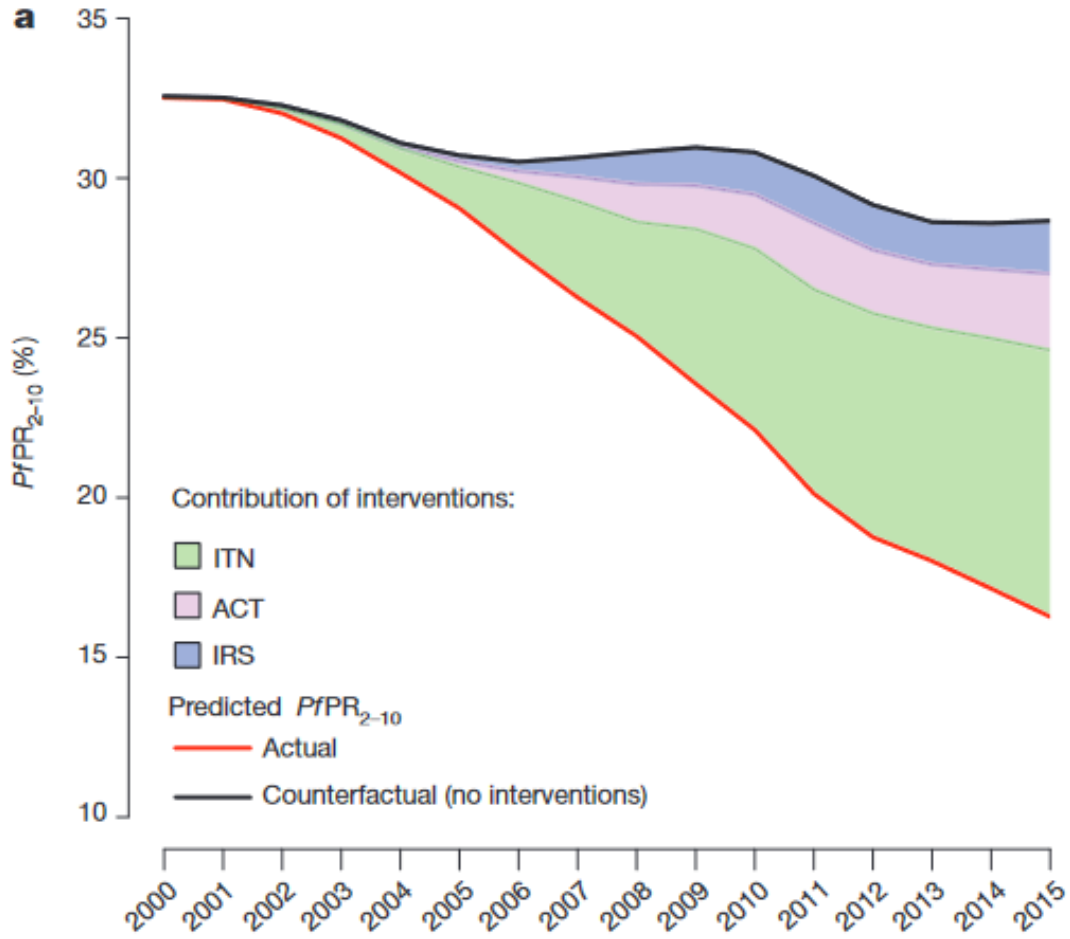

Figure 1.5: Changing endemicity and effect of interventions 2000–2015. Source: Samir Bhatt et al., 2015. Here is represented the predicted time series of population-weighted mean $PfPR_{2-10}$ across endemic Africa. The red line shows the actual prediction and the black line a 'counterfactual' prediction in a scenario without coverage by ITNs, ACTs or IRS. The colored regions indicate the relative contribution of each intervention in reducing $PfPR_{2-10}$ throughout the period.

In populations at risk from malaria, whilst only 7% of households had access to an ITN in 2005, by 2015, 67% had one [4]. The Millennium Development Goals (MDGs) target 6c which aimed to halt and begin to reverse the incidence of malaria by 2015 has been achieved [10-15].

Malaria control stalls?

Despite enormous progress in malaria control since the turn of the millennium, the gains have levelled off with a trend observed over recent years [5]. In 2017, WHO warned that the global response to achieve disease control had stalled [7]. From the 21 countries on track by WHO to achieve their elimination goals, 11 reported increases in indigenous malaria cases since 2015, and five countries reported an increase of more than 100 cases in 2016 compared with 2015 [7]. In many parts of sub-Saharan Africa there was numerous sites where malaria has changed little since the turn of the millennium including Nigeria, Democratic Republic of the Congo, Uganda, Mozambique, Niger and Burkina Faso [5, 7, 12, 16, 17]. The latest evidence from Burkina Faso between 2000 and 2016 was that malaria was increasing in the general population and in the two most vulnerable groups: children under five years old and pregnant women (Figure 1.6). Worryingly, malaria was still the main reason for medical consultation (45.0%), hospitalization (23.1%) and death (17.9%) in public health centres in Burkina Faso [18-20]. Malaria incidence in Burkina Faso is highly seasonal with about 60% of cases occurring within the four months from June to September [21]. The malaria incidence rate is 7.6 per 1000 child days [22] and *Plasmodium* infection prevalence in children was 57.5% in the country in 2017 [23], this is despite high coverage of ITNs, rapid diagnosis and effective treatment with antimalarial. In 2006, the country changed the national malaria treatment policy by adopting artemisinin-based combination therapies and in 2013, seasonal malaria chemoprophylaxis was implemented for children under five years old [24, 25]. A total of 8.4 million ITNs were deployed in 2010 and a further 10.5 million in 2013 resulting in approximately 90% of households owning at least one ITN [26]. More recently, in 2016, an additional 10.3 million ITNs were deployed, raising the coverage rate of households to 97%. Despite this huge investment in ITNs, Burkina Faso is one of the few countries not to have shown a significant association between ITN ownership and a reduction in child mortality [27, 28]. This is a surprising finding since ITNs have been shown to reduce clinical incidence by > 50% in areas of stable malaria [29].

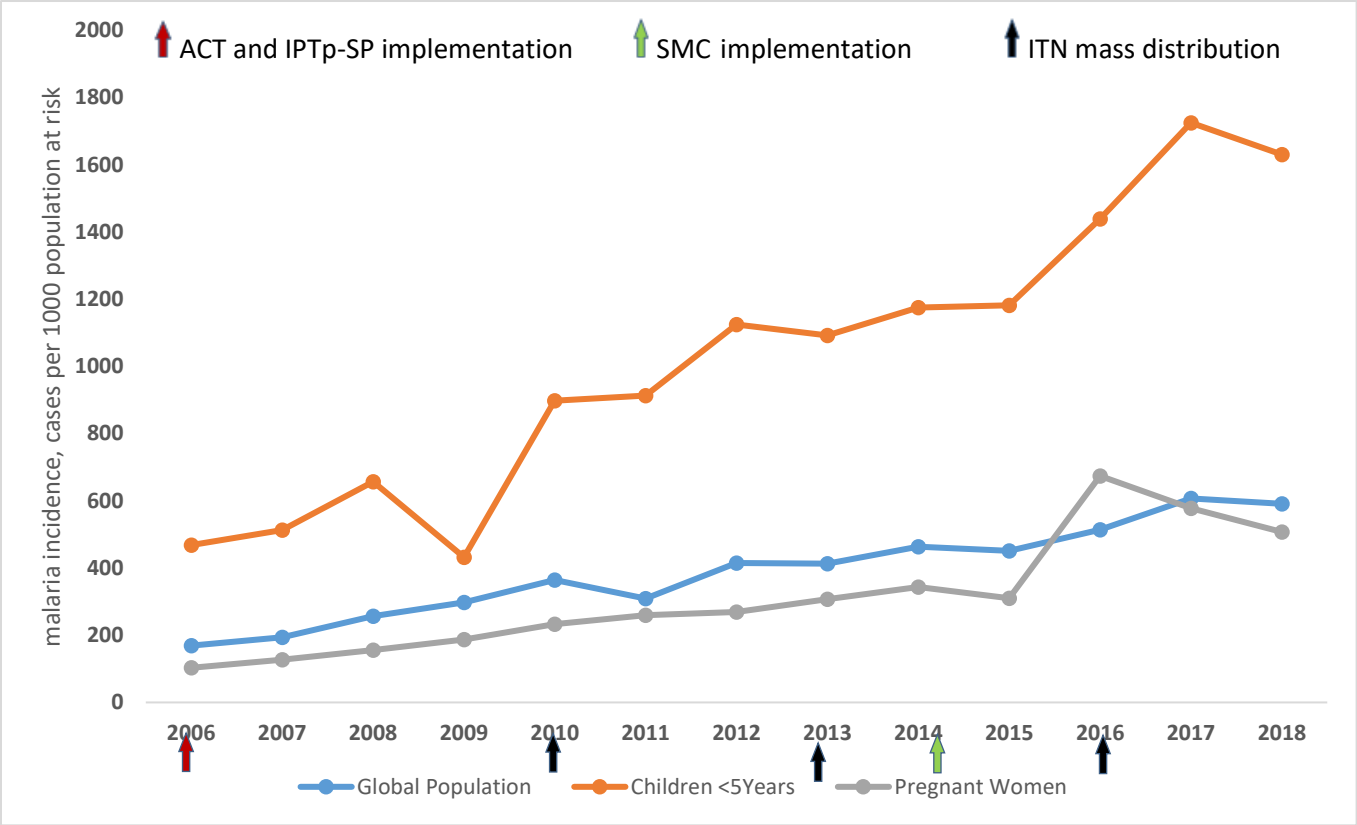

Figure 1.6: Malaria incidence in Burkina Faso between 2006 and 2018 in general population and in two vulnerable groups: children < 5 years old and pregnant women. **Source**: National Malaria Control Program, Burkina Faso

Note: Data from 2019 was missing because of repeated and persistent strikes of health workers and occasional terrorism attacks.

The mechanics of malaria transmission

Hackett's famous quote that 'malaria is comparable to a game of chess, where only few pieces are used to propose an infinite variety of complex situations' [30] captures the essence of malaria [1]. He went on to write that "everything about malaria is so moulded and altered by local conditions that it becomes a thousand different diseases and epidemiological puzzles" [31]. The four main pieces comprising the malaria system are the vector, parasite and human interacting in the environment (Figure 1.7), resulting in a huge variety of malaria transmission across the world. Globally, distinction is made in areas with high or low malaria incidence or malaria free. Some areas experience malaria transmission that lasts for several months or throughout the year, and in others it is very brief attributing to these areas their epidemiological characteristics. The challenge is that to get

effective control and elimination requires a tailored package of interventions for each specific epidemiological pattern [3]. The drivers of malaria operate at a range of spatial scales from coarse to fine. At a global scale, *P. falciparum* transmission is driven by climate and topology that can limit or intensify in the distribution and competence of *Anopheles* vectors [32]; while, at a micro-epidemiological scale in endemic areas, numerous factors influence malaria transmission dynamics including distance to the nearest mosquito breeding site and house construction features [3, 33]. Individual malaria risk may also be associated with human genetic factors, socio-economic status or with behavioural factors [34, 35] including those relating to occupation [35, 36] and travel [37]. Variations in these factors over a small area can result in spatially heterogeneous transmission, resulting in foci or hotspots of malaria infection and targeting hotspots may be a highly efficacious approach for malaria control [38].

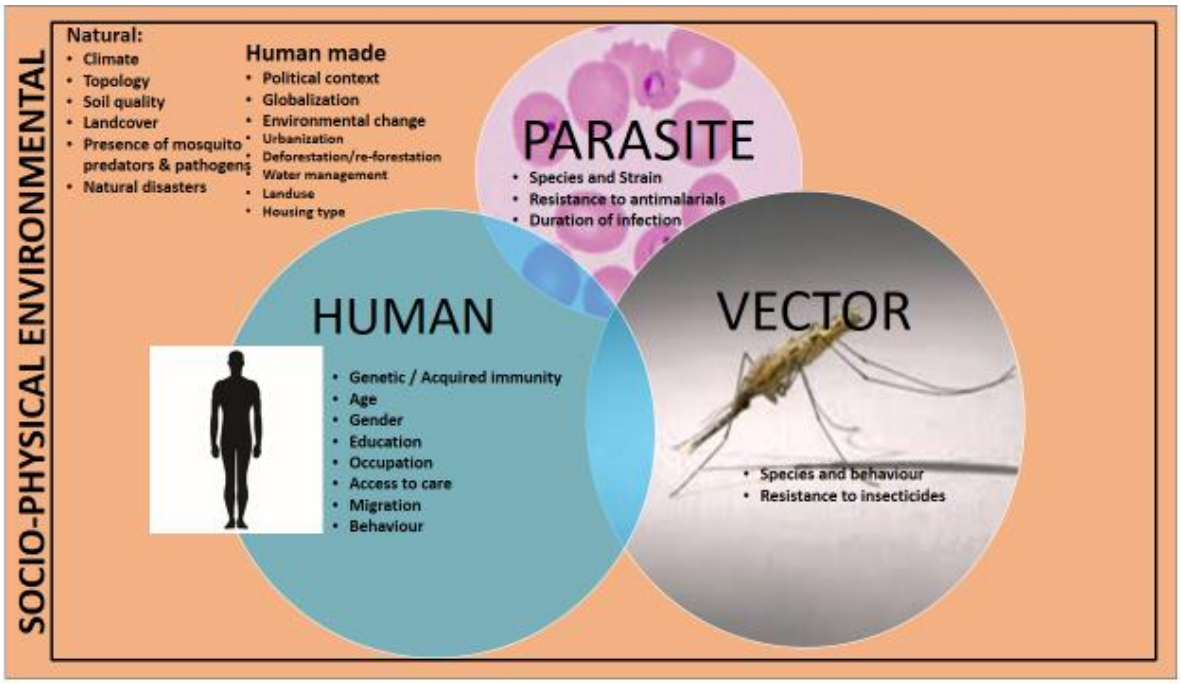

Figure 1.7: Drivers of malaria.

Environmental factors

At a coarse scale, climate dictates where malaria transmission can occur, in particular temperature, rainfall and humidity.

Temperature

Temperature affects the life cycle of the malaria parasite. As the temperature decreases, the number of days necessary to complete the development increases for a given *Plasmodium* species [39]. *P. vivax* and *P. falciparum* have the shortest development cycles and are therefore more common than *P. ovale* and *P. malariae*. Mosquito larvae also develop more quickly at warmer temperatures [40-42]. When it is hot, the time taken to convert a blood meal to eggs shrinks so that the number of eggs laid by mosquitoes increases [43].

Rainfall

The relationship between malaria transmission and rainfall is well established [44] and today, rainfall patterns can help predict malaria incidence [45]. The presence of water is essential for mosquito development. Aquatic habitats for vectors appear during the rainy season and it is for this reason that malaria transmission is highly associated with the pattern of rainfall, with most malaria appearing at the end of the rains, or shortly after.

Relative humidity

In general the survival of vectors is greater at higher humidity than lower ones [46]. In areas of seasonal malaria transmission, water pools dry up during the dry season and the relative humidity drops to levels that raise mosquito mortality. Both temperature and relative humidity affect *An. gambiae* mosquito survival and this associated affects the seasonal pattern of observed mosquito abundance [46].

Malaria endemicity is also affected by climatic and environmental factors, which influence vector proliferation, including rainfall, temperature and vegetation cover. Burkina Faso has a tropical Sudanian climate with a long dry season (October to April) and a short-wet season (May to September). The length of the rainy season and the total annual rainfall allow three climatic zones to be distinguished from the south to the north of the country: i) in the south, there is a Sudanian zone, which has a rainfall of between 1,000 and 1,300 mm over five months. The vegetation consists of open forests and forest galleries along the edges of permanent watercourses. Malaria transmission is intense and lasts at least five months each year; ii) In the centre of the country is a Sudano-Sahelian zone, with rainfall

between 600 and 1000 mm, which falls over three to four months each year. The vegetation consists of shrubs and grass and malaria transmission is of moderate intensity; iii) in the north is the Sahelian zone, characterized by an annual rainfall of between 400 and 600 mm, falling over three to four months. The Sahelian strip is characterized by a tree steppe of balanite and acacia radia with a carpet of grasses on the dunes and along the depressions. Malaria transmission is intense, but brief.

Vectors

The most important vectors in sub-Saharan Africa are members of the *An. gambiae* complex*,* and *An. funestus* complex [47]. The abundance of adult malaria vectors inside people's houses, where most transmission takes place, are associated with several factors [48] such as proximity of aquatic habitats [49], use of indoor vector control interventions like ITNs and IRS, and housing quality [48]. The most efficient vectors are those like *An. gambiae* and *An. funestus* that prefer to feed on humans (anthropophagic), rather than animals, and feed indoors (endophilic) [50]. Their peak night biting occurs between 23.00 and 05.00 h [51].

The recent success of malaria control in sub-Saharan Africa has been achieved by the massive deployment of ITNs and IRS [4], but these gains are threatened by the development of insecticide resistance in vectors [5, 52-54]. Resistance to pyrethroids, currently the only class of insecticides used for treating bednets, is now known spread widely across Africa [55].

Human behaviour

Human behaviour is an important risk factor for malaria. People change the landscape increasing or decreasing the risk of malaria. In Africa, rural development continues to change the physical landscape, increasing mosquito aquatic habitats and biting rates of *An. gambiae* [56]. Development projects including roads, dams, oils pipelines, mining and agricultural development can create aquatic habitats for malaria mosquitoes, leading to the increase in the burden of the disease [3, 57-60]. However, the process of urbanization can though reduce malaria transmission mainly because of fewer aquatic habitats, limiting the numbers of adult mosquitoes [61]. With increased human densities, malaria exposure per capita also decreases [62]. Housing is often of poor quality and the provision of health care and

sanitation is often inadequate [63, 64]. Population movement, for economic reasons or due to climate change, drought, natural disasters, conflict, humanitarian crises and associated deterioration in conditions, might expose individuals to increased malaria risk [65]. These conditions can lead to explosive growth of mosquito vectors and disease incidence increasing and spreading including rural and urban areas.

Adoption of malaria prevention interventions and health-seeking practices in communities govern the effectiveness of interventions such as ITNs, IRS and antimalarials and are important determinants of malaria in geographic areas and among specific population groups [66]. Low socioeconomic status is associated with roughly double the odds of clinical malaria or parasitaemia in children compared with higher socioeconomic status, within a locality [67]. Wealth is seen to be protective against malaria, and is positively associated with other beneficial factors, including better-education, improving housing quality (which reduces mosquito entry in house). Malaria and poverty therefore constitute a vicious cycle for the poorest households [60, 67].

Malaria parasites

The high antigenic diversity of *P. falciparum* allows strains to evade the *An. gambiae* immune system, adapt to mosquito vectors and be an important factor driving malaria transmission [68, 69]. Resistance of *P. falciparum* to artemisinin reported in a few countries in South-East Asia has recently been reported in Africa [70].

Gansane and co-workers in 2021 found a reduction in the efficacy of Artemether-Lumefantrine at day 28 and Dihydro-Piperaquine at day 42 in Nanoro and Gourcy in Burkina Faso, suggesting a change of first-line artemisinin-based combination therapy may be warranted in the country [71].

. The development of resistance to antimalarials poses one of the greatest threats to malaria control.

Human host factors

Individuals vary in their susceptibility to malaria. Most malarial disease, and particularly severe disease with rapid progression to death, occurs in young

children without acquired immunity [72, 73]. However, antibodies to merozoite and infected erythrocytes surface antigens increased following infection in early childhood [72], so that children living in moderate or in high transmission settings develop immunity faster as transmission increases [74]. Severe anaemia, hypoglycaemia and cerebral malaria are features of severe malaria more commonly seen in children than in adults. Pregnant women are also at risk from malaria, because they have weak immunity to malaria infection during their pregnancy [75] and are more attractive for *Anopheles* mosquitoes [76, 77]. Certain population groups evolved genetic factors to protect themselves from malaria and are only infected by some species of malaria parasite. This is the case for people in sub-Saharan Africa whom get infected by falciparum malaria, but not by vivax, making falciparum malaria the most prevalent and serious form of malaria in Africa [5, 9]. Glucose 6 Phosphate Dehydrogenase deficiency confers an advantage against malaria in heterozygous individuals [78]. Sickle haemoglobin is also considered to be protective against malaria, whilst malaria is fatal in homozygous sickle cell disease [79]. Recently researchers have found that gametocyte carriers, are bitten more frequently than those without gametocytes [77, 80, 81].

Conclusion

The decline of malaria in parts of sub-Saharan Africa is threatened by a number of factors including poor coverage of malaria control interventions, risks posed by anomalous climate patterns, and the emergence of parasite resistance to antimalarial medicines and mosquitoes resistant to insecticides. Overall heterogeneous association between climate changes and malaria transmission suggest the importance of location-specific approaches for public health interventions to impact arising from climate change [82]. Analysis of risk factors for malaria will enable development of new vector control tools and approaches, as well as decision support tools to support the activities of malaria control programmes in Africa.

Hypotheses and objectives

Rationale

There has been an unprecedented malaria decline in sub-Saharan Africa between 2000 and 2015, largely attributable to the roll-out of vector control [4]. However, despite all these investments, malaria remains an acute public health problem with children and pregnant women most at risk [5]. Worryingly, malaria has not declined in some areas, with recent reports that malaria control has stalled or the gains have levelled off in some countries in sub-Saharan Africa [5]. In 2019, COVID19 emerged and brings additional challenges to malaria responses worldwide. WHO has already sounded the alarm and is working to revive the fight for malaria control with the "high burden to high impact" (HBHI) approach [12]. Importantly, 11 countries, including Burkina Faso, and nine other countries in sub-Saharan Africa account for approximately 70% of the world's malaria burden [5, 83]. Evidence from Burkina Faso shows that high coverage of ITNs has not decreased malaria and all-cause mortality in children under five years old [27]. WHO country profile data records from Burkina Faso in 2006 and in 2016 were similar [84]. WHO World Malaria Report 2020 recorded similar number of global malaria cases in 2018 and in 2019 (7,875,575 and 7,859,000 millions of cases respectively) [5]. Also, *P. falciparum* prevalence remains high in the north-western region with up to 75.4 % of children aged five to nine years being infected in a community survey conducted in 2013 [23]. The incidence rate of malaria infections in pregnant women in Burkina Faso increased from 310 in 2015 to 800 per 1,000 women-year in 2016 [19]. Since ITNs have been shown to reduce clinical incidence by >50% elsewhere in areas of stable malaria [16], the lack of association between ITN roll-out and malaria in Burkina Faso requires further investigation. Recent surveys from Burkina Faso have shown higher malaria prevalence in children living in traditional houses (70.6%) compared to those living in modern houses (45.5%) [85], but it was unclear whether poor housing simply reflects low socioeconomic status since the wealth index may not have fully captured differences in socioeconomic position [85]. There have been several studies of risks factors for malaria in pregnancy in sub-Saharan Africa, where increased risk is associated with younger age in pregnancy, primigravidae, first trimester of pregnancy infection, non-use of ITNs, lack of education and HIV co-infection [86-89], but few

studies, have evaluated environmental, socioeconomic and intervention risk factors for malaria in pregnancy. The goal of this research project is to identify risk factors for malaria in children and pregnant women in Burkina Faso including individual, socioeconomic and environmental risk factors. Identifying risk factors for malaria in children and in pregnancy could assist in developing interventions to reduce this risk in Burkina Faso and other countries in sub-Saharan Africa.

Research hypothesis

It is hypothesised that malaria in Burkina Faso is less common among children and pregnant women living in modern housing and with high socio-economic status.

Primary objective

To determine risk factors associated with malaria incidence in children aged five to 15 years old and malaria prevalence in pregnant women in rural Burkina Faso.

Specific objectives

- To determine risk factors for clinical malaria in children
- To determine risk factors for malaria infection in pregnant women.

Study outcomes

Epidemiological outcomes

Epidemiological outcomes in children

Incidence of malaria infection detected by microscopy (any level of *P. falciparum* parasitaemia) during active case detection (ACD) or passive case detection (PCD) in children cohort survey.

Epidemiological outcomes in pregnant women

Malaria prevalence in pregnant women defined as the number of pregnant women with positive *P. falciparum* detected by microscopy at each seasonal cross-

sectional survey, one in high seasonal transmission and one in low seasonal transmission.

Human behaviour outcomes
Human behaviour outcomes in children

- Proportion of children who slept under a net the previous night as evaluated by questionnaire/sleeping place inspection by fieldworkers.
- Others malaria prevention skills including mosquito coils, insecticide sprays, traditional or commercial repellents.

Human behaviour outcomes in pregnant women

- Estimate time when pregnant women go to bed at night, when she gets out of bed in the morning and number of times women get out of bed last night as evaluated using a questionnaire administered by a fieldworker.
- Proportion of pregnant women who slept under a net the previous night as evaluated by questionnaire/sleeping place inspection by fieldworker.
- Number of antenatal clinic (ANC) visits and number of intermittent preventive treatment in pregnancy (IPTp) dose coverage by a pregnant women.
- Others malaria prevention technologies used by pregnant women including mosquito coils, insecticide sprays, traditional or commercial repellents.

Environmental risk outcomes in children and pregnant women

- Housing conditions including open or closed eaves, metal or thatched roof, number of rooms, presence of windows, windows number, size and position (next to the door or back to the door), presence of a ceiling, screening and house tidiness; household size.
- Size and proximity of potential aquatic habitats to study households;
- Presence of solid rubbish or trash next to households;
- Presence of alternative hosts close to households.

Socioeconomic risk outcomes in children and pregnant women

- Schooling level and occupation of children's caregivers or pregnant women.

- Specific items owned in households of children's caregivers or pregnant women including mobile phone, television, electricity, and modern bed of children caregivers or pregnant women.
- Children's caregivers or pregnant women are tenants or owners of property?

The research proposal methodology

To test my research hypotheses, I carried out four studies:

1. A cohort study to identify malaria risk factors in children aged five to 15 years old in the Banfora region in south-west Burkina Faso, where there is brief and intense seasonal malaria transmission (**chapter 2**);

2. A cross-sectional survey to identify malaria risk factors across all ages in Banfora region (**chapter 3**);

3. A cohort study in the same study site to determine risk factors for malaria mosquito house entry in study children's houses (**chapter 4**);

4. A cross-sectional surveys in the dry and wet season to identify malaria risk factors in pregnant women in Saponé, central Burkina Faso (**chapter 5**).

Chapter 2

A cohort study to identify risk factors for *Plasmodium falciparum* infection in Burkinabe children: implications for other high burden high impact countries

Adapted from:

Yaro JB, Ouedraogo A, Ouedraogo ZA, Diarra A, Lankouande M, Agboraw E, Worrall E, Toe KH, Sanou A, Guelbeogo WM, Sagnon N, Ranson H, Tiono AB, Lindsay SW, Wilson AL (2020). A cohort study to identify risk factors for *Plasmodium falciparum* infection in Burkinabe children: implications for other high burden high impact countries. *Malaria Journal*, 19, 371.

Abstract

Background

Progress in controlling malaria has stalled in recent years. Today the malaria burden is increasingly concentrated in a few countries, including Burkina Faso, where malaria is not declining. A cohort study was conducted to identify risk factors for malaria infection in children in southwest Burkina Faso, an area with high insecticide-treated net (ITN) coverage and insecticide-resistant vectors.

Methods

Incidence of *Plasmodium falciparum* infection was measured in 252 children aged 5 to 15 years, using active and passive detection, during the 2017 transmission season, following clearance of infection. Demographic, socio-economic, environmental, and entomological risk factors, including use of ITNs and insecticide resistance were monitored.

Results

During the six-month follow-up period, the overall incidence of *P. falciparum* infection was 2.78 episodes per child (95% CI= 2.66-2.91) by microscopy, and 3.11 (95% CI= 2.95-3.28) by polymerase chain reaction (PCR). The entomological

inoculation rate (EIR) was 80.4 infective bites per child over the six-month malaria transmission season. At baseline, 80.6% of children were reported as sleeping under an ITN the previous night, although at the last survey, 23.3% of nets were in poor condition and considered no longer protective. No association was found between the rate of *P. falciparum* infection and either EIR (incidence rate ratio (IRR): 1.00, 95% CI: 1.00-1.00, p=0.08) or mortality in WHO tube tests when vectors were exposed to 0.05% deltamethrin (IRR: 1.05, 95% CI: 0.73-1.50, p=0.79). Travel history (IRR: 1.52, 95% CI: 1.45-1.59, p<0.001) and higher socio-economic status were associated with an increased risk of *P. falciparum* infection (IRR: 1.05, 95% CI: 1.00-1.11, p=0.04).

Conclusions

Incidence of *P. falciparum* infection remains overwhelmingly high in the study area. The study findings suggest that because of the exceptionally high levels of malaria transmission in the study area, malaria elimination cannot be achieved solely by mass deployment of ITNs and additional control measures are needed.

Background

Malaria remains an acute public health problem throughout sub-Saharan Africa with an estimated 213 million cases and 380,000 deaths in 2018 [17]. Despite unprecedented declines in malaria between 2000 and 2015, of which 68% can be attributed to the scale-up of insecticide-treated nets (ITNs) [4], recent years have seen stagnating progress in high burden countries [17]. Reasons for this lack of progress are unclear, but may include incomplete coverage of ITNs, nets in poor condition and malaria vectors resistant to the pyrethroid insecticides used for ITNs. Burkina Faso, along with 10 other high-burden countries in Africa, plus India, has been designated as a High Burden to High Impact country by the World Health Organization (WHO) and Roll Back Malaria Partnership which calls for an aggressive new approach to accelerate malaria control [83]. Burkina Faso has the seventh highest number of malaria cases globally [17], and has seen a rise in the number of malaria cases from 9 million in 2016 to over 11 million in 2017 and 2018 [18, 19, 90].

Despite the known protective efficacy of ITNs, the 2010 ITN universal coverage campaign in Burkina Faso did not decrease malaria and all-cause mortality in children younger than 5 years old, even with 92% of children reportedly sleeping under ITNs [27]. The study site in Burkina Faso has extremely high prevalence and intensity of pyrethroid resistance in malaria vectors [91, 92]. For example, mosquitoes reared from larvae collected in Tengrela village between 2016 and 2018, showed only 2% mortality when exposed to the standard diagnostic dose of deltamethrin of 0.05% designed to kill all mosquitoes [93]. The public health impact of insecticide resistance, however, remains contested [94-97]. Insecticide resistance is only one important factor affecting the efficacy of nets, since ITN coverage, durability and whether people actually sleep under nets, as well as mosquito biting time and behaviour are also important. There is increasing evidence that so-called long-lasting insecticidal nets are not effective for 3 years [98-100], as claimed, and is the reason the term ITN is used. Coverage and system inefficiencies mean that ITNs are unevenly distributed among households and an estimated 50% of ITNs are lost from households after 23 months in Africa [101]. Social and environmental factors, including access to healthcare, socio-economic status (SES) and house construction, found to be protective against malaria in other studies [102], may also impact malaria risk in Burkina Faso.

A cohort study of children was conducted to determine risk factors for *Plasmodium falciparum* infection in an area of persistent and intense malaria transmission in rural Burkina Faso, with high ITN coverage and high levels of insecticide resistance. The study findings may help to identify potential opportunities for improving malaria control in Burkina Faso and other countries in sub-Saharan Africa experiencing persistently high malaria transmission.

Methods

Study site

The study was conducted from June to December 2017 in Banfora Health District, Cascades region, southwest Burkina Faso (Figure 2.1), an area of Sudanian savannah covering 6,295 km^2 and with an estimated population of 407,073 inhabitants [21]. Subsistence farming and animal husbandry are the main

activities. Banfora District has intense seasonal malaria transmission over a six-month period following seasonal rains from May to November [103]. *Plasmodium falciparum* accounts for 90% of cases [21]. The main malaria vector is *Anopheles gambiae sensu stricto* (*s.s.*), but *Anopheles coluzzii* is also found [103]. A universal coverage (defined as one ITN for every two persons at risk of malaria) campaign in 2016 distributed ITNs with permethrin or deltamethrin (Sumitomo Chemical, Vestergaard and BASF) and therefore no new ITNs were distributed by the study. No indoor residual spraying (IRS) was conducted. Since 2014, children under 5 years of age in the study area receive seasonal malaria chemoprevention (SMC) during the transmission season [104].

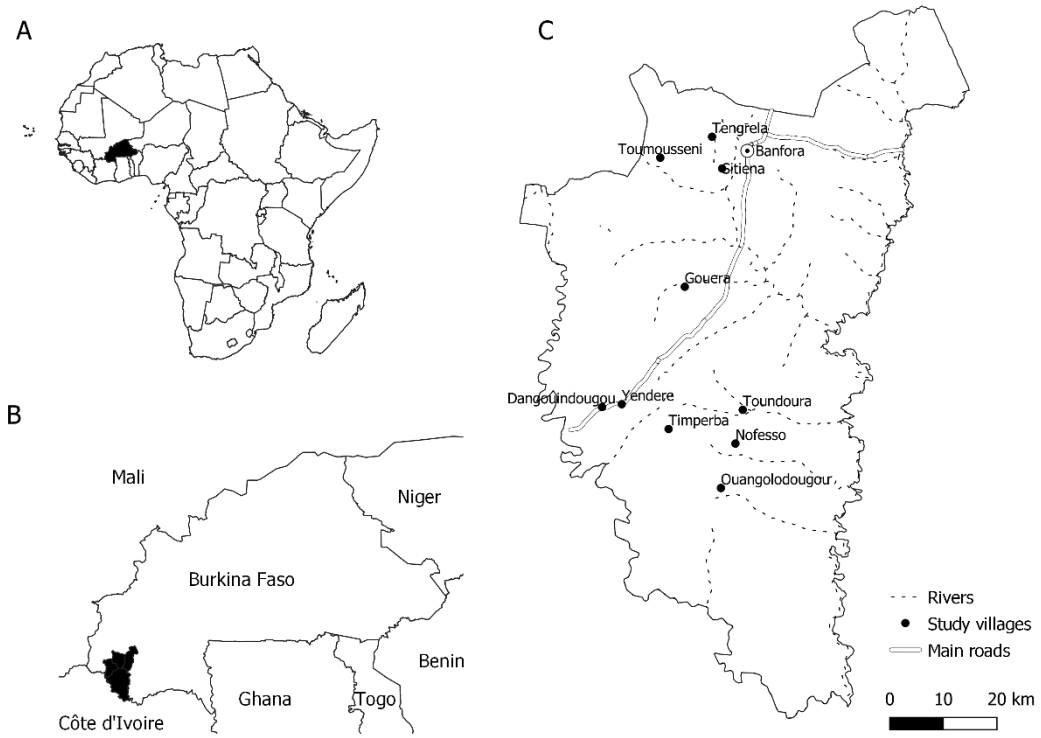

Figure 2.1: Map of the 10 study villages
A) location of Burkina Faso; B) location of study site in Burkina Faso; C) location of study villages in study site.

Recruitment of study cohort

Ten villages were randomly selected from a list of villages in the study area following a two-stage process. Firstly, an area spanning the catchment areas of five health centres was chosen. Each health centre had a catchment radius of

approximately 10 km. Secondly, two villages at least 3 km apart were chosen at convenience from each catchment area, giving a total of 10 villages. Thirty children were randomly selected from each village using the Health and Demographic Surveillance System enumeration list. Children were eligible to participate if they were aged 5-15 years, likely to remain resident in the village for the duration of the study and the caregiver provided informed consent (assent of child if aged 12-15 years). Older children were selected as they have the highest malaria incidence, relatively low immunity and contribute substantially to transmission [105], while children under 5 years were excluded due to roll-out of SMC. Children were not eligible if they were participating in a malaria clinical trial, or had a contra-indication to the artemisinin-combination therapy (ACT), artemether-lumefantrin (AL). At enrolment in June 2017, 300 children provided a blood film. Irrespective of their malaria parasite status, all children received a curative dose of AL (Wellona Pharma Private Limited, Nana Varachha, Surat, India) to clear any existing parasitaemia. Children were revisited 21 days later, at which time two blood slides and a blood spot were taken and examined to ensure parasite clearance. Those with a negative parasite status confirmed by polymerase chain reaction (PCR) were enrolled in the study. Children re-infected in the 21-day period were re-treated with AL and were eligible for enrolment in the study after 28 days once parasite clearance was confirmed using PCR.

Symptomatic and asymptomatic *Plasmodium* infections were recorded using both active and passive detection. Study children were visited at home every two weeks by fieldworkers during the peak transmission season, from July-December 2017. At each visit, fieldworkers measured a child's axillary temperature and prepared two blood films and a filter paper blood spot. Children with an axillary temperature ≥37.5°C or history of fever in the previous 48 hours were advised to visit the local health centre and, if they were a malaria case, treated with AL, following National Malaria Control Programme (NMCP) guidelines [25]. If a child was absent at the time of the visit, the fieldworker made one more attempt to locate them the following day, after which the child was recorded as being absent. Caregivers were encouraged by the study team at enrolment and at fortnightly visits to take their child to the nearest government health centre should the child have a fever or feel unwell. Travel and treatment costs for sick children were reimbursed by the study. Study nurses were posted in the five health centres close to the study villages and

performed rapid diagnostic tests for malaria (SD BIOLINE Malaria Ag P.f/Pan, Abbott Laboratories, Illinois, USA) in study children presenting with an axillary temperature ≥37.5°C or history of fever within the previous 48 hours, and prepared two blood films and a filter paper blood spot. Children diagnosed with clinical malaria received AL [25]. Clinical data were recorded on dedicated study logs and transcribed by fieldworkers who visited each nurse weekly.

Detailed descriptions of the microscopy and PCR and are provided in Appendix 2. In brief, thick blood films were stained with Giemsa (Figure 2.2) and examined under 1,000-fold magnification by experienced microscopists at the *Centre National de Recherche et de Formation sur le Paludisme* (CNRFP) in Banfora. Parasite counts were recorded per high power field and 100 fields counted before a slide was declared negative. Two blood slides from each subject were read separately by two microscopists. Discrepancies in positive and negative reads and parasite counts differing by more than 10-fold between the two reads were resolved by the supervisor.

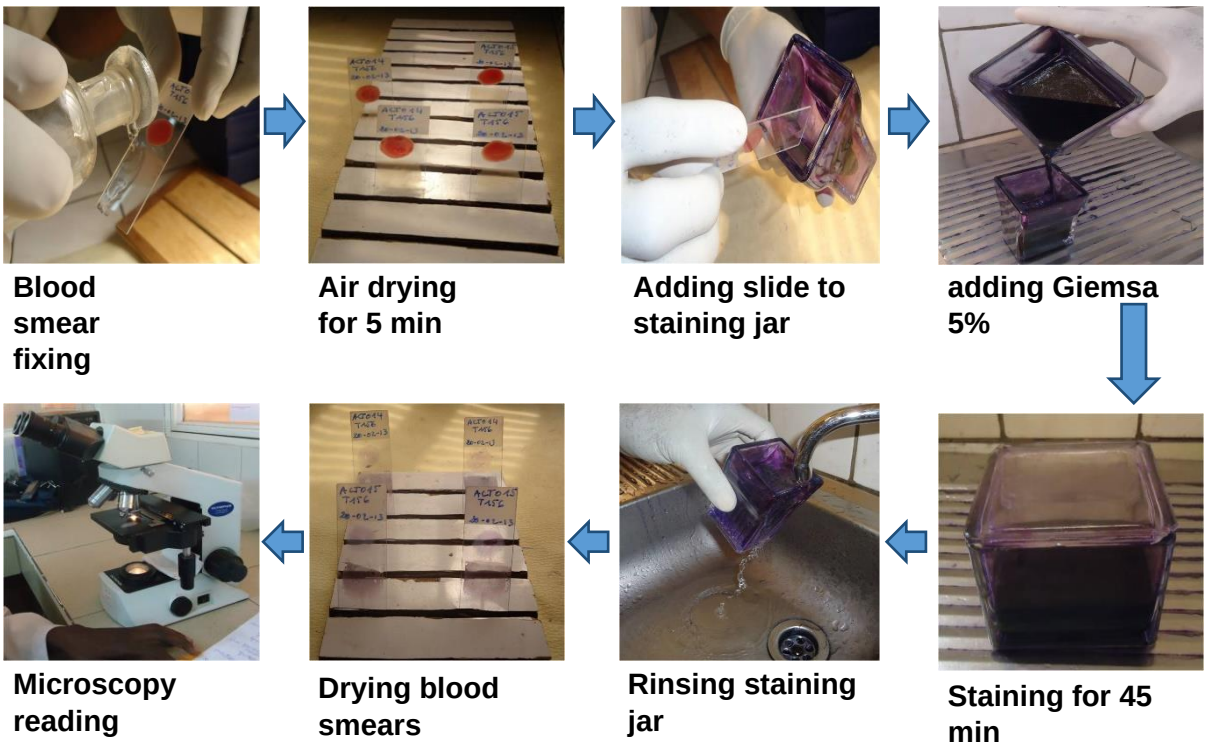

Figure 2.2: Blood smear staining (Source photo - CNRFP)

Filter paper blood samples were analysed for the presence of 18s rRNA gene using PCR [107]. Blood was collected on Whatman filter Papers (Whatman 3 mm, GE Healthcare, Pittsburg, USA) and labelled with patients' study numbers, air-dried, and individually placed into plastic bag marked containing a desiccant to protect against humidity. The bags were stored at room temperature until DNA extraction. Parasite DNA was extracted using Chelex methods [106].

The prepared blood spots were analysed at the CNRFP molecular biology laboratory to confirm or refute the microscopy results. DNA extraction was done using the QIAamp DNA Mini Kit protocol based on the principle of precipitation and column purification of DNA. Nested PCR was used for amplification of *Plasmodium* sp. DNA. Nested PCR consists of performing two successive amplifications. The DNA fragments obtained by PCR were revealed directly by agarose gel electrophoresis using ethidium bromide (BET). Their identification is facilitated by the use of molecular weight markers (Figure 2.3).

The electrophoresis results are interpreted as follows:
Line S: standard molecular weight marker (50-bp).
Line 1: diagnostic band for P. vivax (120 bp).
Line 2: diagnostic band for P. malariae (144 bp).
Line 3: diagnostic band for P. falciparum (205 bp).
Line 4: diagnostic band for P. ovale (800 bp).

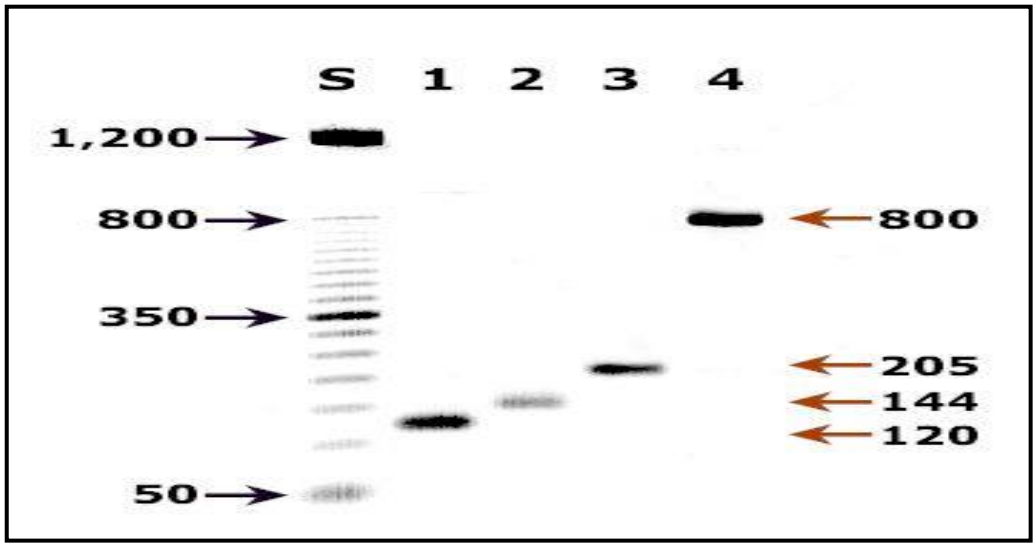

Figure 2.3: Example of agarose gel separation (Snounou et al., 1993).

Follow-up of study cohort

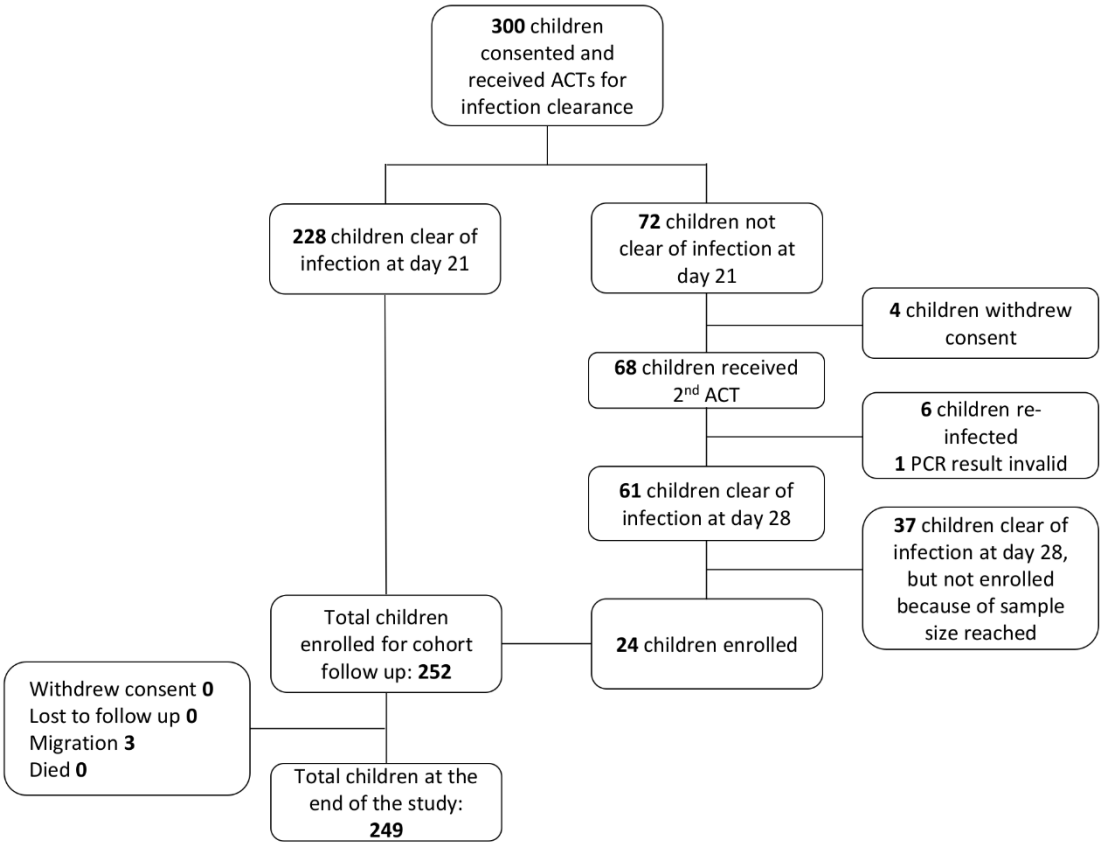

Figure 2.4: Study flowchart

Risk factors

At enrolment, socio-demographic characteristics of the child (age, gender, religion, ethnicity) and their caregiver (education, occupation) were recorded. Questionnaires recorded the presence or absence of large domestic animals (cattle, goats, sheep, donkeys, horses) within 5 m of each study child's house and the materials used to construct the building in which the study child slept, including presence of a metal roof, eaves and door and window screening. The head of household completed a questionnaire on their asset ownership, house construction and other variables, following standard procedures used in the Burkina Faso Malaria Indicator Survey (MIS) [108]. Houses of study children were geolocated using a global positioning system (GARMIN eTrex 20).

Use of an ITN the previous night by each study child and use of spatial or topical repellents were recorded at enrolment and each active visit. Survivorship and integrity of each study child's ITN was measured in July, October and December. Each net was recorded as being in use (i.e., hanging over the study child's sleeping space), in storage, being washed, or lost. Loss was categorized as: i) net given away voluntarily; ii) net stolen; or, iii) net destroyed, discarded or used for alternative purposes. Fabric integrity of the net was assessed by counting the number of holes and their size according to WHO guidance [109]. A weighted sum of hole counts, the proportionate hole index (pHI), was calculated with a pHI of 0-64 categorized as good, 65-642 as acceptable and 643+ as too torn and non-protective [110]. To measure ITN bio-efficacy, 26 bed nets were sampled (at least 2 randomly selected nets per village, except for Sitiena village where 1 net was tested) in October 2017 and stored at +4°C. ITNs taken for testing were replaced with new ones. WHO cone bioassays were performed using the pyrethroid-susceptible Kisumu strain of *An. gambiae sensu lato* (*s.l.*) at the CNRFP insectaries in Banfora using the WHO efficacy requirement of ≥80% mortality [111].

Mosquitoes were sampled with CDC light traps, positioned with the light 1 m above the ground at the foot end of the bed of each study child sleeping under an ITN from 19.00 to 06.00, every four weeks from July to December 2017. In addition, each child's net was systematically searched for mosquitoes between 06.00 and 07.00 every four weeks using a torch. Mosquitoes were identified morphologically using established keys and female *An. gambiae s.l.* typed to species using PCR. The presence of sporozoites in *An. gambiae s.l.* was determined using an enzyme-linked immunosorbent assay. Larval surveys to determine the proximity of anopheline larval habitats to study children's houses were carried out in the vicinity of all 10 villages during September 2017. All types of larval habitats were surveyed including irrigated fields, puddles, muddy foot or hoof prints, streams and ponds.

Phenotypic insecticide resistance was measured using WHO tube tests as per standard procedures [112]. Assays were performed with *An. gambiae s.l.* mosquitoes reared from immatures collected in seven study villages (larvae were not found in the other three villages).

Data management and statistical analysis

Data were collected on android personal digital assistants programmed using KoboCollect and included drop-down boxes and consistency checks to reduce data entry errors. Following cleaning, the dataset was locked and saved in Microsoft Access. An analytical plan was developed prior to data analysis.

The primary outcome was the incidence rate of microscopically confirmed *P. falciparum* infection during the transmission season, detected using active and passive case detection. PCR-confirmed *P. falciparum* incidence rate was a secondary outcome. After ACT treatment, further infections were censored for 28 days since infections during this time were most likely due to recrudescent parasites. Time at risk was also censored for time that study children spent away from their compound should they be found to be absent at the two-weekly home visits. The entomological inoculation rate (EIR) or estimated number of infectious bites per study child during the transmission season was calculated using the formula EIR=*MaSd* where *Ma* is the human biting rate, estimated from the arithmetic mean number of female *An. gambiae s.l.* caught per light trap night across the six-month transmission season, where *S* is the proportion of female *An. gambiae s.l.* found to be sporozoite positive by village and *d* is the number of days in the transmission season (n). Cone bioassay results for the netting pieces from each sampled net were pooled by village and by net type. QGIS Geographic Information System (QGIS Development Team (2019), Open Source Geospatial Foundation Project) was used to determine distances between the child's home and aquatic habitats. Distance to the nearest health facility was determined based on the shortest distance to travel by road. Principal component analysis was used to calculate a SES factor score based on asset ownership and household characteristics. SES factor scores (ranging from -1.8 to 3.2) were ranked and households divided into five equal wealth quintiles (1 poorest, through to 5, least poor).

Mean values were compared using a t-test and proportions compared using Chi-squared tests. Poisson regression models were used to identify risk factors

associated with *P. falciparum* infection incidence rate, adjusting for clustering by village. Risk factors assessed were: child's age, gender, ethnic group, religion, caregiver's education and occupation, wealth quintile and SES factor score, use of ITNs and other personal protection, ITN integrity, number of people sleeping in the room with the study child, housing materials (roof, eaves, wall and floor material, door and window screening), presence of cows, horses, sheep or goats within 5 m of the child's home, Euclidean distance to the nearest aquatic habitat with larval anophelines, distance by road to the nearest health centre, EIR at village level, and percentage mortality of *An. gambiae s.l.* mosquitoes from the village when exposed to 0.05% deltamethrin in a WHO tube test. A multivariate regression model was developed using a forward stepwise approach. Statistical analysis was carried out in Stata 15 (Statacorp, Texas, USA).

Assuming two *P. falciparum* infections per child during the transmission season, an intraclass correlation coefficient of 0.1 (design effect of 3.4), and a loss to follow-up of 10%, the study had greater than 90% power to detect effect sizes of >50% at the 5% level of significance, assuming 50% prevalence of the risk factor of interest in the population using the formula for comparison of two rates [113]. The study is reported following STROBE guidelines [114].

Results

Of 300 randomly selected children, 252 children aged 5 to 15 years old were enrolled in the cohort after confirmation of parasite clearance by PCR (Fig. 2.2). Of these 252 children, 228 were clear of infection at day 21 post-ACT, while an additional 24 children were enrolled following receipt of a second course of ACT due to re-infection. Three children withdrew from the study due to migration. No deaths were recorded among the cohort during the study period.

The mean age of cohort participants was 9.9 years, 52.0% of whom were male (Table 2.1); 38.9% of children were Gouin, 21.8% Karaboro, 11.5% Mossi, 9.1% Turka, 6.3% Fulani, and 4.4% Senoufo. Caregivers were predominantly illiterate (79.0%) and farmers (95.2%). Most sleeping rooms of the children had metal roofs (75.8%), brick walls (57.9%) and cement floors (70.2%). Over half the

children's sleeping rooms had open eaves (54.8%) and the vast majority did not have window screening (96.0%).

Table 2.1: Baseline characteristics of the study cohort

Characteristic		Number (%) N=252
Age at enrolment	5 years to <8 years	76 (30.2%)
	≥8 years	176 (69.8%)
	Age (mean/standard deviation)	9.93 (2.8)
Gender	Male	131 (52.0%)
	Female	121 (48.0%)
Ethnicity	Gouin	98 (38.9%)
	Karaboro	55 (21.8%)
	Mossi	29 (11.5%)
	Turka	23 (9.1%)
	Fulani	16 (6.3%)
	Senoufo	11 (4.4%)
	Others	20 (7.9%)
Reported bed net use	Used bed net usually	215 (85.3%)
	Used an ITN the previous night	203 (80.6%)
Caregiver's education level	Illiterate	199 (79.0%)
	Primary school	45 (17.9%)
	Secondary school or above	8 (3.2%)
Caregiver's occupation	Not working/retired	6 (2.4%)
	Farmer	240 (95.2%)
	Commercial activities / government officer	6 (2.4%)
Eave status of child's sleeping room	Closed	102 (40.5%)
	Open	138 (54.8%)
Roof material of child's sleeping room	Metal	191 (75.8%)
	Thatch	34 (13.5%)
	Other roof type	18 (7.1%)
Wall material of child's sleeping room	Mud	65 (25.8%)
	Brick	146 (57.9%)
	Cement (plastered or painted)	32 (12.7%)
Floor material of child's sleeping room	Cement	177 (70.2%)
	Dirt floor	65 (25.8%)
	Tiles	1 (0.4%)
Window screening of child's sleeping room	Absent	242 (96.0%)
	Present	1 (0.4%)

During the follow-up period, 249 of the 252 children experienced at least one *P. falciparum* infection, as detected by microscopy; 31 children (12.3%) had one *P. falciparum* infection, 139 (55.2%) children experienced two *P. falciparum* infections, 75 (29.8%) experienced three *P. falciparum* infections, and 4 (1.6%)

experienced four *P. falciparum* infections. A total of 550 *P. falciparum* infections were identified using microscopy while 608 infections were identified using PCR. Of the 550 *P. falciparum* infections confirmed using microscopy, 528 (96.0%) were detected using active case detection and 22 (4.0%) detected using passive case detection. Infections detected passively had a higher geometric mean *P. falciparum* density (19,875/mL, 95% CI = 7,896-31,854) compared to those detected through active surveillance (3,744/mL, 95% CI = 2,691-4,797, $p<0.001$). The *P. falciparum* infection incidence rate was 2.78 episodes per child during the six-month transmission season (95% CI= 2.66-2.91) by microscopy, and 3.11 episodes (95% CI = 2.95-3.28) by PCR. Among children suffering from at least one infection, the median time to first infection detected by microscopy was 27 days (range 14 to 123 days).

At baseline, 80.6% of caregivers reported that the study child slept under an ITN the previous night. Reported ITN use the previous night at the baseline survey was greater than 82% across the four lowest SES quintiles compared to the least poor children (quintile 5= 53.2%, $p<0.001$). Most study children's bed nets were either Permanet 2.0 (52.0%) or Olyset net (16.7%), with 85.5% provided by the NMCP and 4.0% purchased on the open market. Sleeping place inspections in July, October and December found 87.8% of children had a bed net hanging over their sleeping place (638 out of 727 observations). The most common reason for not having a net hanging was loss of the net (59/89 observations, 66.3%), while 15.7% (14/89 observations) of nets were stored and 2.2% (2/89 observations) being washed. The proportions lost, stored or being washed did not differ across the three surveys. Of the nets that were reported as being lost, 76.3% (45/59 observations) were destroyed, 22.0% (13/59 observations) stolen and 1.7% (1/59 observations) given to friends or family. It was common for children to share a bed net with siblings and 61.4% of children slept with three or more children. At the last survey in December, 62.2% (155/249) of ITNs were in good condition, 14.5% (36/249) were damaged and 23.3% (58/249) badly torn and non-protective. Net condition did not differ significantly by survey round, although there was a tendency towards net deterioration over the study period. Cone testing of a random sample of 26 children's bed nets gave a mean knockdown at 1 hour of 76.5% (95% CI= 61.9-91.1%) and mortality after 24 hours of 42.2% (95% CI= 18.8-65.6%) for Olyset nets (9 nets, permethrin-treated) and mean knockdown of

85.2% (95% CI= 77.9-92.4%) and 24-hour mortality of 39.9% (95% CI= 27.7-52.0%) for Permanet (17 nets, deltamethrin-treated).

A total of 20,929 mosquitoes were caught from 1,151 trap collections with 16,270 of these being *An. gambiae s.l.*. Highest densities of *An. gambiae s.l.* occurred in August (Figure 2.3). Tengrela village had the highest density of *An. gambiae s.l.* across the season, reaching a peak of 137 *An. gambiae s.l.* per trap /night in August. Of the 7,615 identified to species, 4,101 (53.9%) were *An. gambiae* s.s. and 2,590 (34.0%) *An. coluzzii;* 3.3% of the *An. gambiae s.l.* were sporozoite positive. The overall EIR in the study area was 80.4 infective bites/child over the six-month transmission season. The village-level EIR ranged from 40.8 in Timperba to 191.9 in Tondoura. Monthly systematic searches of study children's bed nets in the early morning did not collect any mosquitoes. *Anopheles gambiae s.l.* were resistant to 0.05% deltamethrin in WHO tube tests with a mean corrected mortality of 52.3% (95% CI= 26.4-78.2%), with values ranging from 27.2% mortality in Toumousseni to 93.0% in Yendere.

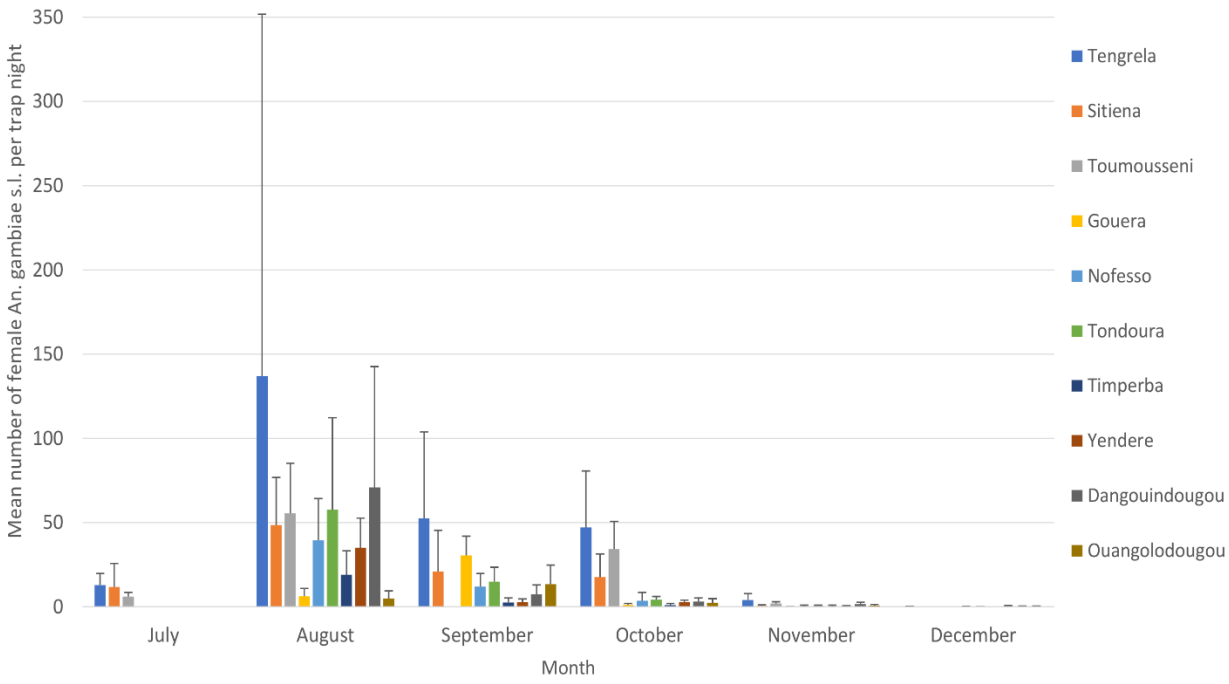

Figure 2.3: Mean number of *Anopheles gambiae* s.l. per trap night in sleeping rooms of study children during the transmission season

The mean number of water bodies with anopheline larvae within 500 m of study houses was 8.7 (95% CI = 7.0-10.3). The average distance by road from the study

house to the nearest clinic was 3.6 km (95% CI = 3.2-4.0), although for the villages of Toundoura and Gouera, which did not have a clinic nearby, the distance was 7.5 km and 9.4 km, respectively.

Correlations between variables were explored and several relationships were found where the correlation was greater than 0.3, which is considered the lower limit for weak associations [238]. There was a weak positive association between floor and wall materials (r=0.3718), between eaves and roof (r=0.3229), occupation and education (r=0.3497), SES and eaves (r=0.3803) and SES and electricity supply (r=0.3012), while there was a weak (negative) association between roof and floor material (r=-0.4138). Correlations suggest that houses with cement or tiled floors also had cement walls; houses with cement walls were more likely to have a metal roof; open eaves were more common in thatched-roofed houses than those with metal roofs; farmers were likely to be illiterate; and richer households were more likely to have electricity. Since these correlations are weak, we do not consider them to have led to exclusion of any important associations in the final model.

Univariate Poisson regression analysis identified two variables associated with incidence of *P. falciparum* infection in the study children and both remained significant in a multivariate model (Table 2.2). Although a small number of children travelled from the study area, this was associated with a 52% increase in the incidence of *P. falciparum* infection compared to children that did not travel (incidence rate ratio (IRR): 1.52, 95% CI: 1.45-1.59, $p<0.001$). The least poor children were found to have a higher incidence of *P. falciparum* infection. A 1 unit increase in the SES factor score was associated with a 5% increase in infection rate (IRR: 1.05, 95% CI: 1.00-1.11, $p=0.04$).

Discussion

An extremely high incidence of *P. falciparum* infection was observed, with only three of the 252 cohort children remaining free from infection during the six-month follow-up period and 86.5% of children experiencing two or more infections. This high incidence of infection, despite regular retreatment with an effective anti-malarial if a child was infected, indicates the high force of infection in the study area and is supported by an estimated EIR of 80.4 infective bites per child during

the transmission season. Similarly, high levels of *P. falciparum* infection and malaria morbidity have been reported from other recent studies in Burkina Faso in areas of high ITN coverage [22, 23, 115].

The assessment of socio-demographic, entomological and environmental risk factors for *P. falciparum* infection did not elucidate any strong associations. It is postulated that this is because the force of infection is so overwhelming in the study area, meaning that all the children were at extremely high risk of infection. Nevertheless, there was an indication of increased malaria risk in the few children that travelled overnight during the study period. Whether staying with family or for farming reasons, these children may be at higher risk of *P. falciparum* infection because they lack or have limited access to malaria prevention including ITNs, and diagnosis and treatment [116]. Overnight travel history was a risk factor for malaria in Uganda in a recent cohort study, with those not using ITNs at particularly high risk [117]. The finding of an association between higher SES factor score and a higher incidence of *P. falciparum* infection was unexpected since it is widely reported that the least poor children are typically at lower malaria risk than the most poor children [102]. The same pattern was also observed when SES quintiles were included in the model. The SES factor score was used in the multivariable model instead of the study quintiles since, unlike quintiles which allocate children into categories, the factor score better reflects the range of values in the dataset. The finding that the least poor children have a higher *P. falciparum* infection risk is not explained by a greater number of passively detected infections in these children. Passive cases contributed only 4% of all cases identified and there were no differences in this proportion across wealth quintiles. *Plasmodium falciparum* infection rates may be higher in the least poor children due to lower ITN use than the most poor children. Only 53.2% of the least poor children were reported to use an ITN the previous night compared to over 82.6% of children in the lowest quintile (most poor).

Table 2.2: Risk factors for *Plasmodium* infection incidence among children aged 5-15 years in Banfora region of Burkina Faso

Variables		Number of children	Number of infections	Time at risk (years)	Rate	**Univariate analysis**		**Multivariate analysis**	
						IRR (95% CI)	P value	IRR (95% CI)	P value
Age (years)	5-7	76	164	31.09	5.28	-	-		
	8-15	176	386	71.40	5.41	1.02 (0.94-1.12)	0.59		
Gender	Male	131	288	52.93	5.44	-	-		
	Female	121	262	49.56	5.29	0.97 (0.90 - 1.05)	0.46		
Ethnic group	Other ethnicity	236	519	95.82	5.42	-	-		
	Fulani	16	31	6.66	4.65	0.86 (0.65-1.13)	0.28		
Caregiver's education	Illiterate	199	443	80.45	5.51	-	-		
	Literate	53	107	22.04	4.86	0.88 (0.76-1.02)	0.09		
Caregiver's occupation	Farmer	240	523	97.39	5.37	-	-		
	Non farmer	12	27	5.09	5.30	0.99 (0.83 - 1.18)	0.89		
Religion	Muslims	170	375	68.97	5.44	-	-		
	Christians	33	67	13.93	4.81	0.88 (0.77 - 1.02)	0.09		
	Animists	49	108	19.58	5.51	1.01 (0.87 - 1.18)	0.85		
SES quintile	Poorest	46	102	20.16	5.06	1.04 (0.99-1.09)	0.14		
	Poor	44	95	18.47	5.14				
	Middle	44	98	18.21	5.38				
	Rich	46	103	19.34	5.33				
	Richest	47	106	17.79	5.96				
SES factor score	1 unit increase	-	-	-	-	1.06 (1.01-1.11)	0.03	1.05 (1.00–1.11)*	0.04
Travel history during the study period	No	246	537	100.93	5.32	-			
	Yes	6	13	1.55	8.39	1.58 (1.31-1.90)	<0.001	1.52 (1.45–1.59)$	<0.001
Slept under bed net previous night	Yes	203	448	83.55	5.36	-	-		
	No	49	102	18.93	5.39	1.00 (0.78-1.29)	0.97		
	≤6	55	124	23.10	5.37	-			

Number of people sleeping in the child room (including child)	6<no.≤12	118	265	46.32	5.72	1.07 (0.94-1.21)	0.33		
	>12	79	161	33.06	4.87	0.91 (0.79-1.04)	0.16		
Bed net integrity (at final survey in December)	Good (pHI: 0-64)	155	331	62.63	5.28	-			
	Damaged (pHI: 65-642)	36	81	15.71	5.16	0.98 (0.83-1.15)	0.76		
	Too torn (pHI: 643+)	58	134	23.52	5.70	1.08 (0.97-1.20)	0.18		
Used other personal protective measures	No	184	407	74.64	5.45	-	-		
	Yes	58	125	24.53	5.10	0.93 (0.81-1.08)	0.36		
Housing: roof of child's sleeping room	Metal	191	420	77.60	5.41	-			
	Non metal	52	114	21.85	5.22	0.96 (0.85-1.09)	0.57		
Housing: floor of the child's sleeping room	Cement/tiles	178	388	72.91	5.32	-	-		
	Dirt	65	146	26.54	5.50	1.03 (0.94-1.13)	0.48		
Housing: wall of the child's sleeping room	Mud	65	151	26.54	5.69	-	-		
	Brick	146	309	59.15	5.22	0.92 (0.83-1.02)	0.12		
	Cement	32	74	13.77	5.37	0.94 (0.83-1.08)	0.39		
Housing: eaves of the child's sleeping room	Open	138	299	56.57	5.29	-			
	Closed	102	229	41.60	5.51	1.04 (0.91-1.20)	0.57		
Cows, horses, sheep or goats within 5m of child's home	Present	169	371	67.80	5.47	-			
	Absent	80	174	33.51	5.19	0.95 (0.83-1.09)	0.46		
Euclidean distance to the nearest positive aquatic habitat	<=300 m	127	266	51.76	5.14	-	-		
	>300 m	125	284	50.72	5.60	1.09 (1.00-1.19)	0.06		
Distance by road to nearest health centre	<=2 km	119	252	49.65	5.08	-	-		
	> 2 km	133	298	52.83	5.64	1.11 (1.00-1.24)	0.06		
EIR (village-level)	-	-	-	-		1.00 (0.99-1.00)	0.08		
% Mortality in WHO tube test with 0.05% deltamethrin diagnostic dose	-	-	-	-	-	1.05 (0.73-1.50)	0.79		
IRR=incidence rate ratio, ***** IRR adjusted for travel history, **$** IRR adjusted for SES factor score									

No association was found between *P. falciparum* infection risk and either of the two entomological variables hypothesized to impact on the primary outcome, namely EIR and insecticide resistance. The lack of association with EIR and vector density is counter-intuitive given it has been demonstrated in several other studies [118, 119], but could arise if vector densities in all villages were sufficiently high to maintain high transmission, or due to outdoor biting. Although levels of insecticide resistance varied between villages, the rate of *P. falciparum* infection was not greater in villages with higher levels of resistance. This finding may be partly due to the relatively small number of villages surveyed for insecticide resistance, and the resulting lack of statistical power, although no evidence of an association between malaria and insecticide resistance has been found in large area studies in the Sudan and Kenya [97]. Despite the high levels of pyrethroid resistance in the study area, no malaria vectors were found under the children's ITN after 1,156 searches. This would imply that the nets are still providing some level of personal protection since a study in The Gambia found malaria mosquitoes under 48.3% of untreated bed nets following 1,584 net searches [120]. Another study conducted in Kenya in an area of pyrethroid resistance found live *An. gambiae* s.l. in holed ITNs, with significantly higher numbers in nets with holes greater than 50 sq cm in size [121]. Although not measured in this cohort, early evening malaria vector biting is found in the study area [122]. This is a time when it is common to find communities active in the peridomestic environment, for example cooking, eating or socializing. In the study site an estimated 85% of exposure to malaria vector bites can be prevented by use of ITNs from 22.00 to 05.00 but early evening biting outdoors and sporozoite rates of 3.3%, mean that residents are still exposed to ~32 infectious bites per person per year even with high ITN compliance [122]. Early evening biting outdoors when people are active in the peridomestic environment is common across Africa [123], and highlights the need for vector control tools that can protect outdoors, such as insecticide-treated 'eave ribbons', attractive targeted sugar baits or larval source management [124-127].

ITN use was high with 80.6% of caregivers reporting that the study child slept under an ITN the previous night at the baseline survey. This is in line with other surveys including the 2017-8 MIS survey, which reported that 76.0% children under 5 years slept under an ITN the previous night [27]. The finding of lower

reported ITN use in the least poor children (quintile 5) was unexpected. Other studies indicate higher ITN use among the least poor, including the 2018 Burkina Faso MIS survey in all ages which shows the least poor (quintile 5) have 1.4-1.6 times the odds of using an ITN the previous night compared to the most poor (quintile 1). It will be important to explore the reasons for the apparent lower ITN use among the least poor children in future studies. Data on ITN ownership or access was not collected as part of this study and so it is not known whether this is the cause of lower ITN use among the least poor children. Studies across sub-Saharan Africa indicate that the net-use gap is primarily driven by intra-household access [128]. Despite the overall high reported bed net use in the study population and considering that the NMCP campaign only took place the year before the study started, there was cause for concern about the durability of the ITNs since only 62.2% of the children's ITNs were in good condition at the final survey. Although a longitudinal survey of the ITNs was not conducted, the finding is supported by studies from other countries. For example, in Tanzania a randomized double-blind prospective evaluation of the lifespan of three ITN products found that the functional survival (ITNs in serviceable condition) was 2 years for Olyset™ and 2.5 years for Permanet® ITNs [99], the two types of ITN delivered by the Burkina Faso NMCP. Bio-efficacy of the sample of children's ITNs evaluated showed low 24-hour mortality of 42.2% for Olyset™ nets and 39.9% for Permanet®, substantially below the ≥80% mortality threshold set by WHO. Thus, there is a great deal of uncertainty about net use and the protective efficacy of ITNs in Burkina Faso. The analysis shows that there was no difference in malaria risk between ITN users and non-users, nor that children sleeping under badly torn nets were more likely to have *P. falciparum* infections than those that slept under good nets.

The study has several limitations. Firstly, and most importantly, the study was probably underpowered to detect small risk factors. The sample size calculation assumed 50% prevalence of risk factors in the study population, while the study children were, in reality, relatively homogeneous with regard to risk factors shown to be important in other studies, for example, house construction. Secondly, while caregivers reported high compliance with ITN use, assessing ITN use is notoriously difficult, and this may have impacted on the ability to identify this as an important risk factor. Indeed, social desirability bias and other forms of error mean that

surveys are likely to substantially overestimate use [129], and objective and unobtrusive tools to measure ITN compliance are not currently available.

Burkina Faso is one of ten sub-Saharan Africa countries designated as a High Burden High Impact country with a response plan, including increased political will, strategic use of data to deploy tools for maximum impact and a multi-sectoral approach. While this study generates useful data on malaria burden in Banfora District, it is clear that additional tools will be needed to reduce this burden. Dual-active ITNs (pyrethroid plus piperonyl butoxide (PBO) or pyrethroid plus chlofenapyr) are now being deployed in the study area. PBO-pyrethroid ITNs have been shown to be more effective in reducing malaria than pyrethroid-only ITNs in areas of pyrethroid-resistant vectors [130, 131], and monitoring of the effectiveness of these dual active ITNs is ongoing in Burkina Faso. Indoor residual spraying (IRS) should also be considered as it has been shown to be effective in reducing malaria in other high-burden countries. In an area of Uganda with an EIR of 176 infective bites/year, three rounds of IRS with the carbamate insecticide, bendiocarb, every six months reduced malaria incidence from 3.3 episodes to 0.6 episodes per person year [132]. Even this effective combination of interventions is, however, insufficient to eliminate malaria, so further interventions are required. SMC is currently being used in 12 sub-Sahelian countries, including Burkina Faso, in children up to 5 years of age, with a protective efficacy of over 50% against parasitaemia in Burkina Faso [104, 133]. A recent trial in Senegal found similarly high reductions in malaria when SMC was used in children aged under 5 years and in children aged 5 to 9 years [134]. Expanding the age range eligible for SMC could, therefore, be effective in reducing malaria further in Burkina Faso. Burkina Faso is also the site of pilot testing of gene drive mosquitoes, which if proven to be efficacious, feasible and acceptable, could be a potential option for malaria control in Burkina Faso.

Conclusions

The study found overwhelmingly high levels of malaria transmission in Banfora district, in southwest Burkina Faso, and the risk factor survey did not identify any risk factors for further investigation to reduce the malaria burden. The findings

have implications for achievement of the ambitious goals set out in the WHO Global Technical Strategy [10]. Malaria elimination in this area of intense seasonal transmission can only be achieved through the use of additional interventions.

Abbreviations

ACT=artemisinin combination therapy, AL= artemether-lumefantrine, CI=confidence interval, CNRFP= Centre National de Recherche et de Formation sur le Paludisme, EIR=entomological inoculation rate, IRR=incidence rate ratio, IRS=indoor residual spraying, ITN=insecticide-treated net, NMCP=National Malaria Control Programme, PBO= piperonyl butoxide, PCR=polymerase chain reaction, pHI= proportionate hole index, SD=standard deviation, SES=socio-economic status, SMC=seasonal malaria chemoprevention, WHO=World Health Organization

Ethics approval and consent to participate

The caregivers of study participants provided informed consent (or assent of child if aged 12-15 years) to participate in the study. Study documents were approved by the Burkina Faso Ministry of Health Research Ethics Committee (Deliberation No 2016-12-137), CNRFP Institutional Bioethics Committee (N°2016/000007/MS/SG/CNRFP/CIB), Durham University Department of Biosciences Ethics Committee (SBBS/EC/MIRA) and Liverpool School of Tropical Medicine Ethical Committee (Protocol number: 16/047). The study was conducted in compliance with principles set out by the International Conference on Harmonization Good Clinical Practice, the Declaration of Helsinki and the regulatory requirements of Burkina Faso.

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Chapter 3

Risk of falciparum malaria infection in an area of intense transmission in Burkina Faso: a community-wide cross-sectional survey.

Abstract

Background

Burkina Faso has one of the highest malaria burdens in sub-Saharan Africa despite the massive deployment of insecticide-treated nets (ITNs). We used a cross-sectional survey to identify risk factors for malaria infection in rural Burkina Faso that might be used to identify and target malaria control measures.

Methods

A cross-sectional survey of 1,199 children and adults (stratified into three age groups, 2 to <10 years, 10 to <30 years, ≥30 years old) was conducted in 10 villages during the peak malaria transmission season in south-west Burkina Faso. *Plasmodium falciparum* infection was diagnosed by microscopy and PCR and data collected on age, gender, ethnicity, socioeconomic status, house construction, presence of animals, use of personal protection and travel history. Binary logistic regression was used to identify risk factors for malaria infection.

Results

Overall *P. falciparum* prevalence detected by microscopy was 32.8%; with 41.9% of children aged 2 to <10 years old, 40.6% in 10 to <30 years old and 16.1% of those > 30 years old infected. Prevalence of symptomatic malaria was 2.7% in the entire study population. 3.3% of the study population had high density *P. falciparum* parasitaemia (>5,000 parasites/μL). Children aged between 5 and 15 years were at highest risk of *P. falciparum* infection. Children aged 5 to <10 years old were at 3.8 times the odds (95% CI= 2.4–6.0, p<0.001) and children aged 10 to 15 years old at 4.3 times the odds (95% CI= 2.6 – 7.1, p<0.001) of *P. falciparum* infection compared to children aged less than 5 years old. Literacy was associated with increased risk of *P. falciparum* infection (OR=1.71, 95% CI= 1.26 – 2.32, p<0.001). There was a weak association between use of an electric fan

(OR=0.55, 95% CI= 0.31 – 0.99, p=0.05) and female gender (OR=0.77, 95% CI=0.58-1.02, 0.07) and decreased risk of *P. falciparum* infection.

Conclusion

Malaria infections were high in all age strata, although highest in children aged five to 15 years, despite universal coverage with ITNs and prompt and effective treatment with antimalarials. Although ITNs provide some protection, additional interventions are required to reduce the burden of malaria in this area of intense transmission. Extension of the eligibility criteria for Seasonal Malaria Chemoprevention may be an option given the burden of infection in school age children.

Introduction

Impressive reductions in malaria have occurred throughout sub-Saharan Africa from 2000-2015 [4]. Progress, however, has not been geographically uniform. In many high-burden countries, parasite prevalence rates and mortality from malaria remain obstinately high despite high coverage with insecticide-treated nets (ITNs), the main preventative measure recommended by the World Health Organisation (WHO) [17].

Burkina Faso is one of the ten countries in the world with the highest burden of malaria and has been designated as a High Burden High Impact country by WHO with calls for a more aggressive approach to malaria control [83]. Despite having national ITN distribution campaigns in 2010 and 2013, the 2014 malaria indicator survey (MIS) found 61% of children aged <5 years old were positive for malaria parasites [135]. In this highly endemic area, the burden of clinical malaria is highest in infants and young children, with modelling showing that 60-70% of cases are in under five year olds in south-west Burkina Faso [136]. Furthermore, data from the National Malaria Control Programme shows that malaria cases have risen from 7,814,634 cases in 2014 [137] to 11,463,808 cases in 2018 [19]. This is surprising since ITN use has been shown to reduce clinical incidence by >50% in areas of stable malaria [95]. The reasons for this control failure may be due to the rise in resistance of vectors to pyrethroid insecticides used to treat ITNs [92], low net usage or lack of net durability or some other factors. A cross-sectional survey was therefore carried out to determine the prevalence of *Plasmodium*

falciparum infection in different age groups and identify risk factors in Banfora Health District in south-west Burkina Faso. This information could be important for developing future malaria control strategies that could be adopted to bring down the burden of malaria.

Methods

Study site

The study was conducted in Banfora Health District, an area of Sudanian savannah in the Cascades region, in south-west Burkina Faso (lying between 10°40' to 10°04'13'' North latitude and 5°01'21'' to 4°46'18'' West longitude). Human settlements are situated across broad valleys inundated with slow-moving rivers and streams. There were 713,059 habitants in 2018 [138] from three major ethnic groups: Gouin, Karaboro and Turka. This is an area of intense seasonal malaria transmission with peaks during the rainy season, from May to November [21], with most cases occurring in September [103]. Farming and animal husbandry are the main activities in rural areas. *Plasmodium falciparum* accounts for 90% of cases [21] and the main malaria vectors are *Anopheles gambiae sensu stricto*, *An. arabiensis*, and, to a lesser extent, *An. coluzzii* [103]. The National Malaria Control Programme (NMCP) undertook an ITN universal coverage campaign (distribution of one ITN for two persons) in 2010, 2013 and 2016 using nets either treated with permethrin or deltamethrin from BASF, Sumitomo Chemical and Vestergaard. No additional nets were distributed by the study team. Indoor residual spraying was not conducted during the study or the preceding 12 months. Since 2014, children under five years old receive seasonal malaria chemoprevention (SMC) on four occasions every month during the transmission season as per WHO recommendations [139].

Study design and procedures

A random sample of 10 villages were selected from a list of villages in the study area using a two-stage process. Five health centres in the study area were chosen with each health centre having a catchment radius of approximately 10 km. The catchment areas of the five chosen health centres were contiguous and 2 villages

randomly selected from each catchment area, giving a total of 10 villages, at least 3 km apart.

An age-stratified cross-sectional survey of both children and adults in three age groups (2 to <10 years, 10 to <30 years, ≥30 years) was carried out to determine the prevalence of *P. falciparum* by microscopy and PCR. The survey aimed to sample a total of 1,200 individuals, 400 from each of the three age strata. 150 study subjects (50 in each age strata) were randomly selected from the Health and Demographic Surveillance System (HDSS) lists of the 10 study villages and entered the screening process. Each study subject was selected from a different household. The 40 first subjects per age strata and per village which met the inclusion and exclusion criteria were enrolled. Participants were eligible to participate if they met the age criteria and provided informed consent but were excluded if they were currently participating in a trial of a malaria vaccine or drug, under chemoprophylaxis (except for SMC) or currently participating in a related cohort study. The survey was carried out shortly after the peak of malaria transmission in 2017, from October to November, when parasite prevalence would be expected to be highest. Fieldworkers visited the study subject at home to obtain written informed consent to participate in the study. After consenting, information was collected on potential malaria risk factors, following a standard questionnaire used in malaria indicator surveys [140]. During the survey, respondents, or their caregivers, gave information on age, gender, socio-economic status, house construction (wall, floor and roof material, eave closure), presence of electricity or functioning fan, presence of animals within 5 m of the household, use of personal protective measures (ITNs, topical and spatial repellents) and travel history in the previous two weeks. Participants were also asked about any fevers within the previous two weeks and treatment seeking for fever, including whether a blood test was performed, and whether antimalarial treatment was given.

During the clinical survey, a finger-prick blood sample was taken from each participant and the axillary temperature measured using an electronic thermometer. Participants with a temperature of ≥37.5°^{c} were considered to be febrile and history of fever within the previous 48 h was recorded. Two blood slides were made, and filter paper blood spots taken for polymerase chain reaction (PCR). A malaria rapid diagnostic test (RDT) (SD BIOLINE Malaria Ag *P.f*/Pan screening test Abbott, Geonggi-do, Republic of Korea) was performed for point-of-care

diagnosis and those with positive RDTs were offered treatment with artemisinin combination therapy according to national guidelines [25]. Thick blood films were stained with Giemsa and examined under 1000-fold magnification by experienced microscopists centrally at Centre National de Recherche et de Formation sur le Paludisme (CNRFP) in Banfora. Parasite counts were recorded per high power field and 100 fields counted before a slide was declared negative. Parasite density was estimated assuming that one parasite per high power field equals 500 parasites/µl. Two blood slides from each subject were read separately by two microscopists. Discrepancies in positive and negative reads and parasite counts differing by more than 10-fold between the two reads were resolved by the supervisor. Filter paper blood samples were analysed for the presence of 18s rRNA gene using PCR. [141]

Human landing catches were carried out outdoors twice a month during the 2017 transmission season in each village. Four households were sampled on one night every month, with a different group of households selected the following month to maximise spatial coverage. Volunteers collected mosquitoes landing on their legs between 19.00 h to 06.00 h. Mosquitoes were typed to species using established morphological keys [122]. Phenotypic insecticide resistance was measured using WHO tube tests as per standard procedures [112]. Assays were performed with *An. gambiae* s.l. mosquitoes reared from immatures collected in seven study villages (larvae were not found in the other three villages during the period of the survey).

Data handling and record keeping

Data were collected on personal digital assistants (PDAs) provided to fieldworkers and programmed with an electronic data capture system, KoboCollect. Each form on the PDA was piloted ten times prior to roll out in the field. Data forms had drop-down boxes and consistency checks to avoid data entry errors. PDAs were uploaded by fieldworkers weekly to a central computer.

Sample size considerations

A random sample of 400 individuals from each of the three age groups (2 to <10 years, 10 to <30 years, ≥30 years) were selected from 10 villages giving a total sample size of 1200 individuals. Assuming a true parasite prevalence ranging between 40 to 60% across the three age groups [142], the study was able to

measure the point prevalence of *P. falciparum* infection by microscopy with a 5% precision at the confidence level of 95% [113].

Statistical Analysis

The primary outcome was *P. falciparum* infection confirmed by microscopy (any level of parasite density). Secondary outcomes were: i) prevalence of symptomatic malaria defined as axillary body temperature ≥ 37.5°C (or history of fever within the previous 48 h) with microscopically confirmed *P. falciparum* infection, ii) prevalence of high-density *P. falciparum* infection (>5,000 parasites/μL) detected by microscopy, iii) prevalence of *P. falciparum* infection detected using PCR and iv) prevalence of sub-microscopic parasitaemia defined as microscopy negative but PCR positive.

Principal component analysis was used to calculate the socio-economic status (SES) factor score (based on asset ownership and household characteristics). SES factor scores were ranked and households divided into 5 equal wealth quintiles (1 being the poorest, through to 5, least poor). Mean values were compared using a t-test and proportions compared using chi squared tests. Parasite prevalence was estimated as the proportion of subjects infected divided by the number of subjects tested. The entomological inoculation rate (EIR) or estimated number of infectious bites per study participant during the transmission season was calculated using the formula EIR=MaSd where Ma is the human biting rate, estimated from the arithmetic mean number of female *An. gambiae* s.l. caught per human landing catches across the six-month transmission season, where S is the proportion of female *An. gambiae* s.l. found to be sporozoite positive by village and d is the number of days in the transmission season. All confidence intervals (CIs) were two-sided computed using the exact method [113]. Logistic regression was used to investigate the association between malaria infection and risk factors. A multivariate model was constructed using a forwards stepwise process [113] and models compared using a likelihood ratio test. Age and gender was retained in all multivariable models *a priori* to control for any potential confounding effects. All analyses were carried out using Stata 15 (Statacorp, Texas, USA).

Ethical consideration

Ethical approval for this study was provided by the Burkina Faso Ministry of Health Research Ethics Committee (Deliberation No 2016-12-137), CNRFP Institutional Bioethics Committee (No2016/000007/MS/SG/CNRFP/CIB), Durham University Department of Biosciences Ethics Committee (SBBS/EC/MIRA) and Liverpool School of Tropical Medicine Ethical Committee (Protocol number: 16/047). The study was conducted in compliance with principles set out by the International Conference on Harmonization Good Clinical Practice, the Declaration of Helsinki and the regulatory requirements of Burkina Faso.

Results

Participant and household characteristics

A total of 1,199 individuals were surveyed, 399 (33.3%) aged 2 to < 10 years, 397 (33.1%) aged 10 to <30 years and 403 (33.6%) aged ≥30 years (Table 3.1). The mean age of the study population was 23.7 years, ranging from 2.1 to 89.9 years old. 55.0% of the study population were female (660/1199), with lower proportions of females in the 2 to < 10 age strata (47.9%) compared to older age strata (53.9% in 10 to <30 years age strata and 63.3% in ≥30 years age strata, $p<0.001$). The most common ethnic groups were Gouin and Turka (51.9%), Karaboro (18.8%), and other ethnic groups including Mossi, Senoufo, Lobi and Fulani (29.3%). The respondents were Muslim (35.4%), Animists (20.3%) and Christians (14.3%). Most study subjects were illiterate (70.5%) and farmers (54.8%), whereas 43.5% of participants were not active either because they were too young or because they were too old to work. 1142 (95.2%) of respondents reported having access to a bednet, with 1,087 (90.7%) saying they slept under a bednet the previous night of the survey. 9.2% of the population reported using a topical repellent in the last week, whether commercial or traditional. Only 6.2% of the study population reported using insecticide aerosols, mosquito coils or other spatial repellents. The study population was sedentary and only 1.8% reported a history of travel in the previous two weeks before the survey.

Table 3.1: Characteristics of participants of the cross-sectional survey

Characteristic	**Overall, N= 1199**	**2 to <10 years, n=399**	**10 to <30 years, n=397**	**> 30 years, n=403**
Age (mean / SD)	23.7 (19.1)	5.9 (2.3)	18.0 (5.7)	46.9 (12.8)
Gender (female %)	660 (55.0)	191 (47.9)	214 (53.9)	255 (63.3)
Ethnicity				
Gouin/Turka (n/%)	622 (51.9)	213 (53.4)	197 (49.6)	212 (52.6)
Karaboro (n/%)	226 (18.8)	73 (18.3)	72 (18.1)	81 (20.1)
Other ethnic group (n/%)	351 (29.3)	113 (28.3)	128 (32.2)	110 (27.3)
Religion				
Muslim	784 (65.4)	255 (63.9)	270 (68.0)	259 (64.3)
Christian	172 (14.3)	60 (15.0)	58 (14.6)	54 13.4)
Animist	243 (20.3)	84 (21.1)	69 (17.4)	90 (22.3)
Education				
Illiterate	845 (70.5)	298 (74.7)	201 (50.6)	346 (86.1)
Literate	353 (29.5)	101 (25.3)	196 (49.4)	56 (13.9)
Occupation				
Farming	657 (54.8)	40 (10.0)	245 (61.7)	372 (92.3)
Commercial and office workers	20 (1.7)	1 (0.3)	6 (1.5)	13 (3.2)
None or retired	522 (43.5)	358 (89.7)	146 (36.8)	18 (4.5)
Used any bednet the previous night	1087 (90.7)	376 (94.2)	346 (87.2)	365 (90.6)
History of travel in the previous 2 weeks	22 (1.8)	9 (2.3)	5 (1.3)	8 (2.0)
Received SMC in previous month	169 (42.4)			
Eave construction of the sleeping room of the subject (212 missing)				
Closed	789 (65.8)	276 (69.2)	273 (68.8)	240 (59.6)
Open	198 (16.5)	41 (10.3)	74 (18.6)	83 (20.6)
Roof construction of the sleeping room of the subject (212 missing)				
Metal	689 (57.5)	217 (54.4)	229 (57.7)	243 (60.3)
Non-metal (Thatch)	298 (24.9)	100 (25.1)	118 (29.7)	80 (19.9)
Sleeping room of subject has a functioning lightbulb installed (213 missing)	763 (63.6)	274 (68.7)	247 (62.2)	242 (60.0)
Sleeping room of subject has a functioning fan	74 (6.2)	34 (8.5)	16 (4.0)	24 (6.0)

Animals tethered within 5 metres of this part of the house (212 missing)	65 (5.4)	27 (6.8)	19 (4.8)	19 (4.7)
Use of topical repellent in last week	110 (9.2)	38 (9.5)	32 (8.1)	40 (9.9)
Use of spatial repellent in the last week (212 missing)	74 (6.2)	22 (5.5)	24 (6.0)	28 (6.9)

Malaria prevalence

Overall *P. falciparum* prevalence detected by microscopy was 32.8% in the study area (Table 3.2). 41.9% of children aged 2 to <10 years were infected with *P. falciparum*, compared to 40.6% of those aged 10 to <30 years and 16.1% of those aged ≥30 years (p<0.001). Further splitting the age groups, *P. falciparum* prevalence was highest among children aged 5 to <10 years old (53.7%) and 10 to < 15 years old (56.7%) compared to other age strata, with these two age groups responsible for 55.0% of the parasite burden in the population (216/393 *P.f* positive participants) (Figure 3.1). Children aged 5 to <10 years old were at 3.8 times the odds (95% CI= 2.4–6.0, p<0.001) and those aged 10 to 15 years old at 4.3 times the odds (95% CI= 2.6 – 7.1, p<0.001) of *P. falciparum* infection compared to children aged less than 5 years old. Prevalence of symptomatic *P. falciparum* malaria (those with fever or reported fever within 48 h with microscopically confirmed parasitaemia) was 2.7% in the entire study population, with no significant differences across the three age strata. 9.0% of children aged 5 to <10 years had high density *P.f* infections (>5,000 parasites/μL), compared to only 1.0% of those aged 10 to <30 years and none of those aged ≥30 years. The *P. falciparum* gametocyte geometric mean density was higher in children aged 5 to <10 years (49.4, 95% CI=29.3 – 83.1) compared to those aged 10 to <30 years (21.6, 95% CI=12.7 – 36.8) or those aged ≥30 years (17.6, 95% CI=12.5 – 24.7).

Table 3.2: Malariometric characteristics of cross-sectional survey participants

Variable		Overall N= 1199	2 to <10 years N= 399	10-30 years N= 397	> 30 years N= 403
***Plasmodium* infection**					
P. falciparum	n (%)	393 (32.8)	167 (41.9)	161 (40.6)	65 (16.1)
P. malariae	n (%)	9 (0.8)	7 (1.8)	2 (0.5)	0 (0)
Mixed infection (P. falciparum + P. malariae)	n (%)	20 (1.7)	14 (3.5)	5 (1.3)	1 (0.2)
P. ovale	n (%)	0	0	0	0
P. vivax	n(%)	0	0	0	0
P. falciparum high density (>5,000 parasites/µL) prevalence, detected by microscopy	n (%)	40 (3.3)*	36 (9.0)	4 (1.0)	0
Geometric mean of *P. falciparum* density per µL	mean, (95%CI)	573.5 (491.0-669.7)	1166.0 (913.7 – 1488.0)	389.9 (318.4 – 477.4)	216.1 (160.3 – 291.3)
Gametocyte prevalence *P.f* (any density)	n (%)	57 (4.8)	29 (7.3)	14 (3.5)	14 (3.5)
Geometric mean *P.f* gametocytes density per µL	mean (95% CI)	31.3 (22.8 – 43.0)	49.4 (29.3 – 83.1)	21.6 (12.7 – 36.8)	17.6 (12.5 – 24.7)
Fever (body temperature ≥ 37.5°C or history of fever in the previous 48h)	n (%)	80 (6.7)	22 (5.5)	30 (7.6)	28 (6.9)
Prevalence of symptomatic *P. falciparum* malaria (fever + microscopy positive)	n (%)	32 (2.7)	12 (3.0)	15 (3.8)	5 (1.2)
Prevalence of asymptomatic *P. falciparum* infection	n (%)	361 (30.1)	155 (38.8)	146 (36.8)	60 (14.9)
* 2/40 were mixed *P.f/P.m* infections					

Of the 422 participants positive for *Plasmodium* parasites by microscopy, 393 (93.1%) were positive for *P. falciparum*, with only 2.1% found to have *P. malariae* and 4.7% mixed *P. falciparum* and *P. malariae* infection.

The EIR in the study area was 188 bites/human subject/season transmission ranging from 0 in Sitiena village to 336 in Dangouindougou village.

23.4% of respondents (280/1199) were found to be PCR positive for *Plasmodium* parasites which was lower than the proportion detected by microscopy. This is likely to be due to lack of amplification during the PCR reaction and therefore we do not present PCR results further here. We don't know why the PCR results were lower than microscopy. This was a surprising result and the laboratory was confident they performed their analysis following their internal protocol with success and quality control was also successfully performed. Because PCR is more

sensitive, we assumed to do not use PCR results through these chapter 3 data analysis process and we kept microscopy data results to complete the analysis as microscopy is a standard diagnosis method.

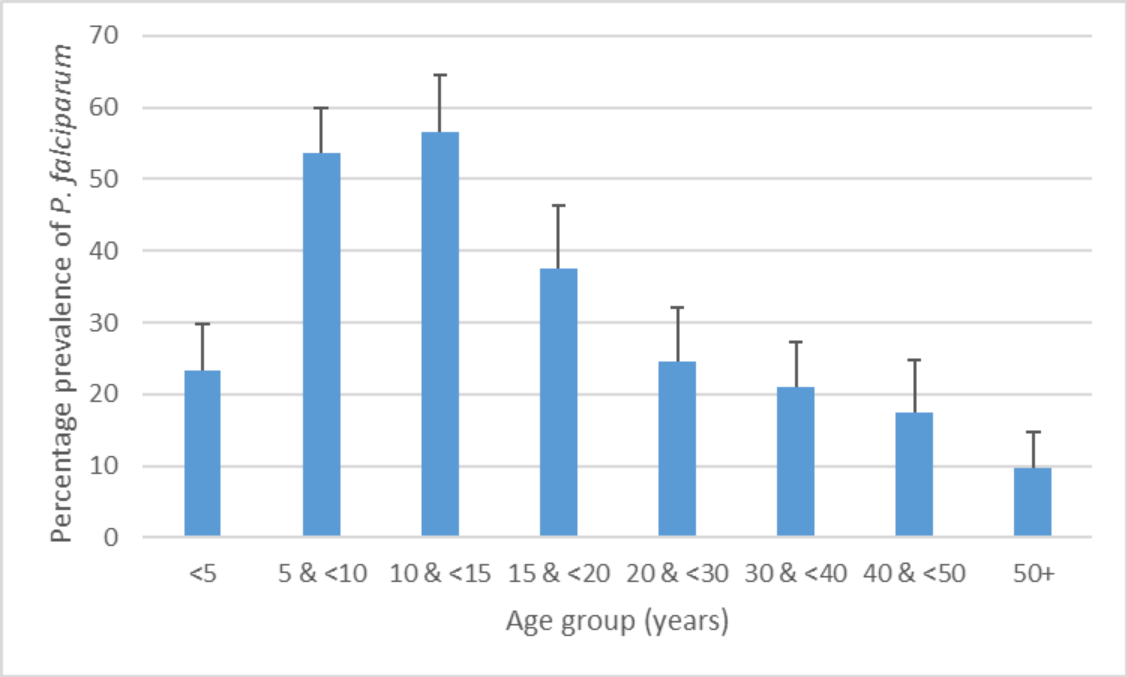

Figure 3.1: Prevalence of *P. falciparum* infection by age group in study population. Error bars are 95% confidence intervals.

Risk factors for malaria infection

Correlations between variables were explored and a weak negative association found between religion and ethnicity (r=-0.4412), while a weak positive association was found between eaves and socio-economic status (r=0.3283). The correlations suggest that Fulani were most likely to be muslims, while richer households were more likely to have open eaves. The latter result was unexpected since we would normally expect richer households to have closed eaves.

In the univariate analysis, literacy (OR=2.26, 95% CI= 1.75 – 2.92, p<0.001) and increasing EIR (OR=1.00, 95% CI=1.00-1.00, p=0.03) were associated with increased risk of *P. falciparum* infection (Table 3). Decreased risk of *P. falciparum* infection was associated with increasing age (1 year increase in age, OR=0.97, 95% CI=0.96-0.97, p<0.001), female gender (OR=0.71, 95% CI=0.56-0.91,

p=0.006) and the use of an electric fan in the sleeping bedroom (OR=0.56, 95% CI= 0.32-0.99, p=0.05). Farming was associated with a lower risk of malaria compared to those with no occupation/retired (OR=0.49, 95% CI=0.39-0.63). No association was found between risk of *P. falciparum* infection and ethnicity, religion, socio-economic status, travel history during the previous 2 weeks, use of a bednet the previous night, roof material, eave status (open or closed), presence of an electric light in the sleeping room, use of topical or spatial repellent, having animals tethered within 5 m of the house and percentage mortality of *An. gambiae* s.l. when exposed to 0.05% deltamethrin in WHO tube tests.

In the multivariate analysis (Table 3.3) literacy (OR=1.71, 95% CI= 1.26 – 2.32, p<0.001) was associated with an increased risk of malaria infection, even after adjusting for age. Age ≥ 30 years (OR=0.27, 95% CI=0.18-0.40, p<0.001) was significantly associated with a decreased risk of *P. falciparum* infection. There was some evidence of an association between sleeping in a room with electric fan (OR=0.55, 95% CI= 0.31 – 0.99, p=0.05) and female gender (OR=0.77, 95% CI=0.58-1.02, 0.07) and decreased risk of *P. falciparum* infection.

We looked for any association between variables and malaria infection risk using corelation table assessment. A table of variables association with other risk factors has been built to check any additional strong association. Table 2 have been added in appendix 3. There was a strong association between distance for all larval habitat and positive larval habitat with malaria risk increase.

Table 3.3: Risk factors for *P. falciparum* infection in study participants

Risk factors	*P. falciparum* prevalence n/N (%)	Univariate analysis		Multivariate analysis	
		Crude OR (95%CI)	P-value	Adjusted OR (95%CI)	P-value
Age (years)					
1 year increase		0.97 (0.96 - 0.97)	<0.001		
2 to < 10	167/399 (42.5)	1		1	
10 to < 30	161/397 (41.0)	0.95 (0.71 – 1.26)	0.71	0.88 (0.63 – 1.22)	0.44
≥ 30	65/403 (16.5)	0.27 (0.19 – 0.37)	<0.001	0.27 (0.18 – 0.40)	<0.001
Gender					
Male	199/539 (36.9)	1		1	
Female	194/660 (29.4)	0.71 (0.56 – 0.91)	0.006	0.77 (0.58 – 1.02)	0.07
Ethnicity					
Gouin and Turka	214/622 (34.4)	1			
Karaboro	65/226 (28.8)	0.77 (0.55 - 1.07)	0.12		
Other ethnic groups	114/351 (32.5)	0.92 (0.69 – 1.21)	0.54		
Education					
Illiterate	232/845 (27.5)	1		1	
Literate	161/353 (45.6)	2.26 (1.75 - 2.92)	<0.001	1.71 (1.26 – 2.32)	0.001
Occupation					
None / retired	217/522 (41.6)	1			
Farmer / pastoral sector	171/657 (26.0)	0.49 (0.39 – 0.63)	<0.001		
Commercial and Office worker	5/20 (25.0)	0.47 (0.17 – 1.31)	0.15		
Religion					
Muslim	253/784 (32.3)	1			
Christian	56/172 (32.6)	1.01 (0.71 – 1.43)	0.95		
Animist	84/243 (34.6)	1.08 (0.80 – 1.46)	0.62		
Socio-Economic Status quintile					
Quintile 1 (lowest)	79/237 (33.3)	1			
Quintile 2 (low)	81/238 (34.0)	1.03 (0.71 – 1.51)	0.87		
Quintile 3 (middle)	84/239 (35.1)	1.08 (0.74 – 1.58)	0.68		
Quintile 4 (high)	72/235 (30.6)	0.88 (0.60 – 1.30)	0.53		
Quintile 5 (highest)	73/237 (30.8)	0.89 (0.61 – 1.31)	0.56		
History of travel in the previous 2 weeks					
No	389/1177 (33.1)	1			
Yes	4/22 (18.2)	0.45 (0.15 – 1.34)	0.15		

Slept under a bednet the previous night					
No	44/111 (39.6)	1			
Yes	348/1087 (32.0)	0.72 (0.48 – 1.07)	0.10		
Eave construction of the sleeping room of the subject					
Closed	258/789 (32.7)	1			
Open	59/198 (29.8)	0.87 (0.62 – 1.23)	0.44		
Roof construction of the sleeping room of the subject					
Metal	229/689 (33.2)	1			
Thatch/mud	88/298 (29.5)	0.84 (0.63 – 1.13)	0.25		
Sleeping room of subject has a functioning lightbulb installed					
No	67/223 (30.0)	1			
Yes	250/763 (32.8)	1.13 (0.82 – 1.57)	0.44		
Sleeping room of subject has a functioning fan					
No	301/913 (33.0)	1			
Yes	16/74 (21.6)	0.56 (0.32 – 0.99)	0.05	0.55 (0.31 – 0.99)	0.05
Animals tethered within 5 metres of the house					
No	298/922 (32.3)	1			
Yes	19/65 (29.2)	0.86 (0.50 – 1.50)	0.61		
Use of topical repellent in last week					
None	355/1089 (32.6)	1			
Yes	38/110 (34.5)	1.09 (0.72 – 1.65)	0.68		
Use of spatial repellent in the last week					
None	290/913 (31.8)	1			
Yes	27/74 (36.5)	1.23 (0.75 – 2.02)	0.40		
EIR (village-level)		1.00 (1.00 – 1.00)	0.03		
% mortality in WHO tube test against 0.05% deltamethrin		1.65 (0.95 – 2.87)	0.08		

Discussion

This study describes the prevalence of *P. falciparum* infection in an area of high and stable malaria transmission [143] with universal ITN coverage since 2010 [144]. This is an area of high malaria transmission where the EIR was 188 infectious bites per human subject over the 6 month transmission season and where children aged 5-15 years have an average of 2.8 episodes of *P. falciparum* infection during the 6 months of the peak transmission season [145]. The study is unusual since most malariometric surveys often focus on the high-risk groups of

children and pregnant women. The overall prevalence of *P. falciparum* infection, detected by microscopy, was 32.8% in all age groups at the end of the malaria transmission season. *P. falciparum* prevalence was, however, highest in children aged 5 to <10 years old (53.7%) and 10 to < 15 years old (56.7%) suggesting these ages are an important reservoir of infection in the community. High infection prevalence in 5-15 year olds may be a result of insufficient and unequal access to ITNs as children grow older and sleep together rather than in the mothers' bed. Anthropological research in the same study area suggests that ITN distributions occur at times of year when family members often moved out of the study area, resulting in insufficient ITNs being provided protection [146]. Studies from other parts of sub-Saharan Africa also report a lower use of ITNs among school-aged children compared to children under 5 years old [147-149] and a sleeping space survey in Uganda found that heads of household sometimes receive priority over children when households have too few nets to cover all members [150]. Age and gender trend in ITN use in sub-Saharan study identify school age children as group of community member which less use ITN with elder aged 50 and more [151]. Another study reported that in malaria endemic countries school-age children are the lest protected with ITN even they are the greatest group of infections reservoir [152]. Also, recent evidence from Uganda reports highest non-adherence of nets use in school-age children, specifically from poorer homes [153]. School-age children, particularly boys may also be more likely to sleep outside, thus increasing their risk of infection [154]. *P. falciparum* prevalence was 23.2% in children aged below 5 years. This compares favourably to the prevalence reported in the 2014 MIS report of 53.3% [108] for the Cascades Region, suggesting that SMC has reduced malaria burden in this group. Early randomised controlled trials of SMC in Burkina Faso demonstrated a protective efficacy of 70% against clinical malaria compared to ITNs alone [155], and quasi-experimental studies of the operational roll-out of SMC show between 50-60% protective efficacy against parasite prevalence [133]. As expected, malaria parasite prevalence and parasite density both decreased in the older age groups, illustrating the impact of acquired immunity [73].

The burden of clinical malaria was relatively low (2.7%) in the study community during this cross-sectional survey and distributed in all age group (3.0% in children aged 2 to <10 years; 3.8% in 10 to <30 years old and 1.2% in 30 years old and

more). This finding suggests the highest prevalence of *P. falciparum* infections was asymptomatic and presents challenges of malaria control since these individuals will not seek care and therefore infections will not be cleared [156]. Few individuals 40 (3.3%) in the survey had high parasite densities (>5000 parasites/µl), and 90.0% of them occurred in children aged 2-10 years old. This result was expected since the burden of malaria is typically higher in the younger age group [23, 157-159]. Overall the *P. falciparum* gametocyte prevalence was low (4.8%), with highest prevalence in children aged 2-10 years old. This finding is much lower to a previous study from Burkina Faso which showed 34.5% prevalence of gametocytes in children aged three to 15 years old [160] and 21.4% all age [161]. Our data recorded microscopy results while others reported qPCR and quantitative-nucleic acid sequence-based amplification (QT-NASBA) analysis results. Also, we may suspect the impact of ACT use. Microscopy results underestimate real gametocytes prevalence.

Literate study participants were found to have 1.7 times the odds of *P. falciparum* infection compared to illiterate participants. This association was unexpected and was present even after adjusting for age (children were more likely to be literate than older study participants due to the introduction of compulsory schooling from ages 6-16 since 2007) [162]. Sleeping in a room with an electric fan was not common in the study population (6.2%), but we found some evidence of an association with use of a fan and reduced *P. falciparum* infection. Ceiling fans or smaller fans may make the sleeping room more comfortable at night, encouraging the use of bednets, as has been found in a study in Ghana [163]. Fans, if they are sufficiently powerful, may also discourage landing and feeding of malaria mosquitoes. Female gender was associated with 0.77 times the odds of *P. falciparum* infection compared to males. In the study area, as found in other studies in SSA, [164] it is common for men to sleep outside at night due to high temperatures with low access to bednets [165]. This is likely to increase infection risk for males compared to females.

We show a high burden of malaria infection despite three ITN universal coverage campaigns in 2010, 2013 and 2016 and malaria case management with effective artemisinin combination therapies. What are the implications of our findings for malaria control in the Cascades region of Burkina Faso? It is well documented that

the burden of malaria shifts to older children as malaria declines [166]. Senegal, another country in the Sahel region with seasonal malaria transmission, has started to implement SMC in children aged up to ten years following the results of clinical trials which showed high protective efficacies against malaria in aged up to ten years [167]. Therefore, this may be an option for Burkina Faso. Implementation of SMC can also have benefits for other age groups. For example, one of the Senegalese trials demonstrated that by implementing SMC to reduce the reservoir of infection, one can reduce the burden of malaria in all age groups, and showed a 26% reduction in incidence of malaria in adults and children too old to receive SMC [167]. Infection risk was highest in the 5-15 year olds who may have insufficient and unequal access to ITNs compared to younger children and those above the age of 15 years. Distribution of additional ITNs could help to increase access for this at-risk group, perhaps using school distributions as a keep-up strategy [168]. Efforts should be made to discourage outdoor sleeping without ITNs, where possible. It may be possible to increase indoor sleeping under ITNs by increasing the thermal comfort of rural African houses using for example, relatively inexpensive solar powered fans [163] or installing two screened windows on opposite walls of the house to encourage cross-ventilation [169]. In the study site, during the hot season, men socialised and slept outside [146] and so indoor sleeping may not be well accepted. Provision of additional ITNs, that could be used outside, during for example funerals when outdoor sleeping is part of local culture [154] may therefore be an option.

Study limitations

Unfortunately, we did not obtain PCR results and so were not able to evaluate sub-microscopic infections. Microscopy results underestimate *P. falciparum* real prevalence in our study findings. There is a need for increase laboratory training and supervision to avoid errors in future studies. We did not observe a relationship between *P. falciparum* infection and bednet use. However, assessment of bednet use through questionnaires is subject to social desirability bias and we lack objective tools for measuring bednet use.

Conclusion

P. falciparum infection in the Cascades Region of Banfora remains high despite universal coverage with ITNs and access to diagnosis and treatment. Our study findings imply that current national strategies for malaria control in Burkina Faso are not sufficient to eliminate the disease. The high prevalence of *P. falciparum* infection in children aged 5 to 15 years suggests that an extension of the age group eligible for SMC may be able to reduce malaria burden substantially.

Chapter 4

Risk factors associated with house entry of malaria vectors in an area of Burkina Faso with high, persistent malaria transmission and high insecticide resistance

Adapted from: Yaro JB, Ouedraogo A, Ouedraogo ZA, Diarra A, Lankouande M, Agboraw E, Worrall E, Toe KH, Sanou A, Guelbeogo WM, Sagnon N, Ranson H, Tiono AB, Lindsay SW, Wilson AL (2020). A cohort study to identify risk factors for Plasmodium falciparum infection in Burkinabe children: implications for other high burden high impact countries. *Malaria Journal*, submitted for publication.

Abstract

Background

In rural Burkina Faso, the malaria vector *Anopheles gambiae* s.l. primarily feeds indoors at night. Identification of factors which influence mosquito house entry could lead to development of novel malaria vector control interventions. A study was therefore carried out to identify risk factors associated with house entry of *An. gambiae* s.l. in south-west Burkina Faso, an area of high insecticide resistance.

Methods

Mosquitoes were sampled monthly during the malaria transmission season using CDC light traps in 252 houses from 10 villages, each house sleeping at least one child aged five to 15 years old. Putative risk factors for house entry of *An. gambiae* s.l. were measured, including socio-economic status, caregiver's education and occupation, number of people sleeping in the same room as the child, use of anti-mosquito measures, house construction and fittings, proximity of mosquito aquatic habitats and presence of animals near the house. Mosquito counts were compared using a generalised linear mixed-effect model with negative binomial and log link function, adjusting for repeated collections.

Results

20,929 mosquitoes were caught, of which 16,270 (77.7%) were *An. gambiae* s.l. Of the 6,691 *An. gambiae* s.l. identified to species, 4,101 (61.3%) were *An.*

gambiae and 2,590 (38.7%) *An. coluzzii*. Having an electricity supply (incidence rate ratio, IRR = 0.4, 95% CI = 0.3–0.7, p = 0.001) and a metal-roofed house (IRR, = 0.6, 95% CI = 0.4–1.0, p = 0.034) were associated with fewer malaria vectors inside the home.

Conclusion

This study demonstrated that there were fewer *An. gambiae* s.l. in homes with electricity and a metal roof compared to those that did not. Brightly-lit, well-built houses with metal roofs may reduce entry of malaria mosquitoes compared to dimly-lit, poorly-built thatched roofed houses.

Introduction

Despite large reductions in the malaria burden across sub-Saharan Africa from 2000-2015 [4], some countries continue to experience extremely high malaria transmission [5]. In Africa, malaria transmission is highly efficient because of the wide distribution of *Anopheles gambiae* s.l., an effective malaria vector that readily feeds readily on people indoors at night, where about 79% of malaria transmission typically occurs [123]. The indoor density of malaria mosquitoes is dependent on numerous environmental and household factors, including the abundance and proximity of aquatic habitats of malaria mosquitoes [48, 170], presence of large domesticated animals who may serve as alternative hosts [171], typology of houses [169, 172], use of anti-mosquito measures in the house [48], the number of residents [173] and variability in the attractiveness of individual people [120] (Figure 4.1).

Burkina Faso is an area of intense seasonal malaria transmission, and cases are increasing [20, 174] despite high coverage of vector control tools, including three national insecticide-treated net (ITN) mass distribution campaigns in 2010, 2013 and 2016 [27]. Resistance to pyrethroids, the main insecticide class used for treating ITNs, is high in *An. gambiae* s.l., and in the study area near Banfora town exposure to ITNs has no impact on the lifelong survival of malaria vectors [93]. New tools are urgently needed to reduce the burden of malaria in Burkina Faso and other countries in sub-Saharan Africa.

Several studies have demonstrated that malaria mosquito house entry can be reduced through simple changes to house design such as closing eaves and

screening windows and doors [175]. The use of personal protective measures such as ITNs and spatial repellents may also reduce transmission [95, 176]. There is a lack of evidence, of whether such methods will reduce house entry of malaria vectors in settings of high insecticide resistance, such as the study site in south-west Burkina Faso. A risk factor survey was conducted to identify variables associated with indoor density of *An. gambiae s.l*. during the malaria transmission season in an area of intense malaria transmission in Burkina Faso. Findings from this study might identify potential opportunities for improving malaria control in Burkina Faso and other countries in sub-Saharan Africa experiencing persistently high malaria transmission.

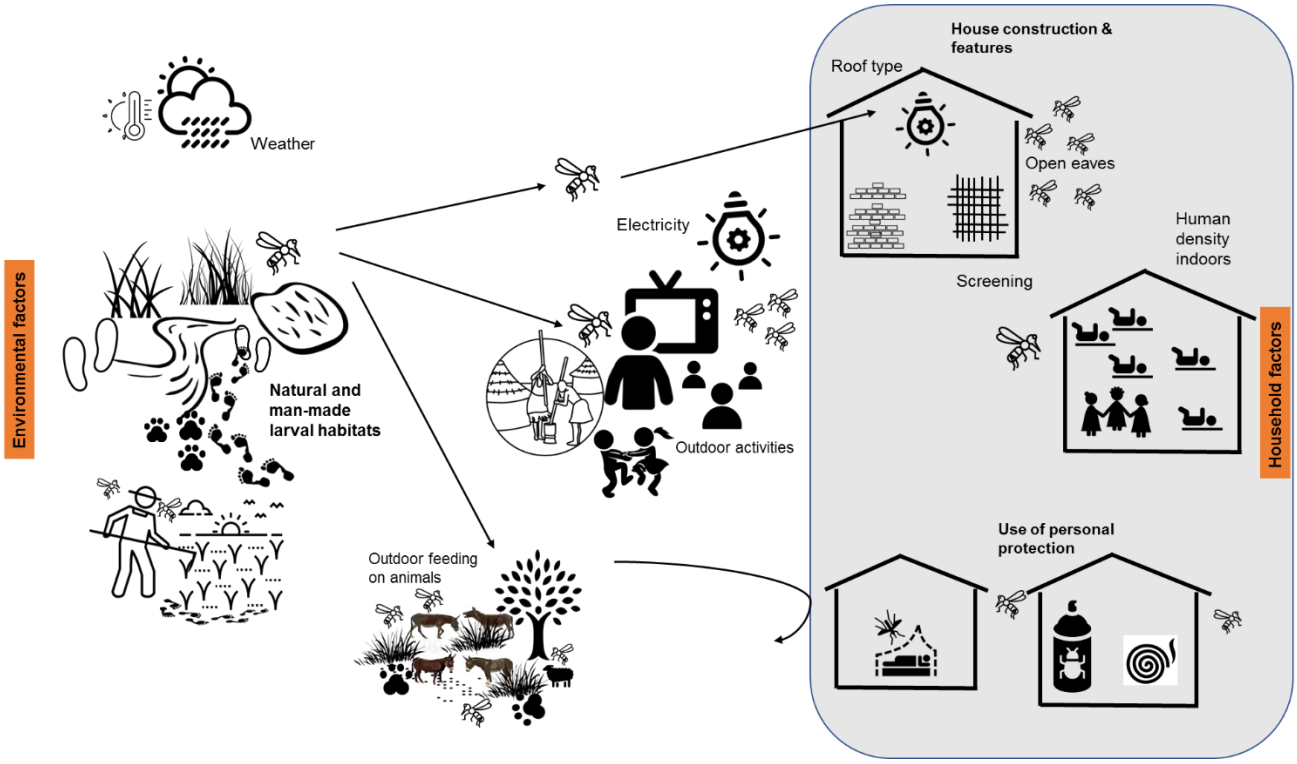

Figure 4.1: Environmental and household factors affecting the abundance of malaria vectors indoors

Methods

Study site

The study was conducted in Banfora Health District, in the Cascades Region, south-west Burkina Faso (Figure 4.2). This is an area of Sudanian savannah covering 6,295 km^2 with an estimated population of 407,073 inhabitants [174]. Malaria

transmission is intense and seasonal, occurring mainly during the rainy season, from May to November [21]. *Plasmodium falciparum* accounts for 90% of cases [177]. The main malaria vectors are *An. gambiae* s.s. and *An. coluzzii* [177]. In 2016, approximately 1 year before this study took place, a universal coverage campaign distributed ITNs with permethrin or deltamethrin (Sumitomo Chemical, Vestergaard and BASF) at a rate of one net for every two people at risk. No additional ITNs were distributed by the study. No indoor residual spraying was conducted. Typically, single room houses each housing a family are organised into compounds, consisting of on average 4 houses, led by a compound head.

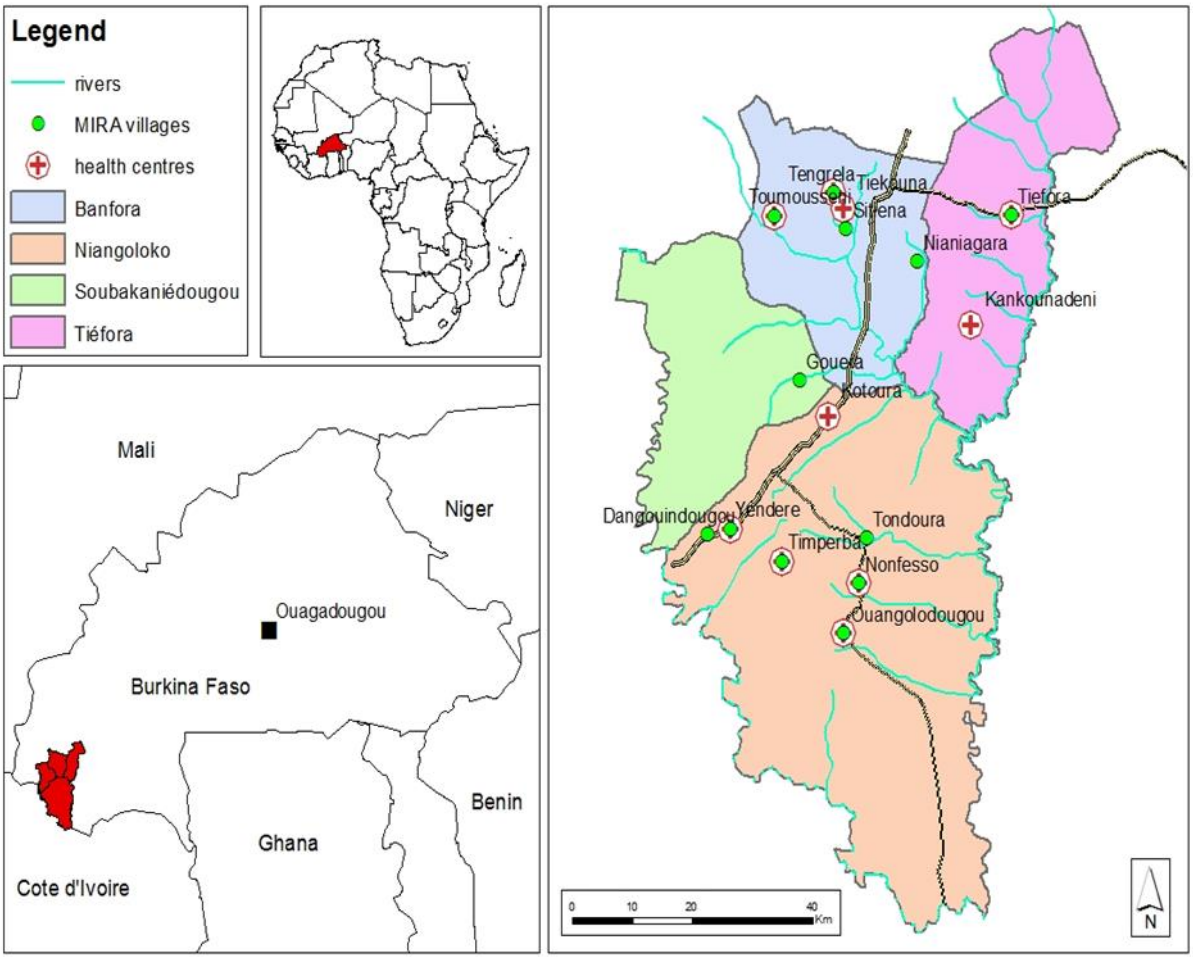

Figure 4.2: Map of study area

Study design

The study was nested in a cohort study of risk factors for *P. falciparum* infection in children aged five to 15 years [145]. This study reports on the household and environmental risk factors associated with the density of *An. gambiae s.l.* in the

children's sleeping room over six months of high malaria transmission from July to December 2017.

Recruitment of study cohort

Sampling and recruitment of the study cohort is described elsewhere [145]. In brief, a random sample of 10 villages were selected from a list of villages in the study area using a two-stage process. Firstly, five health centres in the study area were selected, each with a catchment radius of 10 km. Secondly, two villages, at least 3 km apart, were selected from each catchment area. An enumerated list of children in the study villages was obtained from the Banfora Demographic and Health Surveillance System. From each village, a random sample of 30 children aged five to 15 years were chosen. Each child was selected from a separate house, and, where possible, a separate compound. Children were included in the study if they were of the appropriate age, were likely to remain resident in the village over the duration of the transmission season and the caregiver provided informed consent to participate in the study. 252 children who were successfully cleared of *P. falciparum* infection were included in the cohort study and this current study reports on the entomological surveillance from the children's sleeping rooms.

Entomological surveillance

CDC light traps (John Hock, Gainsville, USA) were used to estimate indoor mosquito densities in the study child's sleeping room. These traps were placed with the bulb 1500 mm above the floor, approximately 500 mm from the foot end of a bed with an ITN occupied by the study child. Houses were sampled from 19.00 h to 06.00 h every four weeks. Typically, houses were sampled once a month, but in some cases two collections were performed. Two villages (Nofesso and Ouangolodougou) were inaccessible for two weeks at the start of the study period due to flooding. Mosquitoes were taken to the laboratory in cool boxes, killed by freezing at −20°C, and identified morphologically using established keys [178]. The presence of circumsporozoites protein (CSP) in *An. gambiae s.l.* were identified using an enzyme-linked immunosorbent assay [179] and *An. gambiae s.l*. females typed to species by PCR [180, 181].

Risk factor assessment

In June, a questionnaire was administered to the caregiver of the study child to collect information on ethnicity, education level and occupation of caregivers, ITN use during the previous night, use of other protective measures (e.g. insecticide knockdown spray, mosquito coils, traditional spatial repellent), number of people sleeping in the room with the child, roof, wall and floor construction of the child's sleeping room, whether the eaves (the gap between the top of the wall) were open or closed, presence of mosquito screening and electricity supply. Information was also collected from the head of the child's household on asset ownership and household characteristics, following standard procedures used in the Burkina Faso Malaria Indicator Survey [108]. The number and type of large domestic animals (cattle, goats, sheep, pig, dog, donkeys or horses) tethered within 5 m of the house was recorded by a fieldworker. The house was geo-located using a handheld global positioning system (GARMIN eTrex 20). Larval surveys were carried out in each village in September, during the peak of the transmission season. All water bodies within 1 km from a village were mapped, including irrigated fields, streams and ponds, puddles, and foot or hoof prints. The presence of anopheline larvae was recorded with a dipper.

Data management and statistical analysis

Data were collected on Android personal digital assistants programmed using the KoboCollect system and included drop down boxes and consistency checks to reduce data entry errors. Following cleaning, the dataset was locked and saved in Microsoft Access. The primary outcome was the number of *An. gambiae s.l*. collected in each child's sleeping room per night. QGIS Geographic Information System (QGIS Development Team (2019), Open Source Geospatial Foundation Project) was used to determine distances between the child's home and aquatic habitats. Principal component analysis (PCA) was used to calculate the socio-economic status (SES) factor score of the head of the child's household. SES factor scores were ranked, and households divided into five equal wealth quintiles, from 1, the poorest, to 5, the least poor. The entomological inoculation rate (EIR) or estimated number of infectious bites per study child during the transmission season was calculated using the formula EIR=*MaSd* where *Ma* is the human biting

rate, estimated from the arithmetic mean number of female *An. gambiae* s.l. caught per light trap night across the six-month transmission season, where *S* is the proportion of female *An. gambiae* s.l. found to be CSP positive by village and *d* is the number of days in the transmission season (n). Mean values were compared using a t-test and proportions compared using chi-squared tests. A generalised linear mixed-effect model with a negative binomial distribution, to account for overdispersion, and log link function was used to identify risk factors associated with the mean number of *An. gambiae s.l.* per catch night per house each month. Risk factors were selected *a priori* based on importance for malaria vector house entry. These were SES quintile, ITN use, use of other protective measures, number of people sleeping in the room with the child, roof, floor and wall material in the sleeping room, eaves (open or closed), electricity supply, presence of large domesticated animals within 5 m of the house and proximity of habitats positive for anopheline larvae. A random effect for study child ID number was used to account for repeated measures on the same house and village was included as a fixed effect. Following univariate analysis, each risk factor with $P<0.1$ was incorporated into a multivariate model which was refined through a process of backwards stepwise elimination using a likelihood ratio test. Interactions were tested between a subset of variables that were thought to be biologically relevant to explore. Means and 95% confidence intervals were calculated. Statistical analysis was carried out in Stata 15 (Statacorp, Texas, USA). The study is reported following STROBE guidelines [114].

Results

As reported elsewhere [145], a total of 20,929 mosquitoes were caught from 1,151 trap collections in the 252 study houses, with 16,270 of these being *An. gambiae* s.l. (77.7%). Of the 6,691 *An. gambiae* s.l. identified to species (excluding 924 lost and non-identified samples), 4,101 were *An. gambiae* s.s. (61.3%) and 2,590 *An. coluzzii* (38.7%). 3.3% of *An. gambiae* s.l. were CSP positive and the overall EIR in the study area was 80.4 infective bites/child over the six-month transmission season. The village-level EIR ranged from 40.8 in Timperba to 191.9 in Tondoura.

The ethnic composition of the study population was Gouin (38.9%), Karaboro (21.8%), Mossi (11.5%), Turka (9.1%), Fulani (6.3%), Senoufo (4.4%) and other

ethnic groups (7.9%) (Table 4.1). Caregivers were predominantly illiterate (79.0%) and farmers (95.2%). 80.6% of caregivers reported that their child slept under an ITN the previous night, while 15.9% reported using mosquito coils and 6.4% insecticide knockdown spray. Children's sleeping rooms were constructed with predominantly brick walls (57.9%), cement or tiled floors (70.6%), metal roofs (75.8%) and open eaves (54.8%). Window screening was rare (0.4%). 67.1% of households had large domestic animals (cattle, goats, sheep, dogs, pig, donkeys or horses) within 5 m of the house. 50.4% of child's households were located within 300 m of an aquatic habitat containing anophelines.

Sleeping spaces in metal roofed houses were more likely to have walls and floors made of finished materials, open eaves, be less crowded and have an electricity supply than thatch roof sleeping spaces. 81.7% of sleeping spaces with a metal roof had a cement or tiled floor compared to 42.3% of those with a thatch roof ($p<0.001$). Metal roof sleeping spaces were also more likely to have brick or cement walls (77.0%) compared to thatch roof sleeping spaces (55.8%, $p<0.001$). Sleeping spaces with a metal roof were also more likely to have open eaves (66.0%) and an electricity supply (51.8%) than sleeping spaces with a thatch roof (23.1% and 28.8% respectively, $p<0.001$ and $p=0.003$). 26.2% of sleeping spaces with a metal roof had more than 12 people sleeping in them compared to 38.5% of sleeping spaces with a thatch roof ($p<0.001$). There was no association between metal roof sleeping spaces and distance from the nearest anopheline larvae positive habitat.

Assessment of linear correlation between electricity or roof type and the other variables included in the model did not identify any correlations, including with SES. As such, we consider that these variables were acting on mosquito house entry independently. That said, we cannot account for unmeasured risk factors or poorly measured risk factors. In this regard, we are aware of the limitations of measuring SES, for example. We have removed the statement in the discussion regarding the potential for electricity to be a proxy for SES.

Univariate analysis of putative risk factors showed that there was an association between *An. gambiae s.l.* abundance and roof and floor materials, electricity supply, and proximity of positive larval habitats (Table 4.2). There was a 40% reduction in the rate of *An. gambiae* s.l. if the child slept in a room with a metal roof (IRR = 0.6, 95% CI 0.4 – 0.9, p = 0.026), increasing to a 60% reduction if

Table 4.1: Characteristics of the study participants and their houses

Characteristic		Number (%) N=252
Socio-demographic characteristics		
Ethnicity	Gouin	98 (38.9%)
	Karaboro	55 (21.8%)
	Mossi	29 (11.5%)
	Turka	23 (9.1%)
	Fulani	16 (6.3%)
	Senoufo	11 (4.4%)
	Others	20 (7.9%)
Caregivers education level	Illiterate	199 (79.0%)
	Primary school	45 (17.9%)
	Secondary school or above	8 (3.2%)
Caregivers occupation	Farmer	240 (95.2%)
	Non-farmer	12 (4.8%)
Number of people sleeping in the child room (including child)	≤6	55 (21.8%)
	7-12	118 (46.8%)
	>12	79 (31.3%)
Use of personal protective measures		
Reported ITN use	Used ITN usually	215 (85.3%)
	Used an ITN the previous night	203 (80.6%)
Use of other personal protection methods	Coils	40 (15.9%)
	Insecticide spray	16 (6.4%)
	Traditional repellent (non-topical)	2 (0.8%)
	None	184 (73.0%)
House construction		
Roof material of child's sleeping room	Metal	191 (75.8%)
	Non-metal (Thatch/mud)	52 (20.6%)
Wall material of child's sleeping room	Mud	65 (25.8%)
	Brick	146 (57.9%)
	Cement blocks (plastered or painted)	32 (12.7%)
Floor material of child's sleeping room	Cement/tile	178 (70.6%)
	Mud	65 (25.8%)
Eave status of child's sleeping room	Closed	102 (40.5%)
	Open	138 (54.8%)
Window screening of child's sleeping room	Absent	242 (96.0%)
	Present	1 (0.4%)
Electricity supply in the child's sleeping room	Present	115 (45.6%)
	Absent	111 (44.0%)
	Missing	26 (10.3%)
Environmental factors		
Presence of large domestic animals within 5 m of the household	Present	169 (67.1%)
	Absent	80 (31.7%)
Proximity to anopheline positive larval habitats	<300 m	127 (50.4%)
	≥300 m	125 (49.6%)

there was an electricity supply in the sleeping room of the child (IRR = 0.4, 95% CI 0.3 – 0.7, p = 0.001). A mud floor was associated with 1.5 times the rate of *An. gambiae* s.l. compared to a cement or tiled floor (IRR = 1.5, 95% CI 1.0 – 2.4, p=0.043). There was 50% increase in the rate of *An. gambiae* s.l. when the child's house was >300 m from a larval habitat containing anopheline mosquitoes (IRR = 1.5, 95% CI 1.0 – 2.3, p = 0.032).

In the final multivariate model, having an electricity supply in the child's sleeping room (IRR = 0.4, 95% CI 0.3 – 0.7 p < 0.001) and a metal roof (IRR = 0.6, 95% CI 0.4 – 1.0, p = 0.034) were associated with fewer malaria vectors indoors (Table 4.2). Inclusion of eave status did not improve the model fit or alter the IRR substantially and an interaction between roof type and eave status was not significant.

Discussion

Our findings demonstrate highly intense transmission of malaria in the study area with a person sleeping without an ITN experiencing a seasonal EIR varying from 40.8 infectious bites per person in Timperba village to 191.9 in Toundoura village [145]. Malaria vector abundance rises in July after the start of the rains in May, reaching a peak in August, before declining to low levels in November and December.

Having a supply of electricity in the sleeping room of the child and a metal roof were both associated with fewer *An. gambiae* s.l. malaria vectors entering houses. Fewer *An. gambiae* s.l. found in houses with electricity may be due to the use of electric lights or fans and this hypothesis requires further investigation. The relationship between electrification and malaria is not well established and there are only a few, low quality studies on this topic, with most indicating a higher risk of malaria given electrification [182-184]. Electrification was associated with a two-fold increase in the odds of clinical malaria in a case control study in Burkina Faso [185]. An increase in malaria may results from mosquitoes being attracted to light. For example, the CDC light trap is thought to be attractive at distances of 5 m [186], and may increase indoor catches of mosquitoes if the light is seen from outside the house [187]. Qualitative studies suggest that outdoor lighting and

ownership of televisions may also increase in malaria risk due to the extension of the period of outdoor activity [154, 188, 189]. Alternatively, there is evidence that lighting is protective against mosquitoes. The disappearance of malaria in England was associated with improvements in housing including better lighting, improved ventilation, drier and more spacious rooms, better ceiled and plastered and less crowded bedrooms [190]. Responses of mosquitoes to light is likely to be more

Table 4.2: Risk factors for *An. gambiae s.l.* abundance in study children's sleeping room

Variable	Mean mosquito density per month (95% CI)	Univariate analysis		Multivariate analysis	
		IRR (95% CI)	P value	IRR (95% CI)	P value
Socio-economic status of child's household					
Poorest	23.0 (10.0 – 36.1)	1			
Poor	14.1 (6.8 – 21.3)	0.8 (0.4 – 1.4)	0.4		
Middle	11.8 (7.7 – 15.9)	0.9 (0.5 – 1.7)			
Rich	11.5 (7.3 – 15.8)	0.7 (0.4 – 1.4)			
Richest	12.4 (5.6 – 19.1)	0.7 (0.3 – 1.5)			
ITN use the previous night					
No	7.1 (4.5 – 9.8)	1			
Yes	15.8 (11.7 – 19.8)	1.2 (0.6 – 2.3)	0.6		
Use of other personal protection measures (insecticide knockdown spray, mosquito coils, traditional spatial repellent)					
No	15.8 (11.4 – 20.2)	1			
Yes	9.6 (6.3 – 13.0)	0.9 (0.6 – 1.6)	0.8		
Number of people sleeping in the same room as the study child					
≤6	13.5 (9.6 – 17.4)	1			
7-12	17.1 (10.3 – 23.8)	1.1 (0.7-1.9)	0.7		
>12	10.4 (7.5 – 13.3)	0.8 (0.4 – 1.3)	0.3		
Roof material of child's sleeping space					
Non-metal	14.3 (8.1 – 20.6)	1		1	
Metal	14.4 (10.3 – 18.4)	0.6 (0.4 – 0.9)	0.03	0.6 (0.4 – 1.0)[$]	0.03
Floor material of child's sleeping space					
Cement/tile	13.3 (9.1 – 17.5)	1			
Mud	17.4 (11.4 – 23.3)	1.6 (1.0 – 2.4)	0.04		
Wall material of child's sleeping space					
Mud	17.4 (11.3 – 23.4)	1			
Brick	13.6 (8.5 – 18.6)	1.0 (0.6 – 1.6)	1.0		
Cement	11.6 (7.0 – 16.1)	0.8 (0.4 – 1.7)	0.6		
Eaves of child's sleeping space					
Open	13.8 (9.8 – 17.7)	1			
Closed	14.9 (9.6 – 20.3)	1.0 (0.6 – 1.6)	0.9		
Electricity supply of child's sleeping space					

No	17.9 (11.1 – 24.7)	1		1	
Yes	11.8 (8.9 – 14.8)	0.4 (0.3 – 0.7)	0.001	0.4 (0.3 – 0.7)[&]	0.001
Presence of large domestic animals near the house					
Yes	14.9 (10.1 – 19.7)	1			
No	12.7 (9.3 – 16.0)	1.1 (0.8 – 1.7)	0.6		
Distance to positive larval habitat					
<300m	9.5 (7.3 – 11.8)	1			
>300m	18.6 (12.4 – 24.9)	1.5 (1.0 – 2.3)	0.03		

IRR: incidence rate ratio, CI: confidence interval, *adjusted for repeated measures and village as fixed effect, [$]adjusted for electricity supply, repeated measures and village as fixed effect, [&]adjusted for roof material, repeated measures and village as fixed effect

nuanced, depending not only on the intensity and frequency of the light, but on the time of day a mosquito perceives the light. For example, *An. gambiae* s.s. exposed to white light for 10 min at the start of the night interrupted feeding activity for two to four hours [191]. The use of electric fans is also likely to reduce collections of mosquitoes by light traps since the powerful air current generated by a fan will prevent or greatly disturb mosquito flight. There may also be other explanations for our finding that are more straightforward. Firstly, the use of electricity, as suggested by Yamamoto and co-workers, may lead to a shift away from use biomass fuels and creation of smoke that would repel malaria vectors from entering the house [185]. Secondly, the use of electricity may simply be a proxy for higher socio-economic status and a more mosquito-proof house, along the lines suggested by James in 1920 [190]. Clearly further research is needed to clarify what is contradictory evidence.

Finding fewer mosquitoes in houses with metal roofs compared with thatched roofs has been reported in several studies, including a Tanzanian study where metal roofed houses had 33% less *An. arabiensis* than thatched-roof houses [192, 193], and a Ugandan study where there were 38-43% fewer *An. gambiae* s.l. in metal-roofed houses [194]. Results are, however, contradictory in other studies. In The Gambia metal-roofed houses were not associated with fewer mosquitoes [171], and in an experimental study, metal roofed houses with closed eaves and mud walls had similar numbers of mosquitoes as thatched-roofed houses with open eaves and mud walls [169]. Whether a metal-roofed house has more or less mosquitoes than a thatched-roof house ultimately depends on how porous the house is to mosquitoes and the extent of ventilation [195]. In general, since metal-roofed houses are hotter before midnight than thatched-roofed houses, metal-roofed houses will generate more carbon dioxide from people sleeping in the houses, and therefore attract more mosquitoes, than cooler thatched-roofed houses [169, 196]. However, metal-roofed houses are often better built, with fewer mosquito entry points, than thatched-roofed houses. In such cases, metal-roofs may simply be a marker for a better quality home that is less porous to mosquitoes.

The lower *An. gambiae* s.l. density in metal-roofed houses compared to thatch houses in this study appeared to be operating independently of eave status, since, thatched roofs were more likely to have closed eaves and an interaction between

eave status and roof type was not significant. A final consideration is that there is evidence that the high temperatures experienced in metal roof houses in the hot humid tropics, increases the mortality of malaria vectors resting indoors during the day [195]. Thus, the reduction in mosquitoes found in metal-roofed houses may be partly due to the higher temperatures experienced indoors.

The study has several limitations. Firstly, both electricity supply and metal-roofed houses are associated with high SES. Adjusting the final model for SES quintile did not impact on the results. Despite this, the SES quintile is a crude measure and there may be other features of wealth that reduce malaria mosquito numbers in houses with electricity supply and metal roofs. Second, ITN use the previous night was assessed by asking the caregiver which may be prone to social desirability bias [129]. The use of an ITN will usually vary over the transmission season, but we only measured use during the baseline survey. This may have impacted on our ability to identify an association between ITN usage and indoor density of malaria vectors.

The cohort study in which this entomological study was nested did not identify strong risk factors for *P. falciparum* infection, with only overnight travel and higher SES factor score being associated with higher rates of *P. falciparum* infection [145]. It is difficult to reconcile the entomological and epidemiological findings and further studies are needed. It is perhaps unsurprising that the risk factors for malaria vector density and *P. falciparum* infection in children differed, since higher indoor vector density does not automatically imply higher infection risk. The indoor density of malaria vectors may be less important in this study area due to the observation of increasing outdoor biting with some studies suggesting ~54% of *An. gambiae* s.l. host seeking outdoors [122]. Research also suggests that the study communities spend more time outside in the peri-domestic environment during peak biting times than previously thought [146].

What are the implications of the study findings for vector control and future research? Increased access to electricity in sub-Saharan Africa raises questions about the impact on vector behaviour (e.g. whether lights attract mosquitoes or leads to mosquito avoidance behaviour), human behaviour (e.g. alteration of time to bed or use of fans) and malaria risk which are complex and yet to be understood. Further research on this topic is needed. The risk factor study also highlights the

potential of improved housing to reduce malaria transmission and supports the results of systematic and multi-country research studies on this topic [85, 102]. Housing improvements tend to be implemented as a package and, in line with this, our study found that metal roof sleeping spaces were more likely to have floors and walls made of finished materials, be less crowded and have an electricity supply than thatch roof sleeping spaces. Improving house construction should be a focus for malaria reduction [197], with increasing evidence in support of screened, self-closing doors, closed eaves, raising buildings off the ground, screened windows on either side of building for ventilation and solid roofs [175]. As well as contributing to the development agenda, there is also evidence that improved housing can reduce risk of other major causes of death in children including diarrhoea, growth failure and anaemia [198]. While other vector control tools such as dual-active ITNs are now being deployed in the study area, the study results highlight the importance of non-insecticidal interventions such as house improvement to increase long-term resilience against malaria and for insecticide resistance management.

Conclusion

This study in south-west Burkina Faso demonstrates reduced indoor density of malaria vectors in houses with electricity and a metal roof. Further research is needed to unpack the implications of electrification and metal roof housing on malaria risk; however, this study adds to the growing evidence base supporting the use of housing improvement against malaria.

Ethics approval and consent to participate

Permission to enter the communities was sought from village leaders. The caregivers of study participants provided informed consent (or assent of child if aged 12-15 years) to participate in the cohort study and for collection of mosquitoes from the child's sleeping room. Study documents were approved by the Burkina Faso Ministry of Health Research Ethics Committee (Deliberation No 2016-12-137), CNRFP Institutional Bioethics Committee (No2016/000007/MS/SG/CNRFP/CIB), Durham University Department of Biosciences Ethics Committee (SBBS/EC/MIRA) and Liverpool School of Tropical

Medicine Ethical Committee (Protocol number: 16/047). The study was conducted in compliance with principles set out by the International Conference on Harmonization Good Clinical Practice, the Declaration of Helsinki and the regulatory requirements of Burkina Faso

Funding

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Chapter 5

Risk factors for *Plasmodium falciparum* infection in pregnant women in Burkina Faso: a community-based malaria cross-sectional survey

Adapted from: Yaro JB, Ouedraogo A, Diarra A, Sombie S, Ouedraogo ZA, Ouedraogo NI, Drakeley C, Sirima SB, Tiono AB, Lindsay SW, Wilson AL (2020). Risk factors for Plasmodium falciparum infection in pregnant women in Burkina Faso: a community-based malaria cross-sectional survey. *Malaria Journal*, submitted for publication.

Abstract

Background

Malaria in pregnancy remains a public health problem in sub-Saharan Africa. Identifying risk factors for malaria in pregnancy could assist in developing interventions to reduce the risk of malaria in Burkina Faso and other countries in the region.

Methods

Two cross-sectional surveys were carried out to measure *Plasmodium falciparum* infection using microscopy in pregnant women in Saponé Health District, central Burkina Faso. Data were collected on individual, household and environmental variables and their association with *P. falciparum* infection assessed using multivariate analysis.

Results

A total of 356 pregnant women were enrolled in the surveys, 174 during the dry season and 182 during the wet season. The mean number of doses of sulphadoxine pyrimethamine for Intermittent Preventive Treatment in pregnancy (IPTp-SP) was 0.4 doses during the first trimester, 1.1 doses at the second and 2.3 doses at the third. Each dose is composed by three tablets of 500mg of sulfadoxine and 25 mg of pyrimethamine. Overall prevalence of *P. falciparum* infection by microscopy was 15.7%, with 17.8% in the dry season and 13.7% in the wet season. 88.2% of pregnant women reported sleeping under an insecticide-

treated net on the previous night. *P. falciparum* infection risk in pregnancy was reduced in those women who reported using an ITN (Odds ratio, OR=0.31, 95% CI 0.12-0.79, p=0.02) and an increasing number of IPTp-SP doses during pregnancy, with each additional dose reducing the odds by 40% (OR=0.59, 95% CI 0.43–0.81, p<0.001).

Conclusion

The prevalence of *P. falciparum* infection among pregnant women remains high in study area although use of IPTp-SP and ITNs were found to reduce the odds of infection. Despite this, compliance with IPTp remains far from that recommended by the National Malaria Control Programme and World Health Organization. Behaviour change communication should be improved to encourage compliance with protective malaria control tools during pregnancy.

Introduction

Malaria in pregnancy remains a major public health problem in sub-Saharan Africa [5], despite the decline in malaria transmission observed throughout the region from 2004-2015 [4]. Burkina Faso is a high burden country and is not experiencing yet declines in malaria and all-cause mortality in children despite high coverage of insecticide-treated nets (ITNs) and prompt and effective treatment with antimalarials [27].

Pregnant women are at high risk from malaria because of their lowered immunity during pregnancy [17, 75]. They are also at increased risk of transmission since they are more attractive to *Anopheles gambiae*, the most important African malaria vector [76], and may leave the safety of their ITNs more often than their non-pregnant counterparts [199]. Infection with *Plasmodium falciparum* can lead to poor outcomes for the mother, the foetus and child, resulting in maternal anaemia, low birth weight, preterm delivery and perinatal mortality [200-203]. Pregnant women, especially those pregnant for the first time (primigravidae), are at increased risk of more frequent and more severe malaria infections [86, 87, 89, 204]. The World Health Organization (WHO) recommends the use of ITNs (distributed free-of-charge at Antenatal Clinic (ANC) visits), intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine pyrimethamine (SP)

and prompt access to diagnosis and effective case management, to prevent and manage malaria risk in pregnancy [139]. According to national guidelines in Burkina Faso, pregnant women are advised to receive at least three doses of IPTp-SP starting from the second trimester, with a minimum interval of one month between doses [25].

The incidence of malaria infections in pregnant women in Burkina Faso in 2014 was 39.2 per 1,000 women-months, and was more than twice as great in primigravids at 88.6 per 1,000 women-months than multigravids [89]. In 2014, another study in the country found that malaria infection was five-fold greater in primigravids than in multigravids [87]. There have been many studies of risk factors for malaria in pregnancy in sub-Saharan Africa, where increased risk was reported to be associated with younger age in pregnancy, primigravidae, first trimester of pregnancy infection, non-use of ITNs, lack of education and HIV co-infection [86-89]. Few, however, have evaluated socioeconomic and environmental risk factors for malaria in pregnancy. For example, recently a number of studies have shown that malaria in children is associated with poor housing [102, 145, 205, 206], but it is not known whether this is also true for pregnant women. The goal of the present study was to identify risk factors for *P. falciparum* infection in pregnancy in Burkina Faso, including potential socioeconomic and environmental risk factors. Identifying risk factors for malaria in pregnancy could assist in developing interventions to reduce this risk in Burkina Faso and other countries in the region.

Methods

Study design

Putative risk factors for *P. falciparum* infection were measured during two cross-sectional surveys, one in the dry season and one in the wet season.

Study site

The study was conducted in Saponé Health District, situated in the central region of Burkina Faso, 30 Km south-west of Ouagadougou, the capital of Burkina Faso (Figure 5.1). In the study area, malaria transmission is intense and highly seasonal [115], with the peak of malaria transmission occurring at the end of the rainy

season (June to October) and markedly reduced transmission during the dry season (December to May) [136]. The main vectors are *Anopheles gambiae* s.s., *An. arabiensis* and *An. funestus,* and *P. falciparum* accounts for more than 95% of all malaria infections [115, 136, 207]. This is a rural area of open Sudanian savannah, where farming is dominant and the major crops grown are sorghum and millet. Houses in the study area are typically constructed with mud walls and floors, with thatched or metal roofs [23].

Surveys

Two cross-sectional surveys were carried out. One at the beginning of the dry season in December 2018 and the second one at the end of the rainy season from September to October 2019. Pregnant women were enrolled through a Demographic Health Surveillance System (DHSS), with home visits in 21 villages in the study area. All women of child-bearing age in the study area were identified and visited at home for pregnancy screening. This approach was adopted, rather than screening at the ANC because ANC attendance is relatively low in the study area, with only 35% of women attending the ANC at least three times [174]. Women thought to be pregnant were referred to the health facilities for a pregnancy test or, if willing to provide urine for a dipstick pregnancy test, fieldworkers performed the test at the woman's home. Women identify as pregnant, but who had not visited their ANC, were referred to the local health facilities. At the ANC, the study protocol and procedures were explained by trained staff to the potential participants in French or the main local language of Moore. Pregnant women were enrolled if aged between 15-40 years, provided written informed consent and agreed with the study procedures, including taking of blood. Pregnant women with a known history of SP allergy or any other medical condition that in the opinion of a study physician may be a threat to her or the foetus were not recruited into the study.

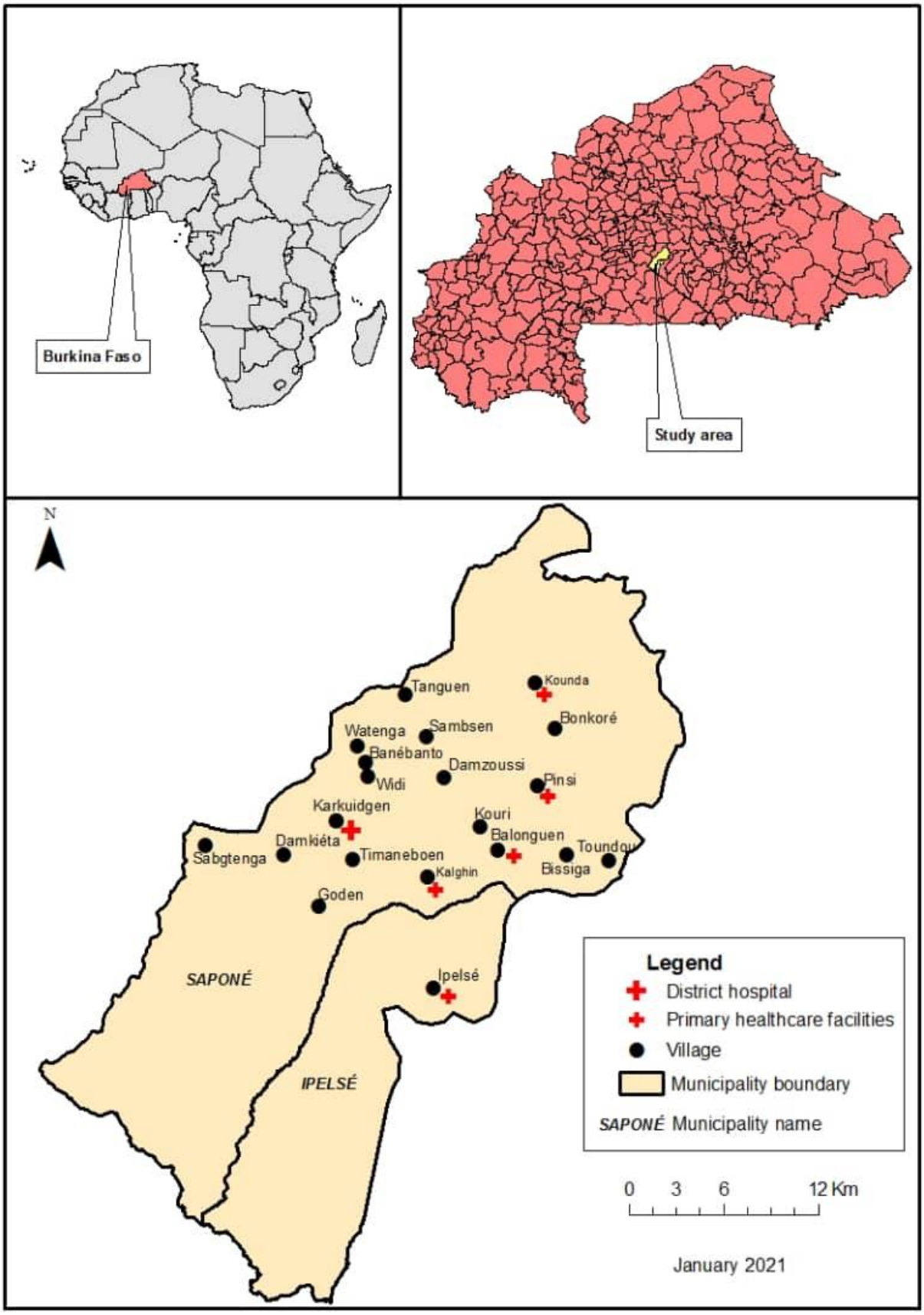

Figure 5.1: The map of study area

Clinical data collection

All study participants completed a questionnaire at enrolment, where demographic data, medical and obstetrical history including previous ANC visits, IPTp doses and use of antimalarials or any other medication within 14 days prior to study enrolment were recorded. For each participant, thick and thin bloods films were performed from finger prick. Smears were air-dried, thin blood films were fixed with methanol and both stained with Giemsa 3%. Slides were then read using light microscope at ×100 (oil immersion objective). One hundred high-power fields (HPF) were examined, and the number of malaria parasites of each species and stage recorded. The number of parasites per microliter of blood was calculated assuming 20 white blood cells per high power field and a fixed white cell count of 8000/µl. Each slide was read by two different lab technicians and if the difference between the two readers was more than 30%, the slide was reexamined by a third reader blinded to the results of the first two readers. The final result was the average of the two closest parasite counts. A slide was considered as negative if no parasite was found after 100 high-power fields (HPF) were examined.

In case of fever (axillary temperature ≥37.5°C or reported fever in the last 24 h) or other symptoms/signs of clinical malaria, a rapid malaria diagnostic test (RDT) (SD BIOLINE Malaria Ag P.f/Pan, Abbott Laboratories, Illinois, USA) was performed. Subjects presenting with clinical malaria (Fever+ RDT positive) were referred to the nearest health facility and treated according to national guidelines [25]. Clinical assessment was performed at home unless there was a specified need to check more about the health status of the volunteer. If this was the case, the woman was referred to the health facility to have deep clinical examination.

Risk factor data collection

Study participants were visited at home by fieldworkers who recorded information about the household, including whether the woman had slept under an ITN the previous night. If the answer was no, the reason why the woman did not use an ITN was recorded. Women sleeping under an ITN were asked about the bed net source and how many times they left their ITN during the night. ITN fabric integrity was also assessed by fieldworker observation and classified as entire/complete, with any hole, or torn. Women were asked to estimate the time they went to bed

and the time they get out of bed in the morning. Social and economic risk factors for malaria were recorded, including ethnicity, education level and occupation, ownership of a radio or mobile phone, estimated distance to the nearest health facility, and use of other protective measures, including mosquito coils, insecticide sprays, traditional spatial repellent or commercial topical repellents.

House construction (metal or thatched roof, presence of open eaves, electricity supply(in sleeping room), household size (number of persons) and the presence of clothes hanging in the sleeping room were recorded. The presence or absence of big domestic animals (donkey, horses, sheep, cows, goats, dogs) and rubbish within 5 m of each study participant household was recorded.

Sample size

The sample size was estimated based on the sample size for frequency of the disease in a population (https://www.openepi.com/SampleSize/SSCohort.htm). To determine malaria parasite prevalence, we assumed a population prevalence of 2.5% in the study area based on previous data of the frequency of malaria in pregnant women in the study area recorded in 2017 [19]. We assumed a 2% precision, with 5% level of significance and 95% confidence limits. Considering housing type as major risk factor (improved housing reduces risk of malaria prevalence by~50%, Odds ratio = 2) [85] and 10% non-response, a sample size of 175 pregnant women was considered necessary for each cross-sectional survey.

Data management and statistical analysis

Data were collected on Android personal digital assistants programmed using Open Data Kit (https://getodk.org/) and included drop down boxes and consistency checks to reduce data entry errors. Following cleaning, the dataset was locked and saved in Microsoft Access and analysed with Stata 15 (Statacorp, Texas, USA).

The primary outcome measure was the prevalence of microscopically confirmed *P. falciparum* infection in pregnant women during each cross-sectional survey. Logistic regression was used to investigate the association between predictor

variables and the primary outcome, adjusting for clustering by village. The multivariable model was constructed using a forwards stepwise process and models were compared using a Wald test.

Results

A total of 356 pregnant women were enrolled in the surveys, 182 during the wet season and 174 during the dry season (Table 1). The mean age of the study participants was 26.9 years, ranging from 15 to 40 years old, and was similar in both surveys. Of these women, 78 (21.9%) were in their first pregnancy, 74 (20.8%) in their second and 204 (57.3%) in their third pregnancy or more. Most women were enrolled in their second and third trimester of pregnancy (37.1% and 30.3% of women where gestational age was recorded). Fewer were enrolled at their first trimester: only 5/78 (6.4%) of primigravidae, 11/74 (14.9%) of secundigravidae and 20/204 (9.8%) of multigravidae were enrolled in their first trimester of pregnancy. 59.0% of women were illiterate and most were farmers (69.9%) or traders (22.1%). 73.1% of primigravidae were literate compared to only 42.5% of those on their second pregnancy and 27.6% of women with two or more pregnancies. 97.5% of study participants were from the Mossi ethnic group. Most women lived in households with three or fewer people 57.9% (206/356). Only 46.3% of women reported having an electricity supply in the sleeping room. Most houses were constructed with metal roofs (95.5%) with 64.6% of houses having hanging clothes inside. Large domesticated animals were common near the house (78.9%), with 45.8% of participants reporting rubbish within 5 m of their households.

Table 5.1: Characteristics of the study participants and households

Variables		Dry season n(%) N=174	Wet season (n/%) N=182	Total n (%) N=356
Age	<20	11 (6.3%)	23 (12.6%)	34 (9.6%)
	20-30	101 (58.1%)	90 (49.5%)	191 (53.7%)
	30-45	62 (35.6%)	69 (37.9%)	131 (36.8%)
Education	Illiterate	107 (61.5%)	103 (56.6%)	210 (59.0%)
	Literate	65 (37.4%)	79 (43.4%)	144 (40.4%)
Occupation	Farmers	115 (66.1%)	134 (73.6%)	249 (69.9%)
	Traders	46 (26.4%)	33 (18.1%)	79 (22.1%)
	Other	11 (6.3%)	12 (6.6%)	23 (6.5%)
Gravidity	Primigravida	31 (17.8%)	47 (25.8%)	78 (21.9%)
	secundigravida	42 (24.1%)	32 (17.6%)	74 (20.8%)
	multigravida	101 (58.1%)	103 (56.6%)	204 (57.3%)
Gestation	1st trimester	19 (10.9%)	17 (9.3%)	36 (10.1%)
	2nd trimester	75 (43.1%)	57 (31.3%)	132 (37.1%)
	3rd trimester	61 (35.1%)	47 (25.8%)	108 (30.3%)
Ethnic group	Mossi	169 (97.1%)	178 (97.8%)	347 (97.5%)
	Fulani	4 (2.3%)	2 (1.1%)	6 (1.7%)
	Other	1 (0.6%)	2 (1.1%)	3 (0.8%)
Roof material of sleeping room	Metal	165 (94.8%)	175 (96.2%)	340 (95.5%)
	Non-metal (Thatch/mud)	7 (4.0%)	6 (3.3%)	13 (3.7%)
Eave status of sleeping room	Closed	*	30 (16.5%)	-
	Open	*	149 (81.9%)	-
Electricity supply in the sleeping room	No	91 (52.3%)	87 (47.8%)	178 (50.0%)
	Yes	72 (41.4%)	93 (51.1%)	165 (46.3%)
Presence of large domestic animals within 5 m of the household	No	31 (17.8%)	37 (20.3%)	68 (19.1%)
	Yes	138 (79.3%)	143 (78.6%)	281 (78.9%)
Presence of solid waste within 5 m of the household	No	90 (51.7%)	98 (53.8%)	188 (52.8%)
	Yes	79 (45.4%)	84 (46.2%)	163 (45.8%)
Household size	<1-3	92 (52.9%)	114 (62.6%)	206 (57.9%)
	4-5	68 (39.1%)	55 (30.2%)	123 (34.6%)
	≥6	8 (4.6%)	13 (7.1%)	21 (5.9%)
Distance to health facility	<3Km	100 (57.5%)	98 (53.9%)	198 (55.6%)
	3-5Km	42 (24.1%)	65 (35.7%)	107 (30.1%)
	>5Km	28 (16.1%)	19 (10.4%)	47 (13.2%)
Hanging clothes in the sleeping room	No	28 (16.1%)	92 (50.5%)	120 (33.7%)
	Yes	140 (80.5%)	90 (49.5%)	230 (64.6%)
* missing data				

The overall prevalence of *P. falciparum* infection by microscopy was 15.7% (56/356), with 17.8% (31/174) during the dry seasonal survey and 13.7%

(25/182) in the wet season survey (p=0.3). The overall geometric mean of parasites density (GMPD) of infected pregnant women was 777.3/µl (95% CI= 496.0-1218.2) (Table 2). GMPD was higher in the wet season (876.2/µl (95% CI= 367.0– 2092.0)) than in the dry season (705.7/µl (95% CI= 444.8–1119.5)). GMPD was higher in women in their first pregnancy (1375.5/µl (95% CI= 720.5–2626.1)) compare to those in their second pregnancy or more ((474.0/µl (95% CI= 260.1– 863.8)). GMPD was higher in women aged under 20 years old than older women, with a GMPD of 3374.7/µl (95% CI= 946.1- 12036.9) among women aged under 20 years, 633.5/µl (95% CI= 368.0-1090.7) among women aged 20-30 and 552.0/µl (95% CI= 204.8-1487.5) among women aged 30 years or more. *P. falciparum* gametocytes carriage was found rare, 6/356 (1.7%) in the survey.

Women had on average received 1.7 doses of IPTp-SP (95% CI= 1.5-1.8) with increasing number of doses according to the trimester of pregnancy (0.4, 1.1 and 2.3 doses at first, second and third trimester of pregnancy, respectively). Secundigravidae were more likely to report taking no IPTp (25.7%) than primigravidae (7.7%) or multigravidae women (11.3%) (p=0.003). Women aged 20-30 years were more likely to report taking no IPTp-SP (17.8%) than women aged under 20 (8.8%) or women aged over 30 years (8.4%) (p=0.003). There was no difference in the proportion of literate and illiterate pregnant women reporting not taking IPTp-SP in this study (p=0.8).

A total of 95.1% of women reported using an ITN in the rainy season survey, compared to 81.5% in the dry season survey (p<0.001). 95.2% of women reported that the National Malaria Control Program provided their ITNs. The mean age of the ITN was 7.9 months (ranged 1 to 48 months) and 89.9% of them were reported to be un-holed. On average women self-reported an estimated time to bed of 20.21 h during the dry season and 20.13 h during the rainy season, and leave the bed at 05.29 h during the dry season and 5:39 h during the wet season. Only 4.5% of pregnant women reporting that they did not leave their ITN until the morning. However, 47.2% of them exited their ITN once or twice a night, and 42.7% exited their ITN three or more times a night. Mosquito coils were used by 19.4% of participants, 5.9% other types of spatial repellent (traditional repellents such as herbs or insecticide sprays) and 9.6% topical commercial repellents.

Multivariable analysis showed that *P. falciparum* infection risk in pregnancy was reduced among pregnant women who used ITNs (Odds ratio, OR=0.31, 95% CI

0.12–0.79, p=0.02) and with use of IPTp-SP, with each additional dose reducing the odds by 40% (OR=0.59, 95% CI 0.43 – 0.81, p=0.001) (Table 5.3).

Correlation of variables with others risk factors have been checked and the summary of the results has been completed in the corelation table 4 in appendix 3 section. Age and gravidity are strongly associated with a reduction in the risk of malaria infection risk while IPTp doses and the gestation are strongly associated with an increased risk of malaria.

Discussion

This study aimed to identify risk factors for malaria infection in pregnant women living in an area of intense and stable seasonal malaria transmission in Burkina [115, 161, 208] with increased pyrethroid resistance in malaria vectors [92] and to identify risk factors for malaria infection. The overall prevalence of *P. falciparum* infection during both surveys was 15.7%. The parasite rates in this study are similar to those recorded in other studies in Burkina Faso (e.g. 18.1% [87]) and other high burden countries in sub-Saharan Africa e.g. 20.1% in Kenya [209] and 21.6% in Ghana [210]. These results suggest that *P. falciparum* malaria infection is common in pregnant women in the community and the burden of *P. falciparum* infection in pregnancy remains high despite use of standard malaria control interventions. The overall geometric mean of parasites density in the study area was 777.3/µl (95% CI 496.0 – 1218.2). Fana and co-workers from Nigeria, another high burden country, recorded a similar mean parasite density of 800/µl [88]. The high parasite densities found in pregnant women results from their decreased immune competence [75, 211]. As expected, the parasite density was higher in primigravidae and secundigravidae compared to those women multigravide and in younger women compared to older women, since younger women are more likely to be primigravid. There was no significant difference in *P. falciparum* prevalence between the wet season (13.7%) and dry season (17.8%). This may be because we conducted the dry season survey in the early stages of the dry season when infections from the end of the rains may be present. Parasite density was, however, higher in the wet season compared to dry season, showing that even in this high burden area, malaria is seasonal in pregnancy [115]. *P. falciparum* gametocytes carriage in pregnancy in this study was lower (1.7%) and was detected by

microscopy. Lower parasites density may result in lower gametocytes identification by microscopy. Evidence of the dynamics of *Plasmodium falciparum* gametocyte carriage in pregnant women under IPT-SP in Benin matched with lower prevalence of gametocytes [212].

Table 5.2: Malariometric characteristics and use of personal protection according to season

Variables	**Dry season** N=174				**Wet season** N=182			
	Primigravidity	**Secundigravidity**	**Multigravidity**	**Total**	**Primigravidity**	**Secundigravidity**	**Multigravidity**	**Total**
	n=31	n=42	n=101		n=47	n=32	n=103	
***P. falciparum* infection**								
Parasitaemia (any level)	7 (22.6%)	10 (23.8%)	14 (13.9%)	31 (17.8%)	7 (14.9%)	2 (6.3%)	16 (15.5%)	25 (13.7%)
Parasitaemia ≥ 1000/µl	4 (12.9%)	2 (4.8%)	4 (4.0%)	10 (5.7%)	5 (10.6%)	1 (3.1%)	6 (5.8%)	12 (6.6%)
**GMPD/µl (95% CI)	1435.7 (412.7 - 4995.2)	738.7 (276.0 - 1976.8)	478.9 (263.7 - 869.7)	705.7 (444.8 – 1119.5)	2925.3 (421.0 - 20325.6)	Low number of observations	469.7 (160.3 - 1376.3)	876.2 (367.0 - 2092.2)
Use of personal protective measures								
Access to ITN	23 (74.2%)	37 (88.1%)	93 (93.0%)	153 (88.4%)	41 (87.2%)	32 (100%)	99 (96.1%)	172 (94.5%)
Used an ITN the previous night	21 (67.7%)	35 (83.3%)	85 (85.0%)	141 (81.5%)	42 (89.4%)	31 (96.9%)	100 (97.1%)	173 (95.1%)
Mosquito coils	8 (25.8%)	9 (21.4%)	24 (24.0%)	41 (23.7%)	10 (21.3%)	3 (9.4%)	15 (14.6%)	28 (15.4%)
Other spatial repellent	1 (3.2%)	0	5 (5.0%)	6 (3.5%)	4 (8.5%)	1 (3.1%)	10 (9.7%)	15 (8.2%)
Commercial repellent (topical)	3 (9.7%)	5 (11.9%)	9 (9.0%)	17 (9.8%)	8 (17.0%)	1 (3.1%)	8 (7.8%)	17 (9.4%)
0 dose of IPTp-SP use during pregnancy	6 (19.4%)	15 (35.7%)	16 (15.8%)	37 (21.3%)	0	4 (12.5%)	7 (6.8%)	11 (6.0%)
1 dose of IPTp-SP use during pregnancy	9 (29.0%)	8 (19.0%)	35 (34.7%)	52 (29.9%)	11 (23.4%)	8 (25.0%)	28 (27.2%)	47 (25.8%)
2 doses of IPTp-SP use during pregnancy	7 (22.6%)	4 (9.5%)	23 (22.8%)	34 (19.5%)	12 (25.5%)	9 (28.1%)	35 (34.0%)	56 (30.8%)

3 or more doses of IPTp-SP use during pregnancy	5 (16.1%)	8 (19.0%)	12 (11.9%)	25 (14.4%)	20 (42.6%)	6 (18.8%)	21 (20.4%)	47 (25.8%)
Mean IPTp dose (SD)	1.4 (1.0-1.8)	1.3 (0.8-1.7)	1.4 (1.2-1.6)	1.4 (1.2-1.6)	2.2 (1.9-2.5)	1.8 (1.3-2.3)	1.8 (1.6-2.1)	1.9 (1.8-2.1)
Use of antimalarial drug two weeks before the survey	3 (10.7%)	6 (15.0%)	18 (18.4%)	27 (16.3%)	7 (14.9%)	2 (6.3%)	10 (9.7%)	19 (10.4%)

Table 5.3: Risk factors for *P. falciparum* infection in pregnant women in Saponé Health District

Factors		*P. falciparum* infection positivity n/N (%) N=356	Univariate analysis			Multivariable analysis		
			Odds Ratio	95% CI	p-value	Odds Ratio	95% CI	p-value
Pregnancy characteristics								
Gestation	1st trimester	10/48 (20.8%)	1					
	2nd trimester	34/132 (25.8%)	1.32	0.90 - 1.94	0.16			
	3rd trimester	7/108 (6.5%)	0.26	0.12-0.58	0.001			
Gravidity	Primigravidae	14/78 (17.9%)	1					
	Secundigravidae	12/74 (16.2%)	0.88	0.48 – 1.64	0.70			
	Multigravidae	30/204 (14.7%)	0.79	0.41 – 1.50	0.47			
Socio-demographic characteristics								
Mean age (years)		-	0.98	0.94-1.02				
Education	No formal education	24/210 (11.4%)	1					
	Literate	31/144 (21.5%)	2.13	1.29 - 3.52	0.003			
Occupation	Farmers	34/249 (13.7%)	1					
	Traders	15/79 (19.0%)	1.48	0.75 - 2.93	0.26			
	Other	6/23 (26.1%)	2.23	0.72 – 6.90	0.16			
Use of personal protective measures								
ITN use the previous night	No	12/41 (29.3%)	1			1		
	Yes	43/314 (13.7%)	0.38	0.18 - 0.81	0.01	0.31	0.12 – 0.79	0.02
Number of IPTp-SP doses		-	0.57	0.41 – 0.80	0.001	0.59	0.43 – 0.81	0.001
Number of IPTp-SP doses	0	13/48 (27.1%)	1					
	1 or more	43/307 (14.0%)	0.44	0.17 – 1.15	0.09			
Mosquito coils	No	46/286 (16.1%)	1					
	Yes	9 /69 (13.0%)	0.78	0.48 - 1.29	0.33			
Other spatial repellent	No	54/335 (16.1%)	1					

	Yes	2/21 (9.5%)	0.55	0.09 – 3.53	0.53			
Commercial repellent (topical)	No	51/320 (15.9%)	1					
	Yes	4/34 (11.8%)	0.70	0.24 – 2.04	0.52			
Distance to nearest health centre	<3km	26/198 (13.1%)	1					
	3-5km	19/107 (17.8%)	1.43	0.73 – 2.78	0.30			
	>5km	10/47 (21.3%)	1.79	0.66 – 4.83	0.25			
Use of antimalarial drug during the last two week before the survey	No	48/302 (15.9%)	1					
	Yes	5/46 (10.9%)	0.65	0.32 – 1.31	0.23			
House characteristics and construction								
Household size	<4	31/206 (15.0%)	1					
	4≤no.<6	20/123 (16.3%)	1.10	0.58 - 2.07	0.78			
	≥6	4/21 (19.0%)	1.33	0.36 - 4.91	0.67			
Roof material of sleeping room	Metal	52/340 (15.3%)	1					
	Thatch or mud	3/13 (23.1%)	1.66	0.62 - 4.42	0.31			
Electricity supply in sleeping room	No	33/178 (18.5%)	1					
	Yes	21/165 (12.7%)	0.64	0.37 – 1.11	0.11			
Clothes hanging in sleeping room	No	16/120 (13.3%)	1					
	Yes	38/230 (16.5%)	1.29	0.58 – 2.84	0.53			
Asset ownership								
Own a radio	No	24/127 (18.9%)	1					
	Yes	31/224 (13.8%)	0.74	0.42 – 1.30	0.29			
Own a mobile phone	No	8/56 (14.3%)	1					
	Yes	47/295 (15.9%)	1.14	0.62 – 2.09	0.68			
Environmental factors								
Season enrolled	Dry season	31/174 (17.8%)	1					
	Rainy season	25/182 (13.7%)	0.73	0.41 - 1.31	0.30			
Presence of large domestic animals within 5 m of the household	No	10/68 (14.7%)	1					
	Yes	45/281 (16.0%)	1.11	0.48 – 2.55	0.81			
Presence of solid waste within 5 m of the household	No	30/188 (16.0%)	1					
	Yes	24/163 (14.7%)	0.91	0.50 – 1.64	0.75			

between the wet season (13.7%) and dry season (17.8%). This may be because we conducted the dry season survey in the early stages of the dry season when infections from the end of the rains may be present. Parasite density was, however, higher in the wet season compared to dry season, showing that even in this high burden area, malaria is seasonal in pregnancy [115]. *P. falciparum* gametocytes carriage in pregnancy in this study was lower (1.7%) and was detected by microscopy. Lower parasites density may result in lower gametocytes identification by microscopy. Evidence of the dynamics of *Plasmodium falciparum* gametocyte carriage in pregnant women under IPT-SP in Benin matched with lower prevalence of gametocytes [212].

Overall, 91.3% of pregnant women owned an ITN, with 88.2% reporting using an ITN the night before the survey. This is similar to other surveys from Burkina Faso; in the Banfora Region, 80.6 % of surveyed children reported sleeping under an ITN the previous night [145]. The high reported ITN use is encouraging, although accurately determining net use is challenging and reporting can be susceptible to response bias [129, 213, 214].

The study found that IPTp-SP and ITNs are highly effective interventions for preventing malaria infection during pregnancy. For each additional dose of IPTp reported as being received by women, the odds of malaria infection fell by 40%. Relatively few women, however, took three or more doses of IPTp-SP (20.2%) which is recommended by the NMCP and WHO [5, 25]. We also found lower use of IPTp-SP among women aged 20-30 compared to other age groups and among secundigravidae compared to other women. This suggests that women in their second pregnancy may be more compliant with ANC attendance and malaria prevention than women in their first pregnancy or later pregnancies. This finding contrasts with another study in Burkina Faso that found compliance with IPTp-SP in adolescent women to be more problematic due to structural constraints (e.g. social position and household labour requirements) and needs (e.g. anonymity in the health encounter) [215]. Numbers of secundigravidae women were relatively small and so this finding requires further exploration.

ITNs associated with 69% reduction in the odds of *P. falciparum* infection, which is higher than other studies have found [216]. This indicates that ITNs are protective against malaria in pregnancy despite high levels of insecticide

resistance present in Burkina Faso [92, 217-220]. This contrasts with findings from a cohort study in children aged 5-15 years in south-west Burkina Faso which showed no difference in malaria risk between ITN users and non-users [145], and in all age in a community-wide survey in Banfora region (Yaro, J.B. et *al*., Unpublished).

Women reported going to bed at 20.21 h during the dry season and 20.13 h during the rainy season. This finding contrasts with a study by Guglielmo and co-workers who reported that 100% of females in south-west Burkina Faso (sample of 211 and 695 females observed in two villages) were outdoors until 22.00 h, after which point women started to move indoors to bed [146]. It may be that pregnant women tend to go to bed earlier and so those using ITNs are more likely to be protected from vectors biting during the early evening which has been observed in Burkina Faso [146]. Increased malaria risk in Human including pregnant women who go to bed later has been observed in other studies in sub-Saharan Africa [154, 188, 221].

Our study has a number of limitations. Firstly, the sample size was probably not large enough to identify minor risk factors in this study and future investigations may help improve the sample power. Secondly, ITN ownership and use was self-reported and subject to social desirability bias and we lack objective tools for measuring bednet use in this study. Microscopy technology used for *Plasmodium falciparum* diagnosis may underestimate the real community prevalence of parasite level. Future bio-molecular technology may be useful for undetectable asexual and sexual parasite fractions [3, 222].

What are the implications of this research for control of malaria in pregnancy in Burkina Faso? Behaviour change communication is necessary to ensure high ANC attendance and compliance with IPTp-SP and ITN use. Messages need to be tailored to the different vulnerable groups of women. For example, we found lower IPTp-SP compliance among women aged 20-30 than the other age groups. As it this common in sub-Saharan Africa, pregnant women are often unaware that they are pregnant and so do not attend or are unwilling to attend an ANC in the early stages of pregnancy. An association between early ANC attendance and a higher average number of IPTp-SP doses has been demonstrated in several studies [223-225]. One option to increase IPTp-SP coverage is community delivery by

community health workers, rather than ANC. This delivery route has been shown in a clinical trial in Burkina Faso to increase IPTp-SP compliance from 2.1 to 2.8 doses in the community delivery study arm with no apparent decrease in ANC attendance [226]. Also, evidence of a community-based multicentre cluster-randomized controlled trial from The Gambia, Burkina Faso and Benin reported a lower risk of placenta malaria, anaemia at delivery, and low birth weight associated with increasing number of IPTp-SP doses given during pregnancy and recommend this as a priority of strategies for malaria control in pregnancy [227].

Conclusion

The prevalence of *P. falciparum* infection among pregnant women remained high despite wide deployment of ITNs, access to IPTp-SP and prompt and effective treatment with ACTs. Nonetheless, women who took IPTp-SP and use ITNs during their pregnancy were at much reduced risk of being infected by malaria. These findings suggest that IPTp-SP and ITNs use are effective at reducing malaria infection in pregnant women living in malaria high burden countries, but that research is needed to increase uptake of IPTp-SP use.

Ethics approval and consent to participate

Study participants provided informed consent before they were enrolled in the study. The caregivers of study participants aged <20 years provided informed consent (while participants provided assent). Study documents were approved by the Burkina Faso Ministry of Health Research Ethics Committee, CNRFP Institutional Bioethics Committee, the London School of Hygiene and Tropical Medicine ethical Committee and Durham University Department of Biosciences Ethics Committee. The study was conducted in compliance with principles set out by the International Conference on Harmonization Good Clinical Practice, the Declaration of Helsinki and the regulatory requirements of Burkina Faso.

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Chapter 6

Discussion

Overview and summary of findings

The goal of this thesis was to determine risk factors associated with malaria infection and transmission in rural Burkina Faso, a high burden, persistent malaria infection and high insecticide resistance area, in children, all ages and pregnant women. Identification of the drivers which impede disease control may help guide malaria control strategies in Burkina Faso as well as sub-Saharan African countries.

Chapter 1 summarises the background and literature review on malaria burden in the world, in sub-Saharan Africa and Burkina Faso. It was about the mechanisms of disease transmission including all major factors involve the physiopathology: environment factors with temperature, rainfall, humidity, vectors and human behaviours; malaria parasites and the high antigenic diversity of *Plasmodium falciparum*; human host factors affecting an individual's susceptibility to malaria. This chapter also displays the research hypothesis and the study outcomes after the study methodology for data collection, analysis and scope.

Despite enormous progress in malaria control since the turn of the millennium, substantial worries persist in malaria cases in the WHO Region of Africa [5]. The World Malaria Report in 2020 recorded that the gains achieved between 2000 and 2015 have levelled off over recent years. The global trends in malaria incidence rate (cases per 1000 population at risk) stalled from 2015 to 2019 [5]. From the same report, 29 countries accounted for 95% of malaria cases globally in the world, with Nigeria (27%), the Democratic Republic of the Congo (12%), Uganda (5%), Mozambique (4%) and Burkina Faso (3%) accounted for about 51% of all cases globally [5]. The eleven countries including Burkina Faso are on track to keep focus on the first wave of the approach to high impact malaria control following World Health Organization and Roll Back Malaria partnership to end malaria guiding principles [83]. The latest evidence from Burkina Faso is that

malaria was increasing in the general population and in the two most vulnerable groups: children less than five years old and pregnant women (Figure 1.6) from 2000 to 2018. There were 7,859,000 malaria cases reported 2019, slightly fewer than 7,875,575 cases in 2018. However, the accuracy of the statistics collected in 2019 have been challenged because of the long and persistent strikes of health workers and from occasional terrorism, so that sometimes record data in 2019 was a repeat data of 2018. Has malaria control in Burkina Faso stalled? Worryingly, malaria was still the main reason for medical consultation (41.3%), hospitalization (21.4%) and death (16.4%) in public health centres in Burkina Faso in 2018 [174]. Malaria incidence in Burkina Faso is highly seasonal with about 60% of cases occurring within the 4 months from June to September [21]. The malaria incidence rate is 7.6 per 1000 child days [22] and Plasmodium infection prevalence in children was 57.5% in the country in 2017 [23]. A total of 8.4 million ITNs were deployed in 2010 and a further 10.5 million in 2013 resulting in approximately 90% of households owning at least one ITN [26]. More recently, deployment of an additional 10.3 million ITN in 2016 and 12 million in 2019 raised the global coverage rate of households to 85% (https://www.afro.who.int/fr/news/lutte-contre-le-paludisme-au-burkina-faso-campagne-de-distribution-de-12-millions-de-milda-aux).
Despite this huge investment in ITN coverage, Burkina Faso is still one of the few countries not to have shown a significant association between ITN ownership and a reduction in child mortality [27, 28]. This situation is surprising since elsewhere, ITN use have been shown to reduce clinical incidence by > 50% in areas of stable malaria [29].

To elucidate the numerous factors that influence malaria transmission dynamics in this study area, this thesis challenges was to look for effective control packages of interventions for this specific epidemiological pattern that could help for malaria control in Burkina Faso and all others endemic sub-Saharan countries.

Chapter 2 summarises the malaria incidence of *P. falciparum* in children aged five to 15 years old, the entomological inoculation rate (EIR), the use of ITNs, the main malaria vector (*Anopheles gambiae*) resistance to insecticide, the ITNs used resistance to pyrethroids and the risk associated with *P. falciparum* for children in Burkina Faso.

In fact, in this study area, malaria infection incidence remains overwhelmingly high. During the six-month follow-up period, covering the high malaria transmission season in the study site, the overall incidence of *P. falciparum* infection, was 2.78 episodes per child (95% CI= 2.66-2.91) by microscopy, and 3.11 (95% CI= 2.95-3.28) by PCR. The entomological inoculation rate (EIR) was 80.4 infective bites per child over the six-month malaria transmission season. However, a high level of 80.6% of children were reported as sleeping under an ITN the previous night, although at the last survey, 23.3% of their nets were in poor condition and considered no longer protective. We do not find an association between the rate of *P. falciparum* infection and either EIR (Incidence Rate Ratio, IRR: 1.00, 95% CI: 1.00 –1.00, p=0.08) or mortality in WHO tube tests when vectors were exposed to 0.05% deltamethrin (IRR: 1.05, 95% CI: 0.73–1.50, p=0.79). The significant association was only found with the travel history of child out of the study area during the survey (IRR: 1.52, 95% CI: 1.45–1.59, p<0.001) and the increasing socio-economic status of the child caregivers were associated with an increased risk of *P. falciparum* infection (IRR: 1.05, 95% CI: 1.00–1.11, p=0.04). These study findings suggest that because of the exceptionally high levels of malaria transmission in the study area, malaria elimination cannot be achieved solely by mass deployment of ITNs and additional control measures are needed.

Chapter 3 aimed to identify the prevalence of *P. falciparum* infection in all age strata in the study community-based screening and the risk factors for *P. falciparum* infection in rural Burkina Faso that might be used to identify and target malaria control measures in this area of high burden, persistent malaria infection and high insecticide resistance as well in all high burden malaria countries in sub-Saharan.

In this survey findings, malaria infections were high in all age strata, although highest in children aged 5 to 15 years, despite universal coverage with ITNs and prompt and effective treatment with antimalarials. The overall *P. falciparum* prevalence detected by microscopy was 32.8%, with 41.9% of children aged 2 to <10 years old, 40.6% in 10 to <30 years old and 16.1% of those > 30 years old infected. Symptomatic malaria cases were found represented 2.7% in the entire

study population and 3.3% had high density *P. falciparum* parasitaemia (>5,000 parasites/μL). Children aged between 5 and 15 years were the group at highest risk of *P. falciparum* infection in the community. Those aged 5 to <10 years old were at 3.8 times the odds (95% CI= 2.4–6.0, p<0.001) and those aged 10 to 15 years old at 4.3 times the odds (95% CI= 2.6 – 7.1, p<0.001) of *P. falciparum* infection compared to children aged less than 5 years old. Literacy was associated with increased risk of *P. falciparum* infection (OR=1.71, 95% CI= 1.26 – 2.32, p<0.001). However, there was a weak association between use of an electric fan (OR=0.55, 95% CI= 0.31 – 0.99, p=0.05) and female gender (OR=0.77, 95% CI=0.58-1.02, 0.07) and decreased risk of *P. falciparum* infection. 1142 (95.2%) of respondents reported having access to a bednet, with 1,087 (90.7%) saying they slept under a bednet the previous night of the survey. 9.2% of the population reported using a topical repellent in the last week, whether commercial or traditional. Only 6.2% of the study population reported using insecticide aerosols, mosquito coils or other spatial repellents.

Our study findings imply that current national strategies for malaria control in Burkina Faso are not sufficient to eliminate the disease. The high prevalence of *P. falciparum* infection in children aged 5 to 15 years suggests that an extension of the age group eligible for SMC may be able to reduce malaria burden substantially.

Chapter 4 aimed to identify the risk factors associated with house entry of *An. gambiae* s.l. in rural Burkina Faso, an area of high burden, persistent malaria infection and high insecticide resistance. This study demonstrated that there were fewer *An. gambiae* s.l. in homes with electricity (incidence rate ratio, IRR = 0.4, 95% CI = 0.3–0.7, p = 0.001) and houses with metal roofs (IRR, = 0.6, 95% CI = 0.4–1.0, p = 0.034) than those that did not. In fact, there are some implications of the study findings for vector control and future research. The increased access to electricity in sub-Saharan Africa raises questions about the impact on vector behaviours (e.g. whether lights attract mosquitoes or leads to mosquito avoidance behaviour), human behaviour (e.g. alteration of time to bed or use of fans) and malaria risk which are complex and yet to be understood. The risk factor study also highlights the potential of improved housing to reduce malaria transmission and supports the results of systematic and multi-country research studies on this

topic. Well-lit and well-built houses with metal roofs may reduce entry of malaria mosquitoes compared to poorly lit, poorly built and thatched roofed houses. While other vector control tools such as dual-active ITNs are now being deployed in the study area, the study results highlight the importance of non-insecticidal interventions such as house improvement to increase long-term resilience against malaria and for insecticide resistance management.

Chapter 5 was aimed to assess the prevalence of *P. falciparum* in pregnancy in both the wet and dry seasons, the compliance of pregnant women to intermittent preventive treatment in pregnancy (IPTp), the use of ITNs in pregnancy, pregnant women behaviour at night time and the risk associated with *P. falciparum* infection for pregnant women in Burkina Faso, an area of high burden, persistent malaria infection and high insecticide resistance. Overall compliance with IPTp was two mean doses during the survey with increasing doses during pregnancy (0.4, 1.1 and 2.3 doses respectively at first, second and third trimester of pregnancy). The overall prevalence of *P. falciparum* infection by microscopy was 56/356 (15.7%), with 31/174 (17.8%) and 25/182 (13.7%) respectively in dry and wet season. Primigravidae and secundigravidae have carried 54.8% of *P. falciparum* infection in dry season and 36.0% in wet season. In the previous night of the survey, 88.5% of pregnant women reported they slept under an insecticide-treated net (ITN). During the night, 47.2% of pregnant women reported getting out of their ITN one or two times, while 42.7% did it more than two time a night. *P. falciparum* infection risk in pregnancy was reduced in those women who reported using an ITN (Odds ratio, OR=0.31, 95% CI 0.12-0.79, p=0.02) and an increasing number of IPTp-SP doses during pregnancy, with each additional dose reducing the odds by 40% (OR=0.59, 95% CI 0.43–0.81, p<0.001).

This study findings highlight persistent high prevalence of *P. falciparum* infection among pregnant women in study area although use of IPTp-SP and ITNs were found to reduce the odds of infection. Despite this, compliance with IPTp remains far from that recommended by the National Malaria Control Programme and WHO. Behaviour change communication should be improved to encourage compliance with protective malaria control tools during pregnancy.

Table 6.1: Summary of significant risk factors for *P. falciparum* infection in this study. Where RR= risk ratio and OR= odds ratio.

Outcome	Study design	Study population	Risk factors	Odds ratio/Rate ratio	95 % CI	p value
Malaria incidence	cohort study	children aged 5 to 15 years old	SES factor score 1 unit increase	RR : 1.05	1.00 - 1.11	0.04
			Travel history during the study period	RR : 1.52	1.45 - 1.52	<0.001
Malaria infection risk	cross-sectional survey	community-wide (all ages)	Literacy	OR : 1.71	1.26 - 2.32	0.001
			sleeping room of subject have a functioning fan	OR : 0.55	0.31 - 0.99	0.05
House entry of *An. gambiae*	cohort study	children aged five to 15 years old	Metal roof materials of child sleeping room	RR : 0.6	0.4 - 1.0	0.03
			Electricity supply for child sleeping room	RR : 0.4	0.3 - 0.7	0.001
Malaria infection prevalence	cross-sectional survey	pregnant women aged 15 to 40 years old	ITN use	OR : 0.31	0.12 – 0.79	0.02
			Use of IPTp-SP doses 1 dose increased	OR : 0.59	0.43 – 0.81	0.001

Table 6.1 summarizes significant risk factors associated with malaria infection and transmission in the study.

The assessment of socio-demographic, entomological and environmental risk factors for *P. falciparum* infection did not elucidate any strong associations. This may be because of the huge force of infection in the study area which meant that all the children were at extremely high and persistent risk of infection. The finding of higher SES factor score was associated with higher incidence of *P. falciparum* infection was unexpected since it is widely reported that the least poor children are typically at lower malaria risk than the most poor children [67]. Quintiles were used to allocate children into categories, while the factor score was better reflected

the range of values in the dataset has been used in the multivariable model. *P. falciparum* infection rates may be higher in the least poor children due to lower ITN use than the most poor children. For example, only 53.2% of the least poor children were reported to use an ITN the previous night compared to over 82.6% of children in the lowest quintile (most poor). Nevertheless, ITN use was only associated with protection against malaria infection in pregnant women. Additional ITN is attributed free of charge to pregnant women during their ANC visits by the Ministry of Health. This second attribution of ITN is usually considered by pregnant women as their own comparatively to the national campaign one which was considered for the whole family. During the ANC visits, health workers sensitized pregnant women to use their ITN. This innovative strategy of additional free of charge ITN in pregnancy maybe introduce in children by planning for a school net additional programme through primary schools net distribution associated with health workers and teachers actions of children sensitization for these ITN use. Similar evidence has been implemented with proven for feasibility and effective strategy of household ITN high coverage in Tanzania [228].

However, the increased access to electricity highlight the questions about the impact on vector behaviour (e.g. whether lights attract mosquitoes or leads to mosquito avoidance behaviour), human behaviour (e.g. alteration of time to bed or use of fans) and malaria risk which are complex and yet involved confounders and need to be understand. Our study found that child sleeping in houses with metal roofs reduce mosquito house entry. However, metal roof houses are more likely to have floors and walls made of finished materials and have electricity supply with sometime electric fan supply than thatched-roofed houses. Although, higher SES is associated with owning or living in improve housing conditions with better commodities supply (well house construct with metal roof materials, electricity supply, electric fan supply, television owner, etc). Future research is needed clear state about benefits of electricity in improving housing conditions regarding the risk of malaria infection and transmission.

Study limitations

This study has several limitations. Firstly, and most importantly, the study methodologies (cohort study and cross-sectional surveys) used to assess risk factors were subject to bias and confounders that could reduce the quality of study

results during the surveys [229-231]. Risk factors assessed in our study did not mean causality and the reported associations highlight the possible interaction between factors assessed and malaria vectors or *P. falciparum* infection. The associations reported may be influenced by both the dependent variable (malaria infection) and independent variables (risk factors assessed). I'd try to reduce confounders by randomly selected subject in cohort study in children (chapter two) and in the community-wide cross-sectional study (chapter three). Selection bias with the study participants' selection has been fairly good enough managed in chapter two, chapter three and chapter four, where study participants' were randomly selected after study villages' random selection from demographic and health surveillance system (DHSS) of Banfora Health District. In chapter 5, we also selected our study participants' based on the DHSS from Sapone Health District, however, pregnant women recruitment was community-based and the sample may not be representative of the larger community because pregnant women were not randomly selected regarding their women age or parity, gestational age nor household locations. All the studies suffered from recall bias, where the age of the ITNs may be imprecise. While caregivers reported high compliance with ITN use in their children, assessing ITN use is notoriously difficult to assess and this may have impacted on our ability to identify this as an important risk factor in all age in community or more specifically in children. Indeed, social desirability bias and other forms of error mean that surveys are likely to substantially overestimate use [130], and we lack objective and unobtrusive tools to measure ITN compliance.

Secondly, the cohort study in chapter two was probably underpowered to detect small risk factors in children. The sample size calculation assumed 50% prevalence of risk factors in the study population aged five to 15 years old in cohort study, while the study children were, in reality relatively homogeneous with regards to risk factors shown to be important in other studies, for example, housing construction. Risk for mosquitoes' house entry study was a nested study in the cohort study in children. The sample size was probably not large enough to identify minor risk factors. The risk in pregnant women study was deduced from a research protocol on pregnant women. Our study was a cross sectional and the sample size was not sufficiently large to detect all the small risk factors, but sufficient to carry out the survey.

Thirdly, I was unable to collect the real information about ITN used by study participants in all studies. Information's used to assess ITN ownership and use was collected by fieldworkers based on the questionnaire. This may subject to social desirability bias with lack objective tools for measuring bednet use in this work [129, 232, 233]. However, ITN use assessment was associated with *P. falciparum* infection reduce in only pregnant women target group. Do the additional ITN provided to pregnant women during their ANC visits may have impacted on our ability to identify it was an important risk factor. Future exploration for better understanding is needed.

Fourthly, we did not obtain PCR results for parasites real estimates and so were not able to evaluate sub-microscopic infections in the community-wide in chapter three. We miss to assess the community-wide disease level and rising up the need for increase laboratory training and supervision to avoid errors in future studies, at community all age level.

Future direction and wider applicability of this research

Burkina Faso is one of eleven sub-Saharan African countries designated as a High Burden High Impact country with a response plan including increased political will, strategic use of data to deploy tools for maximum impact and a multi-sectoral approach [83]. While this study generates useful data on malaria burden in Banfora District, it is clear that additional tools will be needed to reduce the disease burden. Dual-active ITNs (pyrethroid plus piperonyl butoxide or pyrethroid plus chlofenapyr) are now being deployed in the study area. PBO-pyrethroid ITNs have been shown to be more effective in reducing malaria than pyrethroid only ITNs in areas of pyrethroid-resistant vectors [130], and monitoring of the effectiveness of these dual active ITNs is ongoing in Burkina Faso [234]. IRS should also be considered as it has been shown to be effective in reducing malaria in other high burden countries [235]. In an area of Uganda with an EIR of 176 infective bites/year, three rounds of IRS with the carbamate insecticide, bendiocarb, every six months reduced malaria incidence from 3.3 episodes to 0.6 episodes per person year [132]. But even this effective combination of interventions is insufficient to eliminate malaria so further interventions are required. SMC is currently being used in 12 sub-Sahelian countries including Burkina Faso in

children up to five years of age, with a protective efficacy of over 50% against parasitaemia in Burkina Faso [133, 236]. A recent trial in Senegal found similarly high reductions in malaria when SMC was used in children aged under five years and in children aged five to nine years [134]. Expanding the age range eligible for SMC up to 15 years old could therefore be effective in reducing malaria further in Burkina Faso. Burkina Faso is also the site of pilot testing of gene drive mosquitoes which, if effective and acceptable, could constitute another option for malaria control in Burkina Faso [237].

Recommendations for malaria control in Burkina Faso forward into policy and practice for others high burden, persistent malaria infection and high insecticide resistance sub-Saharan Africa

The study found predominantly high and persistent levels of malaria transmission in Burkina Faso despite the large coverage of ITN, and the risk factor survey did not identify much risk factors for further investigation to reduce the malaria burden in this high burden country. This lack of impact of ITN may be due to the intense level of transmission of malaria during the high transmission season. The lack of association with EIR and vector density was also surprising, but could arise if vector densities in all villages were sufficiently high to maintain high transmission, or due to outdoor biting associated human extended night time activities. Despite the high levels of pyrethroid resistance in the study area, no malaria vectors were found under the children's ITN after more than 1000 searches [145]. This would imply that the nets are still providing some level of personal protection.

The findings have implications for achievement of the ambitious goals set out in the WHO Global Technical Strategy. Malaria control in this area of intense seasonal transmission and subject of increased insecticide resistance cannot be achieved solely by massive ITN deployment. Additional strategy of intervention are needed though the use of additional interventions including dual-active ITNs and IRS with non-pyrethroid insecticides, extension of SMC in children under five years to school-age children, those aged five to 15 years old, promotion of ITN use in school-age children and pregnancy and specifically communication for IPTp-SP use

in pregnancy, and housing condition improvement such as well constructed house with metal roof, windows screen and electricity supply as well electric fan.

SMC use could be extended to children aged 5 to 15 year old, to help reduce malaria burden in community. This school-age children should be a new group age of interest in the community regarding malaria control.

Conclusion

The study found overwhelmingly high levels of malaria transmission in study area highlighting Burkina Faso as high burden of high and persistent insecticide resistance area despite the best available treatment, particularly for *P. falciparum* malaria, artemisinin-based combination therapy (ACT) and sulfadoxine-pyrimethamine (SP) for intermittent preventive treatment in pregnancy since 2006, the massive ITN deployments since 2010 and the use of seasonal malaria chemoprevention in children since 2016. School-age children age five to 15 years old are more at risk (three to four times increase) compare to other age group in the community. Higher SES children, those who travelled out of the study area during the survey and people literates were more at risk for *P. falciparum* infection. There was evidence of lack of impact of ITN universal coverage in children in Burkina Faso and insecticide resistance spreading throughout study sites in Banfora. However, well-lit house with metal roof materials, electricity supply, as well electric fan are associated with fewer mosquitoes' in houses. Also, IPTp-SP and ITNs use are effective at reducing malaria infection in pregnant women living in malaria high burden countries, even research is needed to increase uptake of IPTp-SP use. Our findings have implications for achievement of the ambitious goals set out in the WHO Global Technical Strategy for malaria 2016 – 2030 [10] will not be achieved with solely massive ITN deployments and other additional strategy is needed. Even risk factors assessment doesn't mean causality, major risk associations highlight potential contribution for malaria control. Evidences that additional interventions to contribute to accelerate malaria control in Burkina and other high burden countries may be oriented on research questions which need to answer as well ITN real use, SMC extension in school-age children impact on *P. falciparum* prevalence in community, electricity real benefit in the context of country development, house contribution with the types of structured house which would be effectiveness for malaria control in Burkina Faso and other high burden

countries in sub-Saharan Africa. Because education of young people was strongly associated with malaria infection risk, Burkina Faso Government may take account people education in the disease control strategies to impact significantly mosquitoes' abundance and the infection risk reduce. The onset of Coronavirus disease (COVID-19) pandemic bring new challenges for fragile health systems and strong, adequate and preventive actions should be implemented to deal with the emergency and avoid a setback in the fight against malaria. Robust political commitment in high burden countries will be key to success.

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Appendix 1.

Table 1: mode of action of drugs in the study

Drug name	**Sulfadoxine - pyrimethamine**	**Artemether - lumefantrine**
indication	Sulfadoxine, a sulphur medicine, and pyrimethamine combination is used to treat malaria (uncomplicated malaria). This medicine may also be used to prevent malaria in people who are living in, or will be traveling to, an area where there is a chance of getting malaria.	Artemether - lumefantrine combination is an antimalarial used to treat acute, uncomplicated malaria infections due to *Plasmodium falciparum* in patients of 5 kg bodyweight and above. It is not typically used to prevent malaria or treat severe malaria. It's an effective and well-tolerated malaria treatment, providing high cure rates even in areas of multi-drug resistance. It has been shown to be effective in geographical regions where resistance to chloroquine has been reported.
Mode of action	Pyrimethamine is an antiparasitic drug. It prevents the growth and reproduction of parasites. Sulfadoxine is a sulfa drug that fights bacteria in the body. The combination of pyrimethamine and sulfadoxine is used to treat malaria, a disease caused by parasites. Pyrimethamine selectively inhibits the plasmodial form of dihydrofolate reductase, reducing the production of folic acid required for nucleic acid synthesis in the malarial parasite	Artemether is administered in combination with lumefantrine for improved efficacy. Artemether has a rapid onset of action and is rapidly cleared from the body. It is thought that artemether provides rapid symptomatic relief by reducing the number of malarial parasites. Lumefantrine binds to hemin produced during hemoglobin breakdown, preventing detoxification to crystalline malaria pigment (hemozoin). During the same process, the perOXide group in artemether binds to heme and releases toxic free-radicals.

Description	Each tablet contains 500 mg N^1-(5,6-dimethoxy-4-pyrimidinyl) sulfanilamide (sulfadoxine) and 25 mg 2,4-diamino-5-(p-chlorophenyl)-6-ethylpyrimidine (pyrimethamine). Each tablet also contains cornstarch, gelatin, lactose, magnesium stearate and talc.	Each tablet contains 20 mg of artemether and 120 mg of lumefantrine. Each tablet is supplied as yellow, round, flat tablets with beveled edges and scored on one side.
Sides effects	Nausea, vomiting, diarrhoea, fatigue, feeling of fullness, dizziness, headache, mouth sore, skin rash.	Headache, dizziness, fever, cough, feeling weak or tired, muscle pain, tenderness, or weakness, joint pain, vomiting, loss of appetite.

Appendix 2. Microscopy and PCR procedures

Microscopy

Blood sampling

For each study subject, slides for thick and thin blood smears were made. During this study, the materials used for data collection were chosen to minimise the risks to participants in the study. Thus, for all blood samples, sterile single-use equipment was used (vaccinostyl, lancet). The sampling site was systematically disinfected and protected with an alcohol swab before and after sampling.

Preparation of thick & thin smears

Thick blood smears were made from 4 drops of peripheral blood collected from the fingertip by pricking with a vaccinostyl. The finger was cleaned with cotton wool soaked in 70°C ethanol before pricking, and the first drop of blood wiped off with clean toilet paper (Fig. 1). The thick drop and the blood smear were made on the same slide. The slide was identified by writing the patient's anonymous code, date and day of collection with an indelible marker;

- Make a thick spread with the drops intended for the thick drop, spread the blood with the corner of another clean slide by mixing the 3 drops, until a homogeneous spread of about 1.5cm in diameter is obtained;

- For the thin smear, hold the slide with one hand so that the drop is facing you. For the blood smear drop, hold the slide with one hand so that the drop is pointing towards you. With the other hand, place the edge of a clean slide just in front of the blood smear drop;

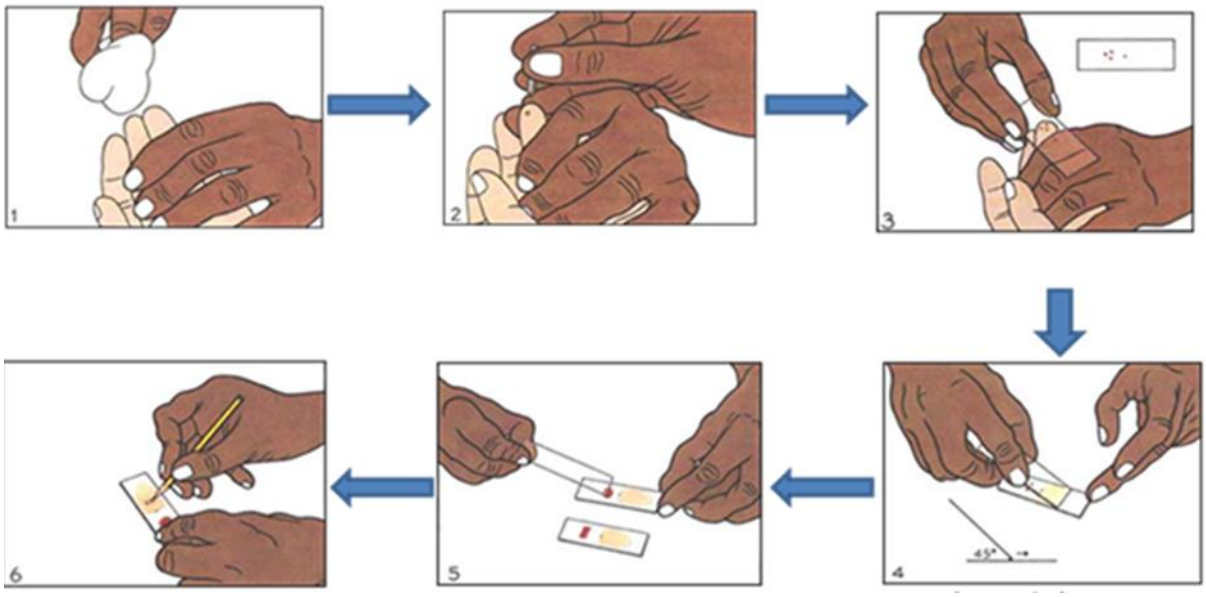
- Slide the slide towards you until it touches the drop of blood;

- Allow the blood to spread along the edge of the slide and then lift the slide to an angle of approximately 45°C;

- Push the slide to the end of the spreader blade in a smooth, even motion;

- Allow the smear to dry completely and with a pencil, transfer the same information written in permanent marker on the slide.

1- Finger cleaning *2- Fingertip incision* *3- Deposit 4 drops of blood*



6- Blood smear identification *5- Making the blood drop* *4- Preparing the blood smear*

7- Appearance of the thick drop / blood smear

Figure 1: Steps in making a thin and thick blood smear (World Health Organization, 1991)

Blood spot preparation

For each subject included in the study, blood samples were taken on filter paper from the fingertip. After collection, the spots were dried in a dust-free, well-ventilated area; they were then packed with silica gel in plastic bags and sent to the CNRFP Molecular Biology laboratory for genotyping (Fig. 2).

Blood smear fixation *Drying 5mn* *put the slide in the staining box* *Add Giemsa 5%*

Microscopy reading *Drying the slides* *Rinsing the slides with a tap* *Coloration 45mn*

Figure 2: Staining of slides with GIEMSA (Photo CNRFP)

1-Finger cleaning *2-Fingertip incision* *3-Doing the spot*

4-Drying the spot *5- spot packaging*

Figure 3: Preparation of a PCR spot on filter paper (Photo CNRFP)

Laboratory analysis

The immuno-parasitology and molecular biology laboratories are where the samples collected were processed and analysed.

Microscopy reading

Staining of slides

The smears on the prepared slides were fixed with methanol (thick drops are not fixed) and then dried for about 5 minutes. The slides were then immersed in a 6% diluted Giemsa solution for 35 minutes, rinsed with buffered water and dried (Fig. 3).

Preparation of buffered water at pH 7.2

Na2HPO4 1 g

K2HPO4 0.8 g

Distilled water 1 L

Mix well and adjust the pH to 7.2 using a pH meter

Preparation of the 6% Giemsa solution

Pure Giemsa 12 ml

Buffered water 188 ml

This gives a total volume of 200 ml of Giemsa solution for 20 slides.

Microscopic reading of slides

Giemsa-stained slides were read under a microscope with a 100X objective and oil immersion. Parasite densities (DP), expressed as the number of parasites per microlitre (µl) of blood were calculated using the following method: the number of parasites per microlitre of blood in a thick drop is determined in relation to the number of leukocytes fixed at 8,000 per microlitre of blood. We used a manual counter. To obtain the number of parasites/µl (DP), we count in parallel a number n of parasites for a number X of leukocytes, i.e. the following formula:

$$DP = n \times \frac{8000}{X}$$

If the number of parasites of asexual forms n ≥ 10, at least X = 200 leucocytes are counted and the formula is applied:

$$DP = n \times \frac{8000}{200} = n \times 40$$

If the number of parasites of asexual forms n < 10 or in case of presence of sexual forms, the parasite density is made on the basis of 1000 leucocytes counted.

$$DP = n \times \frac{8000}{1000} = n \times 8$$

If the number of asexual parasites n > 1000 before reaching 200 leukocytes, stop the count after reading the current field.

If no parasite growth forms are found in 100 microscopic fields, the slide is declared negative.

Slides with a defective thick drop were examined only at the thin smear level and the parasite density calculated from the smear. Estimating an average of 200 red blood cells (RBCs) per microscopic field and 4,000,000 red blood cells per microlitre of blood, the PD expression is as follows:

$$DP = \frac{Nombre\ de\ GR\ \ parasites \times 4\,000\,000}{Nombre\ de\ champs\ examin\ és \times 200}$$

The smear was also used to identify the plasmodial species.

PCR (Polymerase Chain Reaction) analysis

The prepared blood spots were analysed at the CNRFP molecular biology laboratory to confirm or refute the microscopy results. The procedure used in PCR diagnosis was as follows: extraction of DNA, amplification of the extracted DNA and electrophoresis of the amplified DNA on agarose gel.

DNA extraction using the QIAamp® Kit (QIAGEN)

The extraction was done according to the QIAamp DNA Mini Kit protocol based on the principle of precipitation and column purification of DNA. It is described as follows by the supplier:

- Cut 3 pellets of 3 mm diameter from a drop of dried blood using a pair of scissors or a hole punch and transfer them to 1.5 ml Eppendorf tubes;

- Add 180 µl of ATL buffer, incubate for 10 minutes at 85°C and then centrifuge briefly to recover the droplets accumulated in the cap;

- Add 20 µl of Proteinase K, mix by vortexing, incubate for 1 hour at 56°C then centrifuge briefly;

- Add 200 µl of Buffer AL to each sample, mix vigorously for 15 seconds and incubate for 10 minutes at 70°C. Centrifuge briefly to recover the droplets accumulated in the cap. This step corresponds to the lysis of the red blood cells;

Note:

For effective lysis, it is important to mix the samples immediately and vigorously with AL buffer to obtain a homogeneous solution.

Never add QIAGEN protease directly to AL buffer.

A white precipitate may form after the addition of Buffer AL. This precipitate will mostly dissolve during the incubation time. This precipitate does not affect the QIAamp procedure or downstream applications.

- Add 200µl of absolute ethanol (96-100%) to the sample and mix for 15 seconds by vortexing. Centrifuge briefly; the DNA will be a precipitate;

- Carefully place the resulting mixture into the QIAamp column (installed in a 2ml collection tube) without wetting the edge. Close the cap and centrifuge for 1 minute at 8000 rpm;

- Transfer the QIAamp column to a new 2ml collection tube (provided) and discard the tube containing the filtrate;

Note:

Close each column to prevent aerosols from forming during centrifugation.

Centrifugation is performed at 8000 rpm for 1 minute to limit the noise of the centrifuge.

If the solution has not completely passed through the membrane, centrifuge a second time at high speed until the column is completely empty.

- Carefully open the QIAamp column and add 500 µl of Buffer AW1 diluted with ethanol (19 ml Buffer AW1 plus 25 ml ethanol) to each sample without wetting the rim. Close the cap and centrifuge for 1 minute at 8000 rpm;

- Transfer the column to a new 2 ml collection tube (provided) and discard the tube containing the filtrate;

- Open the QIAamp column and add 500 µl of Buffer AW2 diluted with ethanol (13 ml Buffer AW2 plus 30 ml ethanol) to each sample without wetting the rim. Close the cap and centrifuge for 3 minutes at maximum speed 13,000 rpm;

To avoid any risk of recovery of the AW2 buffer, empty the collection tube containing the filtrate and return the QIAamp column to the old 2 ml collection tube. Refuge for 3 min at maximum speed of 13 000 rpm.

- Transfer the QIAamp column to a clean 1.5 ml tube. Discard the old collection tube containing the effluent. Carefully open the QIAamp column and add 150 µl of buffer AE or distilled water to the column. Incubate for 2 minutes at room temperature (15o to 25oC) and then centrifuge for 3 minutes at 8000 rpm;

- Discard the QIAamp column and close the 1.5 ml tubes containing the extracted DNA which should be stored at +4oC.

Amplification of specific sequences from the extracted DNA

Nested PCR was used for the amplification of Plasmodium sp. DNA sequences.

Nested PCR involves performing two successive amplifications (the second using the products of the first). It improves the specificity and efficiency of the PCR. Described by Mullis and his colleagues in 1985, PCR is based on the replication of DNA "in vitro" (Kaplan and Delpech, 1993). It allows the amplification of DNA sequences in a specific manner and a considerable increase in the quantity of DNA initially available. It requires knowledge of the sequence of the regions that delimit the DNA to be amplified. These sequences will be used to synthesise complementary oligonucleotide primers that will be used to delimit the portion of DNA to be amplified.

Principle

The principle of this molecular technique is to repeatedly use one of the properties of DNA polymerases, which is the ability to synthesise a complementary strand of DNA from a pair of primers. The reaction consists of a number of cycles, each of which includes three steps: denaturation, hybridisation and elongation.

All the elements necessary for the reaction are contained in a tube which will be subjected to the different temperatures corresponding to each stage of the PCR. These temperature cycles are performed automatically by a program in a thermal cycler.

Reconstitution or suspension of primers at 100 µM

As the primers are supplied in lyophilized form and in nmol, the following formula is applied to obtain stock solutions at 100 µM concentration: where,

Co = Concentration in nmol of the primer lyophilisate

Ve = Volume of sterile distilled water in µl to be added to the lyophilisate = Co x 10

This gives a primer stock solution of 100 µmol/L.

Dilution of the primer stock solution to 10 µM

- To obtain a total volume of 100 µl of working solution, simply take 10 µl of the 100 µM primer stock solution and make up with 90 µl of sterile distilled water, i.e. a 1/10 dilution.

NB: All reagents are stored at -20oC

Components of the amplification mixture

Reagents :

10 X PCR-Buffer

50 mM Magnesium Chloride

dNTP: deoxyribonucleotide triphosphate

Taq polymerase

Sterile distilled water

Primers :

First amplification: rPLU5&6

Second amplification: rFAL1&2, rMAL1&2, rVIV1&2, rOVA1&2

Procedures

First amplification

First label the tubes required for the PCR reaction and then prepare the mixture (reaction mix) according to the total number of samples to be processed, as shown in Table below:

Table : Volumetric composition of the amplification mix

Reagents	Co	Cf	vol/reaction	Mix x (n+3) µl
PCR Buffer	10X	1X	2	
$MgCl_2$	50mM	2mM	0,8	
DNTP	2mM	0,125mM	1,25	
rPLU5	10µM	0,250µM	0,5	
rPLU6	10µM	0,250µM	0,5	
Taq	5UI	0,4UI	0,1	
H_20	-	-	13,85	
Total			19	
DNA			1	

Co : initial Concentration; Cf : final Concentration

For each manipulation, a calculation of the volume necessary for each reagent is essential. This calculation is always done on n+3, n being the number of samples to be analysed and the 3 additional samples are composed as follows 1 negative control consisting of sterile distilled water (water for injection); 1 positive control consisting of a recognised positive extract. The third additional sample avoids having an insufficient volume for the last sample due to possible pipetting losses.

A volume of 19 µl of Mix was dispensed into each tube. Then 1 µl of DNA was added to each tube to give a total volume of 20 µl. The tubes were then immediately placed in the thermocycler for the 1st amplification following a pre-recorded program described as follows:

1. Initial denaturation at 95°C (5 min); 2. Hybridisation at 58°C (2 min); 3. Extension at 72°C (2 min); 4. Initial denaturation at 94°C (1 min); 5. 24 cycles of step 2 to 4; 6. Hybridisation at 58°C (2 min); 7. Final extension at 72°C (5 min); 8. 20ºC indefinite time for completion of amplification.

Second amplification

Label the tubes for the second amplification by transferring the previous numbers to the new tubes (Table II).

Table II : Volumetric composition of the amplification mix

Reagents	Co	Cf	vol/reaction	Mix x (n+3) µ
PCR Buffer	10X	1X	2	
$MgCl_2$	50mM	2mM	0,8	
DNTP	2mM	0,125mM	1,25	
rFAL1/rMAL1/rOVA1/rVIV1	10µM	0,250µM	0,5	
rFAL2/rMAL2/rOVA2/rVIV2	10µM	0,250µM	0,5	
Taq	5UI	0,4UI	0,1	
H_20	-	-	13,85	
Total			19	
DNA			1	

A volume of 19 µl of MIX was dispensed into each tube. Then 1 µl of the product of the first amplification was added to each tube. The tubes were then placed in the thermocycler for the 2nd amplification following the program pre-entered in the thermocycler as follows:

1. Initial denaturation at 95°C (5 min); 2. Hybridisation at 58°C (2 min); 3. Extension at 72°C (2 min); 4. Initial denaturation at 94°C (1 min); 5. 30 cycles of step 2 to 4; 6. Hybridisation at 58°C (2 min); 7. Final extension at 72°C (5 min); 8. 20ºC indefinite for completion of amplification.

Agarose gel electrophoresis of the amplification product

The DNA fragments obtained by PCR are in such quantity that they can be revealed directly by agarose gel electrophoresis using ethidium bromide (BET). BET is an intercalant that slips between the bases of nucleic acids. It fluoresces orange under short-wave UV illumination of approximately 300 nm. As the migration speed is dependent on the molecular weight, the DNA fragments are separated according to their size expressed in base pairs. Their identification is facilitated by the use of molecular weight markers.

Preparation of T.B.E. (Tris-Borate-E.D.T.A. 0,5X)

T.B.E. is the electrolyte or migration buffer. It is also used for the preparation of the migration gel. Its composition for 1 litre of solution is as follows

Reagents	Quantity
Tribase [(Trihydroxyméthyl) aminométhane $C_4H_{11}NO_3$]	54 g
EDTA ($C_{10}H_{14}N_2NaO_8$ $2H_2O$) (0,5M)	20 ml
Boric acid (BH_3O_3)	27,5 g
Distilled water qsp	1L

Preparation of the gel

Agarose is a purified agar-based polymer and is available in powder form. The weight of agarose to be used and the volume of buffer required for gel formation are determined by the size of the fragments to be separated.

- Prepare the gel mould containing the combs according to the number of samples to be processed. The comb is used to mark the footprint of the DNA deposition wells.

Prepare a 1.5% agarose gel as follows

- Dissolve 1.5 g of agarose in 100 ml of 0.5X TBE in an Erlenmeyer flask

- Boil this mixture in a microwave oven for 3 minutes,

- Cool to approximately 45oC, add 3 µl of Ethidium Bromide at 10mg/ml to the boiled agarose. Ethidium bromide binds to the hydrogen bridges of the nucleic acids, making the DNA bands UV fluorescent.

- Cast the gel into the mould and wait for it to solidify

Migration

- Immerse the gel in the migration vessel containing 0.5X TBE which is the migration buffer.

- Place approximately 2 µl of the molecular weight marker in the first well.

- Mix 10 µl of each sample with the loading buffer and place in the wells.

- Migrate at 100 volts for 20-30 minutes.

Revealing and interpreting electrophoresis results

Revealing the amplification is done by electrophoretic migration with 10L of the amplification product on a 1.5% TBE agarose gel containing 1.5% ethidium bromide in order to demonstrate the effective presence of each species on the basis of band size. Reading is done under UV light.

A good reaction is indicated by the presence of bands. In this case, the conformity of the size of the expected product as well as the negative and positive controls must be assessed.

The electrophoresis results are interpreted as follows:

Line S: standard molecular weight marker (50-bp).

Line 1: diagnostic band for *P. vivax* (120 bp).

Line 2: diagnostic band for *P. malariae* (144 bp).

Line 3: diagnostic band for *P. falciparum* (205 bp).

Line 4: diagnostic band for *P. ovale* (800 bp).

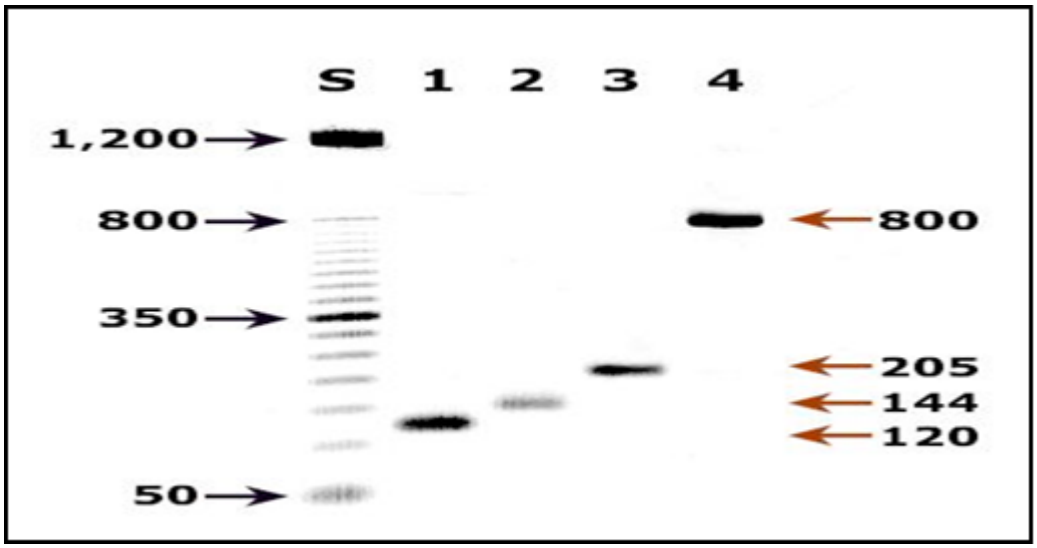


Figure 4 : Example of agarose gel separation (Snounou et al., 1993).

Appendix 3. Table 1: Linear correlations between risk factors for malaria related to

	Sex	Age	Ethnicity	Wall	Ground	Roof	Eaves	Religion	Education	Occupation	Distance to all larval habitat	Distance to positive larval habitat	Use of ITNs*	Electricity supply	Integrity of ITNs*	Presence of house-hold animals	SES#	House hold size
Sex	1.0000																	
Age	0.0039	1.0000																
Ethnicity	-0.0973	-0.0039	1.0000															
Wall	-0.098	-0.0449	-0.061	1.0000														
Ground	-0.0566	0.0222	-0.1949	0.3718	1.0000													
Roof	0.0197	0.0652	0.2813	-0.1964	-0.4138	1.0000												
Eaves	0.0141	0.0127	-0.1173	0.0904	0.0683	-0.3229	1.0000											
Other protection	-0.0671	-0.1069	-0.0086	0.2911	0.2124	-0.116	0.0839											
Religion	-0.0159	0.0708	-0.1659	0.1188	-0.0363	-0.0438	0.0175	1.0000										
Education	-0.1596	-0.0479	-0.1175	0.0255	0.0899	-0.0301	0.1357	0.0536	1.0000									
Occupation	-0.0625	-0.0158	-0.0737	-0.0187	0.0557	0.023	0.0927	-0.0202	0.3497	1.0000								
Distance to all larval habitat	0.1008	0.0152	0.2117	-0.0371	-0.0074	0.0694	-0.2212	0.0104	0.0046	0.0653	1.0000							
Distance to positive larval habitat	0.0719	-0.0212	0.2177	0.0305	0.0618	-0.0511	-0.1511	-0.0145	-0.0064	0.0311	0.7037	1.0000						
Use of ITNs*	0.0948	0.0877	-0.0496	-0.0278	-0.0343	-0.1288	0.1146	0.0733	0.0898	0.0956	0.1438	0.1883	1.0000					
Electricity supply	-0.0533	0.0212	0.2727	-0.1826	-0.212	0.1883	-0.2445	0.0025	-0.107	-0.0955	0.0707	0.043	-0.2799	1.0000				
Integrity of ITNs*	-0.0801	0.0463	0.0876	-0.0265	-0.0642	0.0526	-0.1903	-0.0203	0.0614	0.0323	0.1756	0.0793	-0.0257	0.1946	1.0000			
Presence of household animals	-0.1076	-0.0145	0.1088	0.1656	-0.0201	0.077	-0.0417	0.1602	0.0425	0.0085	-0.051	-0.0828	0.0413	0.0058	-0.0308	1.0000		
SES#	-0.0305	-0.0242	-0.0001	0.034	0.0297	0.053	-0.3803	0.1946	-0.0602	-0.0642	0.0602	-0.0693	-0.185	0.3012	0.1921	0.1586	1.0000	
Household size	-0.0384	-0.0014	0.0081	-0.2003	0.0368	0.1198	-0.1796	-0.0429	-0.019	-0.0841	-0.0867	-0.0473	-0.0859	0.0059	0.0499	-0.0172	0.0385	1.0000

**ITN=Insecticide treated net, #SES=socioeconomic status*

Variable codes: Sex (male=0, female=1), age (5-7=0, 8-15 years=1), ethnicity (other ethnicity=0, Fulani=1), wall (mud=0, brick=1, cement=2), ground (dirt floor=0, cement/tiles=1), roof (metal=0, non-metal=1), religion (Muslim=0, Christians=1, Animists=2), education (illiterate=0, literate=1), occupation (farmer=0, non-farmer=1), distance to all larval habitat (<=300 m=0, >300 m=1), Distance to positive larval habitat (<=300 m=0, >300 m=1), Use of ITNs (no=0, yes=1), Electricity supply (no=0, yes=1), Integrity of ITNs (good=0, damaged=1, too torn=2), Presence of household animals (no=0, yes=1), SES (poorest=0, poor=1, middle=2, rich=3, richest=4), eaves (closed=0, opened=1) .

Table 2: Linear correlations between risk factors for malaria infection related to chapter 3

	Ethnicity	Religion	Education	Occupation	SES#	Use of ITNs*	Presence of electricity	Presence of electric fan	Presence of household animals	Use of other protection**	Type of roof	Eaves
Ethnicity	1.0000											
Religion	-0.4412	1.0000										
Education	-0.0405	0.0474	1.0000									
Occupation	0.0389	-0.0701	-0.3081	1.0000								
SES#	0.1040	-0.2181	0.0532	0.0860	1.0000							
Use of ITNs*	-0.1176	0.0688	-0.0768	-0.0377	-0.1357	1.0000						
Presence of electricity	-0.1352	0.0903	-0.0107	-0.1045	-0.2560	0.1585	1.0000					
Use of electric fan	0.0013	-0.0529	-0.0371	-0.0213	-0.0198	0.0401	0.1428	1.0000				
Presence of household animals	0.0072	-0.0271	-0.0423	-0.0372	0.0010	-0.0263	0.0308	0.1207	1.0000			
Use of other protection	-0.0022	-0.0525	0.0781	0.0185	0.0415	-0.0409	0.0040	0.0606	0.0307	1.0000		
Type of roof	-0.0623	0.0054	-0.0940	0.0334	0.0215	0.0608	-0.0606	-0.0217	0.0753	-0.0892	1.0000	
Eaves	0.1498	-0.2659	-0.0011	0.0751	0.3283	0.0104	-0.2954	-0.1009	-0.0358	0.0054	0.0236	1.0000

**ITNs=Insecticide treated net, #SES=socioeconomic status*

*** Use other protection= use of mosquito coil, insecticide spray, traditional repulse*

Variable's characteristics: Ethnicity (others ethnicity=0, Fulani=1), religion (Muslim=0, Christian=1, Animists=2), education (illiterate=0, literate=1), occupation (farmer=0, commercial=1, none/retired=2), Socio-economics status (poorest=0, poor=1, middle=2, rich=3, richest=4), use of ITNs (No=0, yes=1), use of electric fan (No=0, yes=1), Presence of electricity (No=0, yes=1), Presence of household animals (No=0, yes=1), Use of other protection (No=0, yes=1), type of roof (metal=0, non-metal=1), eaves (opened=0, closed=1).

Table 2: Linear correlations between risk factors for malaria infection related to chapter 3

	Ethnicity	Religion	Education	Occupation	SES#	Use of ITNs*	Presence of electricity	Presence of electric fan	Presence of household animals	Use of other protection**	Type of roof	Eaves
Ethnicity	1.0000											
Religion	-0.4412	1.0000										
Education	-0.0405	0.0474	1.0000									
Occupation	0.0389	-0.0701	-0.3081	1.0000								
SES#	0.1040	-0.2181	0.0532	0.0860	1.0000							
Use of ITNs*	-0.1176	0.0688	-0.0768	-0.0377	-0.1357	1.0000						
Presence of electricity	-0.1352	0.0903	-0.0107	-0.1045	-0.2560	0.1585	1.0000					
Use of electric fan	0.0013	-0.0529	-0.0371	-0.0213	-0.0198	0.0401	0.1428	1.0000				
Presence of household animals	0.0072	-0.0271	-0.0423	-0.0372	0.0010	-0.0263	0.0308	0.1207	1.0000			
Use of other protection	-0.0022	-0.0525	0.0781	0.0185	0.0415	-0.0409	0.0040	0.0606	0.0307	1.0000		
Type of roof	-0.0623	0.0054	-0.0940	0.0334	0.0215	0.0608	-0.0606	-0.0217	0.0753	-0.0892	1.0000	
Eaves	0.1498	-0.2659	-0.0011	0.0751	0.3283	0.0104	-0.2954	-0.1009	-0.0358	0.0054	0.0236	1.0000

**ITNs=Insecticide treated net, #SES=socioeconomic status*

*** Use other protection= use of mosquito coil, insecticide spray, traditional repulse*

Variable's characteristics: Ethnicity (others ethnicity=0, Fulani=1), religion (Muslim=0, Christian=1, Animists=2), education (illiterate=0, literate=1), occupation (farmer=0, commercial=1, none/retired=2), Socio-economics status (poorest=0, poor=1, middle=2, rich=3, richest=4), use of ITNs (No=0, yes=1), use of electric fan (No=0, yes=1), Presence of electricity (No=0, yes=1), Presence of household animals (No=0, yes=1), Use of other protection (No=0, yes=1), type of roof (metal=0, non-metal=1), eaves (opened=0, closed=1).

poor=1, middle=2, rich=3, richest=4), use of ITNs (No=0, yes=1), use of electric fan (No=0, yes=1), Presence of household animals (No=0, yes=1), Use of other protection (No=0, yes=1), type of roof (metal=0, non-metal=1), eaves (open=0, closed=1).

Table 3 of correlations between risk factors for malaria related to chapter 4

	Sex	Occupation	ethnicity	Religion	education	Socio-economic status	Use of insecticide treated net	Other protection	Eaves	Roof	Electricity supply	Use of electric fan	Household animals
Sex	1.0000												
Occupation	-0.0759	1.0000											
ethnicity	0.0057	0.0650	1.0000										
Religion	-0.0118	-0.1110	-0.1540	1.0000									
education	0.1162	-0.3091	-0.1226	0.0599	1.0000								
Socio-economic status	-0.0146	0.0775	0.0209	-0.3167	0.0187	1.0000							
Use of insecticide treated net	0.0215	0.0172	-0.0457	0.1080	-0.0944	-0.1276	1.0000						
Other protection	-0.0421	0.0611	-0.0473	-0.0531	0.1006	0.0142	-0.0759	1.0000					
Eaves	-0.0374	0.1137	0.0321	-0.2959	-0.0213	0.3498	0.0145	-0.0076	1.0000				
Roof	0.0578	0.0248	0.1968	0.0922	-0.1295	-0.1123	0.1302	-0.1202	-0.1231	1.0000			
Electricty supply	-0.0122	-0.0708	-0.0899	0.1335	-0.0214	-0.2726	0.0937	0.0227	-0.3710	0.0477	1.0000		
Use of electric fan	-0.0264	-0.0259	-0.0549	-0.0934	0.0481	0.0084	0.0039	0.0190	-0.1132	0.0069	0.1339	1.0000	
Household animals	0.0995	-0.0475	-0.0100	-0.0023	-0.0603	-0.0595	0.0450	0.0369	-0.0608	0.0777	0.0644	0.1785	1.000
Insecticide resistance	-0.0564	-0.0881	-0.0731	0.1804	0.1332	-0.0140	0.0479	0.0631	-0.1208	-0.3769	0.0208	-0.0539	-0.0736

Categorical variables: sex (male=0, female=1), occupation (farming=0, commercial and office workers=1, none or retired=2), ethnicity (Gouin/Turka=0, Karaboro=1, other ethnic group=2), religion (Muslim=0, Christian=1, Animist=2), education (illiterate=0, literate=1), SES (lowest=0, low=1, middle=2, high=3, highest=4), other protection (topic repellent=0, spatial repellent=1), eaves (closed=0, open=1), roof (metal=0, thatch/mud=1), electricity supply (no=0, yes=1), use of electric fan (no=0, yes=1).

There is no correlation between electricity supply and roof

Table 4 of correlations between risk factors for malaria related to chapter 5

	Age	IPTp doses	Gestation	Gravidity	Use of ITNs*	Education	Occupation	House hold Size	Electricity supply	House hold animals	Clothes hanged	Solid wastes	Out bed night	Roof	Eaves
Age	1.0000														
IPTp doses	-0.1486	1.0000													
Gestation	0.0993	0.5675	1.0000												
Gravidity	-0.7601	0.1164	-0.0806	1.0000											
Use of ITNs*	0.2397	-0.1801	-0.194	-0.2599	1.0000										
Education	-0.4745	0.135	0.0568	0.348	-0.1702	1.0000									
Occupation	-0.2388	0.0816	0.1882	0.1938	-0.1917	0.3637	1.0000								
HouseHould size	0.3704	-0.0126	0.1188	-0.3304	0.0774	-0.1338	-0.0483	1.0000							
Electricity supply	-0.0896	0.1589	0.1938	0.1371	0.0631	0.2131	0.0866	0.1523	1.0000						
Household animals	0.086	-0.0367	0.061	-0.1756	0.2161	-0.0819	0.0826	0.1137	-0.0963	1.0000					
Clothes hanged	0.1336	-0.0094	0.0868	-0.1246	0.0422	-0.1246	0.0725	0.0227	-0.1024	0.1803	1.0000				
Solid wastes	0.048	0.1171	0.1986	0.031	0.1107	0.069	0.1264	0.0556	0.0747	0.1799	0.1588	1.0000			
Out bed night	0.1026	0.1102	0.1897	0.0195	0.0317	-0.2633	-0.1631	0.0756	-0.0926	-0.0222	0.0258	0.0744	1.0000		
Roof	0.0697	0.0496	0.0783	-0.0989	0.049	-0.0989	-0.1263	-0.0007	-0.2409	0.1126	0.1349	0.0674	-0.0317	1.0000	
Eaves	0.0688	0.0858	-0.0097	-0.123	0.0188	-0.0724	-0.0574	0.0533	0.1884	0.1421	-0.1541	-0.1469	-0.0663	-0.2556	1.0000

*ITNs = Insecticide treated net

A linear relationship between variables has been computed to identify correlation of variables with others risk factors have been performed and the summary of the results is below in the corelation table (table 4), inserted in appendix 3 section.

Variable's code:

Age (30-40=0, 20-30=1, <20=2) years old

IPTp (0 dose=0, 1 dose=1, 2 doses=2, 3 doses or more=3)

Gestation (3rd trimester=0, 2nd trimester=1, 1st trimester=2)

Gravidity (multigravida=0, secundigravida=1, primigravida=2)

Use of ITNs (no=0, yes=1)*

Education (illiterate=0, literate=1)

Occupation (farmer=0, trader=1, others=0)

Household size (1-3=0, 4-5=1, ≥6=2)

Electricity supply (no=0, yes=1)

Household animals (no=0, yes=1)

Clothes hanged (no=0, yes=1)

Solid wastes (no=0, yes=1)

Out bed night (<2=0, 2 or more=1)

Roof (metal=0, non-metal=1)

Eaves (closed=0, open=1)

A linear relationship between variables has been computed to identify correlation of variables with others risk factors have been performed and the summary of the results is below in the correlation table (table 4), inserted in appendix 3 section.

Correlations between variables were explored and negative association was found between education and age (r=-0.4745), gravidity and age (r=-07601) and household size and gravidity (r=-0.3304). On the other hand, positive correlations were found between gestation and intermittent preventive treatment in pregnant number of doses (r=0.5675) and household size and age (r=0.3704). Correlations suggest that younger pregnant women were better educated and had fewer births than older women; Pregnant women were more likely to be multigravida and were lived in households with large people and IPTp doses increased in women with more children more advanced pregnancies; the larger the household, the greater it contained younger and multigravids. The effects on our study results overall model imply that major risk factors of interest were gestation and it's association with IPTp doses (eg. for each additional dose of IPTp reported as being received by women, the odds of malaria infection fell by 44%).