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Low birthweight and infant growth among children in Papua New Guinea - effect of malaria and other infectious diseases during childhood.

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Abstract

Globally, young children continue to die or fail to thrive from treatable and preventable causes including low birthweight (LBW), childhood undernutrition (wasting, stunting, underweight) and infectious diseases. Reducing the burden of these are an essential pillar of global child health and survival targets. These global trends are reflected in Papua New Guinea (PNG), a resource constrained setting that continues to observe high rates of illness and death in young children with LBW, childhood undernutrition and infectious diseases (especially malaria, pneumonia and diarrhoea) continuing to be the leading causes. Improving child health and survival in PNG requires evidence about the risk factors for LBW, sub-optimal growth and infectious diseases, as well as the inter-relatedness of risk factors, that can inform policies and identify appropriate interventions and strategies. This thesis aims to address critical knowledge gaps in this area and provide insights to inform interventions and strategies aimed at reducing low birthweight, childhood undernutrition and malaria in young children in PNG and globally.

LBW is caused by a multitude of factors which are often inter-related and with that, it is often difficult to distinguish between independently direct and indirect effects. Moreover, current interventions targeted at preventing LBW generally assume a single dominant cause overlooking the inter-relatedness of risk factors and the possibility of factors exerting joint effects on LBW. These are difficult to establish with widely used standard statistical methods and have therefore been rarely investigated. Using structural equation modelling, we showed intermittent preventive treatment of malaria during pregnancy with sulphadoxine-pyrimethamine (SP) plus azithromycin (AZ) to be independently directly associated with reduced probability of LBW. Unexpectedly, anaemia at enrolment was also directly associated with reduced probability of LBW. Maternal undernutrition at enrolment was independently directly associated with increased probability of LBW. No significant indirect associations between risk factors and LBW were established.

After birth, children in lowlands PNG experience high rates of malaria, pneumonia and diarrhoea alongside undernutrition. Infections and undernutrition are believed to have a bi-directional relationship and whilst the effect of undernutrition on risks of illness and death is well known, little is known about the effects of malaria, pneumonia and diarrhoea on growth faltering, particularly among PNG children. By using multivariable regression and distributed

lag models for data analysis, we observed malaria pneumonia and diarrhoea to have a differential impact on child growth. The effect of acute malaria on child growth was observed to be long-term while pneumonia and diarrhoea had short-term effects lasting up to 3 months.

Of these three diseases, malaria was once ranked the top leading infectious cause of childhood morbidities and mortality in PNG, especially in lowlands PNG. The nationwide scale-up of malaria control interventions significantly reduced overall malaria transmission between 2008 and 2014 but a detailed understanding of the impact of this changing transmission on the epidemiology and risk profile of malaria infections and disease due to the two main species in young children was lacking. By analysing three consecutive longitudinal child cohorts (1-5-year-old children) conducted over the period of improved control (2013), we observed a differential impact of improved control on *P. falciparum* and *P. vivax*. Additionally, we showed that with declining malaria transmission, burden of malaria infections and illness were highly spatially localised to areas that had the highest burden prior to scale-up, highlighting potential hotspots of transmission.

Collectively, the findings from this thesis provide important insights for improving child health in PNG and globally.

Summary

It is widely recognised that the earliest years of a child's life are the most critical period. The foundations for their future health, growth and development are believed to be established during this period with substantial evidence linking adversities in first 1000 days to poor respective outcomes later in life with intergenerational influences. Preventing adversities and promoting adequate nutrition and nurturing care during this period are therefore crucial to ensuring a healthy and promising start to a child's life. Like Jawaharlal Nehru, the first Indian Prime Minister once quoted, "Children are like buds in a garden and should be carefully and lovingly nurtured, as they are the future of the nation and the citizens of tomorrow".

Globally, low birthweight, childhood undernutrition - which comprises stunting (too short for one's age), wasting (too thin for one's height) and underweight (too light for one's age) - and infectious diseases (especially malaria, pneumonia and diarrhoea) have consistently been the leading causes of deaths and suboptimal health, growth and development among young children. Reducing the burden of these health problems is therefore an urgent global priority making them an essential part of global child health and survival goals or targets. Achieving these goals will require detailed understanding of these causes to be able to identify appropriate interventions and strategies that can prevent them.

The global trends for child health are also reflected in PNG where child death rates remain alarming with more than 40 under-5 deaths per 1, 000 livebirths and approximately 15% of neonates are born low birthweight each year. The prevalence of stunting remains very high, with almost 50% of children aged under 5 years old are stunted. Notably, this has remained unchanged for over three decades. In addition, the country is experiencing an increase in the burden of wasting, which was extremely rare but is becoming common (~14%). Malaria, pneumonia and diarrhoea continue to be the major contributors to childhood morbidities and mortality. In resource constrained settings such as PNG, where availability and accessibility to affordable and quality resources for the care of LBW newborns is limited, preventing the occurrence of LBW is the top priority. The high burden of infectious diseases alongside undernutrition presents challenges for the long-term health, growth and development of PNG children. Reducing the burden of infectious disease and undernutrition is therefore of highest priority in PNG. By combining various unique cohorts with advanced statistical methods, this

thesis aimed to investigate LBW, undernutrition and infectious diseases in detail such that the information and knowledge generated may provide important insights that can inform policies that may contribute to improving child health and survival in PNG and globally.

Chapter 1: In this introductory chapter, the background and rationale for the work undertaken in this thesis is provided. This includes a review on the global situation for child health, providing an overview of the burden and trends in child mortality and suboptimal health, growth and development in the context of existing challenges and priorities.

We give an overview of malaria with a brief description of the life cycle of malaria to highlight the unique biological differences between *P. falciparum* and *P. vivax*, the two predominant Plasmodium species investigated in this thesis. The changing epidemiology of malaria in PNG is as a result of the nationwide scale-up of malaria control interventions is described. Although malaria affects all age groups in PNG, we focused this review in line with the maternal and child health focus of the thesis, highlighting some of the common consequences of malaria including malaria in pregnancy and the associated mechanisms through which malaria may cause adverse birth outcomes. Severe anaemia during childhood as mechanism through which malaria may cause child mortality is also described. An overview of LBW highlights the mechanisms via which intrauterine growth restriction and preterm delivery may occur leading to LBW, as well as the challenges LBW presents given its complex causality. Lastly, we give an overview of child undernutrition, its burden, causes and consequences.

Chapter 2: In this chapter the methodology of the trials and field studies from which the data was sourced is described alongside their key findings. A detailed description of the analytical methodology applied is provided, including the fitting a distributed lag model in R. An overview of structural equation modelling (SEM) methodology employed in chapter 3 of this thesis is given.

Chapter 3: Reducing the burden of LBW has been a global priority for the past 3 decades, with global efforts to implement interventions that target some of the causes of LBW. Yet, it has been recently reported by World Health Organisation (WHO) that the rate of decline in the past two decades has plateaued, with 15% of newborns still born too small each year. This means that every year, 1 in 7 of the newborns will face the highest risk of dying within the first month of their life or are at increased risk of having poor motor, social and emotional

development as well as being at risk of various adult onset diseases including hypertension and diabetes. Moreover, if the rate of decline in LBW continues at the current rate, the global target of a 30% reduction in LBW between 2012 and 2025 will most certainly not be met. As such there is an urgent need for actions that can accelerate the rate of decline in LBW and the first critical step is to gain a detailed understanding of the causes of LBW which ultimately can inform appropriate strategies and interventions.

The causes of LBW are multifactorial, but these factors are also often inter-related, i.e., they are likely to influence each other to contribute to LBW both directly and indirectly. For instance, malaria infection in pregnancy can result in LBW directly but it can also cause anaemia which in itself is a risk factor for LBW. The numerous interventions that are currently implemented to reduce LBW target specific causes, generally assuming a single dominant cause. This clearly does not take into consideration the inter-relatedness of these factors and the possibility of joint effects in causing LBW. Developing novel interventions to adequately address LBW will entail a detailed insight into the inter-relationships between factors causing LBW. Although clearly a high priority, there is currently very limited knowledge on this subject. Very often, standard regression models have been employed to investigate the association between the risk factors and LBW but the challenge with using these methods is that they are unable to distinguish between direct (unmediated effect of a risk factor on an outcome) and indirect effects (part of the mechanism through which a risk factor causes an outcome is mediated through another risk factor, e.g. malaria causes anaemia which cause LBW).

In Chapter 3, using data from a large intermittent preventive treatment of malaria in pregnancy (IPTp) trial conducted in PNG, we performed structural equation modelling to examine the relationships among selected modifiable risk factors to establish direct and indirect associations between these factors and LBW. A structural equation modelling approach utilizes various types of models including regression, path and factor analysis to model relationships among variables and allows the direct and indirect effects of variables to be distinguished.

The risk factors that were investigated included the IPTp intervention with SPAZ, maternal undernutrition, anaemia, iron deficiency, peripheral microscopically confirmed malaria infections (any species of any density) and inflammation as indicated by increased C-reactive protein (CRP) and/or ∝-1-acid-glycoprotein (AGP) - all of which were measured at the first

antenatal visit. Of these factors, anaemia and IPTp were associated with a significantly reduced probability of delivering a LBW baby. The opposite was observed for maternal undernutrition with all the observed associations manifesting through direct rather than indirect pathways. No statistically significant associations, direct or indirect, were observed for malaria infections, iron deficiency and inflammation in this analysis. These findings provide invaluable insight into some of the most prevalent causes of LBW, such as maternal undernutrition. The data suggests that improving maternal health and nutrition is likely to improve birth outcomes independent of the other factors investigated in this analysis. The IPTp intervention also appeared to improve birthweight via mechanisms independent of factors that were measured factors in this analysis. Although we could not establish mechanisms through which IPTp may be improving birthweight hence reducing the risk of LBW in our analysis, it is apparent that it can be an important intervention that can concurrently address multiple factors in improving birth outcomes.

Chapter 4: Besides LBW, infectious diseases and inadequate nutrition during early childhood are major contributors to suboptimal growth during early childhood. Suboptimal growth in utero and early childhood is recognised as an important cause of child morbidity and mortality as well as being linked to long-term consequence.

Childhood undernutrition and infectious diseases are a common co-occurrence, particularly in resource poor countries such as PNG. Whilst they share many common causes, such as inadequate access to land or to education among many others, they are also postulated to influence each other. It is well documented that undernutrition increases a child's susceptibility to infectious diseases and that the child's risk of dying from infectious causes is heightened if the child has underlying undernutrition. The inverse of this relationship has also been documented in some settings and was initially met with substantial scepticism. However, there has been an increase in evidence linking diarrhoeal illness in particular, to undernutrition (especially stunting), whilst associations between other infectious diseases and risk of undernutrition are inconsistent.

In PNG, data on these associations is limited and yet the there is a persisting high burden of both infectious diseases and childhood undernutrition, with rates of stunting unchanged over the past three decade and an increasing burden of wasting. It was therefore a high priority to explore the influence that these infectious diseases have on the nutritional status of children in

Chapter 4. Using data from Intermittent Preventive Treatment for malaria in infants (IPTi) trial conducted on the north coast of PNG, we employed multivariate statistical methods to examine the associations of acute episodes of malaria, pneumonia and diarrhoea with childhood undernutrition. The potential delayed effects of these diseases were investigated using distributed lag models.

Over the study period, a total of 8902 illness episodes were recorded and 26% of these episodes were due to malaria, a third were due to pneumonia and 21% were due to diarrhoea. Rates of acute co-morbidities between the three were below 11%. As anticipated, stunting was the most prevalent form of undernutrition with 61% of the children experiencing stunting at least once during the study. Children were classified as underweight in approximately 16% of the measurements, whilst wasting was very rarely observed in this cohort (~6%).

Malaria, pneumonia and diarrhoea were observed to have a differential impact on child growth. A significant association was observed between pneumonia and risk of a child being underweight. No such association was observed for malaria or diarrhoea. No significant relationships were observed between any of these three diseases and the risk of stunting. When the lag effects of these diseases on child growth were examined, the effect of malaria on child growth appeared to be delayed, manifesting up to 6 months after an acute episode. The effects of pneumonia and diarrhoea were short-term, lasting up to 3 months after the acute episode. Evidence generated in this Chapter provides useful insights to guide the development of integrated policies addressing infectious diseases and undernutrition, thereby contributing to reducing the burden of undernutrition in PNG and other similar settings.

Chapter 5: Until the early 2000s, malaria was the leading infectious cause of childhood morbidities and mortality in PNG. However, beginning 2004, the country joined in the global fight against malaria by progressively scaling up a suite of malaria control interventions including long-lasting insecticide-treated bed-nets (LLINs), rapid diagnostic tests (RDTs), artemisinin combination therapy (ACTs) and primaquine for vivax radical cure. Between 2005 and 2014, mortality due to malaria, incidence of both severe and acute uncomplicated clinical malaria and the prevalence of both *P. falciparum* and *P. vivax* infections substantially declined. It was therefore of great interest to investigate the impact of this declining transmission on the epidemiology and risk profile of malaria infections and disease due to the two main species in young children, who are the most vulnerable group. This is the focus of Chapter 5. Evidence

generated will also provide insight into the contribution of malaria to high rates of suboptimal child growth in PNG.

In this chapter, 3 consecutive longitudinal child cohorts (1-5-year-old children) conducted in the same study area across the period of malaria control scale up - prior (2006), during (2008) and after five years of improved control (2013) – were analysed. A differential impact of improved control on *P. falciparum* and *P. vivax* was observed; an immediate reduction in *P. falciparum* infections and clinical episodes as well as *P. vivax* clinical episodes but not *P. vivax* infections. A decline in the burden of *P. vivax* infections was only evident in the study that was conducted 5 years after the scale up of malaria interventions in the area suggesting that the currently implemented interventions may still be effective even for *P. vivax,* but the effect may not be immediately visible. Additionally, I showed that with declining malaria transmission, burden of malaria infections and clinical episodes were highly spatially localised to areas that had the highest burden prior to scale-up, highlighting potential hotspots of transmission. The findings indicate that effective and sustained malaria control reduces exposure to malaria infections and disease during early childhood, which is likely to positively impact child growth and development.

Chapter 6: In this chapter, a summary of all the main findings of this thesis is provided and the relevance or significance of this work in the field are discussed. The results described in this thesis expand on the existing knowledge on the commonest causes that continue to affect young children and provides important insights for improving child health in Papua New Guinea and globally.

Declaration

This is to certify that

i. this thesis comprises only my original work towards the degree of Doctor of Philosophy, except where indicated in the Preface;
ii. due acknowledgment has been made in the text to all other material used;
iii. this thesis is less than 100, 000 words in length, exclusive of tables, figures, references and appendices

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February 2020

Preface

The work presented in this thesis used data collected from several studies conducted in Papua New Guinea by PNG Medical Institute of Medical Research in collaboration with University of Melbourne, Walter and Eliza Hall Institute and Case Western Reserve University. All the analyses were conducted by the author unless indicated.

In chapter 3, we used data that was collected in a large pregnancy intervention trial (named IPTp study), conceived and designed by my supervisors Professor Stephen Rogerson and Professor Ivo Mueller. The implementation of the study including the supervision of data and sample collection and the general coordination of the study was supervised by 4 research clinicians; Dr. Sarah Hanieh, late Dr. Regina Wangnapi, Dr. Holger Unger and myself. The data used in chapter 4 was collected as part of a large infant intervention trial (named IPTi study) that was supervised by Dr. Nicolas Senn and Dr. Patricia Rarau. Associate Professor Leanne Robinson supervised reading of peripheral blood slides, results of which have been used in all the chapters of this thesis. She and I led the implementation of one of the child cohorts (2013 cohort). Dr Anna Rosanas-Urgell and Dr Eline Kattenberg supervised the analysis of samples using PCR assays for detection of malaria infections (chapter 5). Dr. Stephan Karl, James Cook University, performed analysis on malaria genotype data to create dataset of new malaria parasite clones that were used in estimation of molecular force of blood-stage infections (chapter 5). Dr. Thomas Obadia, Pastur Institut, Paris assisted in estimating the molecular force of blood-stage infections and provided the description of the methods involved (chapter 5). Dr Sophie Zaloumis, University of Melbourne provided statistical support in all my analyses, assisted me with analysis of distributed lag models, generating figures and interpretations of the different models and results.

Chapter 5 was published in a peer reviewed journal (Ome-Kaius, M., Kattenberg, JH., Zaloumis, S., Siba, M., Kiniboro, B., Jally,S., Razook, Z., Mantila, D., Sui, D., Ginny, J., Rosannas-Urgell, A., Karl, S., Obadia, T., Barry, A., Rogerson, SJ., Laman, M., Tisch, D., Felger, I., Kazura, JW., Mueller, I., Robinson, LJ. Differential impact of malaria control interventions on *P. falciparum* and *P. vivax* infections in young Papua New Guinean children. BMC Medicine, DOI: 10.1186/s12916-019-1456-9). I was the first author and I performed data collation, cleaning and analysis and manuscript writing. The senior authors were Robinson,

LJ., and Mueller, I. Obadia, T., and Zaloumis, S. provided assistance in analysis, Kattenberg, JH, Siba, P., Kiniboro, B., Jally, S., Razook, Z., Mantila, D., Sui, D., Ginny, J., Rosannas-Urgell, A., Karl, S were involved in data collection and generation; and all authors were involved in review and editing of the manuscript.

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First and foremost, I would like to thank my supervisor, Associate Professor Leanne Robinson for taking me on as her PhD student. She has been an amazing friend and an incredible mentor who has been a source of my motivation, constantly providing me with support and guidance in all aspects of my PhD. Her patience, continuous encouragement and her trust in me have allowed me to get through some of the most difficult times during my PhD and get to the end of this journey. I will be forever grateful. I would also like to thank my co-supervisor Professor Ivo Mueller for his constant support, encouragement and patience. Professor Mueller provided excellent guidance and critical and thorough reviews of my statistical analyses and my writing. I was always learning something new from him in our discussions and he made statistics and epidemiology so easy to understand. I learnt a lot from him. I would also like to sincerely thank my co-supervisor Professor Stephen Rogerson for his support and his guidance. Professor Rogerson always provided critical and thorough review of my work and was always quick to send me back feedback on my work for which, I am very grateful. I will forever be grateful to my co-supervisor Dr. Sophie Zaloumis who taught me statistics and who guided me through every stage of my analyses. Although I love statistics, it did not come easy to me and I was so fortunate to have Dr. Zaloumis who was patient and encouragingly helped me through understanding my analyses and interpreting my results. I want to extend my gratitude to my PhD advisory committee Professors Melanie Bahlo and Harin Karunajeewa for their support.

Enormous thanks to Dr. Stephan Karl, Dr. Thomas Obadia and Dr. Leila Larson for providing statistical assistance in this work. I am indebted to Zahra Razook and Jessica Brewster for their help in the lab. My heartfelt thank you to Mrs. Natalie Senzo for her daily support which made life much easier for me. Also, many thanks to Sue Davy who has been very supportive throughout the past 4 years.

I was so fortunate to be part of an amazing group, the Robinson lab, namely Desmond Gul, Shazia Ruybal, Rachael Farquhar and Samuel McEwen who have been great support and for the friendship and the laughter which was always a good medicine for stress relieve. I want to also thank the Mueller lab past and present members especially, Yi Wan Quah, Zoe Liu, Sarah Charnaud, Cristian Koepfli, Jessica Brewster, Ramin Mazhari, Emily Eriksson, Rhea Longley and Caitlin Bourke who have been like a family to me for the past 4 years. Their friendship

will be forever cherished. Many thanks to Abebe Fola, Dulcie Lautu and Patricia Rarau for their support and friendship.

I could not have done this without the support of my family and friends and I am very thankful for their confidence in me, their constant encouragement and their love. My dearest mum and brother Henry, you left just before I could finish and celebrate with you this long journey we started together. Thank you for always being there for me and for loving me. Special thank you to my daughter and my husband who have been by my side every step of the way.

Finally, I would like to thank the participants and the communities in Papua New Guinea for their involvement in the studies. Many thanks to the everyone at PNG Institute of Medical Research and the collaborating international institutions especially WEHI, University of Melbourne and Case Western Reserve University who have contributed to the design and implementation of these studies. Special thanks to the IPTp, IPTi and the child cohorts study teams for their hard work.

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Abbreviations

ACT	artemisinin-based combination therapy
ADI	active detection of infection
AGP	∝-1-acid-glycoprotein
AIDS	acquired immunodeficiency syndrome
AL	artemether lumefantrine
AQ	of amodiaquine
AS	artesunate
AZ	azithromycin
CFI	comparative fit index
CI	confidence interval
CQ	chloroquine
CRP	C-reactive protein
dhfr	dihydrofolate reductase
dhps	dihydropteroate synthase
DLM	distributed lag model
ELISA	enzyme-linked immunosorbent assays
EVT	extravillous trophoblasts
GCP	Good Clinical Practice
GEE	generalised estimating equations
Hb	haemoglobin
HIV	Human Immunodeficiency Virus
IL	interleukin
IPTi	Intermittent Preventive Treatment in Infants
IPTp	Intermittent Preventive Treatment in Pregnancy
iRBC	infected red blood cell
IUGR	intrauterine growth restriction
LBW	low birthweight
LDR-FMA	ligase detection reaction/fluorescent microsphere assay
LM	light microscopy
LMICs	low-middle-income countries
LLINs	long-lasting insecticide treated nets
lrtest	likelihood ratio test

MDG	Millennium Development Goal
$_{mol}$FOB	molecular force of blood-stage
MRAC	Medical Research Advisory Committee
MUAC	mid upper arm circumference
OR	odds ratio
ORS	oral rehydration salts solution
PCR	polymerase chain reaction
PE	protective efficacy
Pf	Plasmodium falciparum
PlGF	Placental Growth Factor
PNG	Papua New Guinea
PTD	Preterm delivery
PYAR	per-year-at-risk
RBC	red blood cell
RDT	Rapid diagnostic test
RMSEA	root mean square error of approximation
RR	risk ratio
RTIs	reproductive tract infections
SDG	Sustainable Development Goal
sEng	soluble endoglins
sFlt-1	soluble fms-like tyrosine kinase-1
SAM	severe acute malnutrition
SEM	structural equation modelling
SP	Sulphadoxine Pyrimethamine
STIs	sexually transmitted infections
TLI	Tucker–Lewis index
TNF	tumour necrosis factor
U5MR	Under-5 mortality rate
WBC	white blood cells
WFL	weight-for-length
WFH	weight-for-height
WLSMV	weighted least square mean and variance adjusted

1 Chapter 1: Introduction

Over the past two decades, global child mortality has substantially declined (Figure 1.1) with the total number of child deaths dropping from 14.2 million in 1990 to 6.2 million in 2018. Among children under 5 years of age, the age group that often bears the highest burden of all child deaths, the mortality rate (U5MR) decreased by 59% from 93 to 39 deaths per 1, 000 live births (United Nations Inter-Agency Group for Child Mortality Estimation (UN IGME) 2019). These reductions reflect the substantial progress that has been made in child health and survival with most of these reductions attributable to fewer deaths due to infectious diseases, including lower respiratory infections, diarrhoeal diseases, measles and malaria (G. B. D. Child Mortality Collaborators 2016). However, these gains have not been uniform across regions and countries; half of the global under-5 mortality are concentrated in only five countries: India, Nigeria, Pakistan, the Democratic Republic of the Congo and Ethiopia with the first two alone accounting for about a third (United Nations Inter-Agency Group for Child Mortality Estimation (UN IGME) 2019). Overall, these countries together with other low- and middle-income countries (LMICs) contribute almost all (~98%) of the global under-5 child deaths.

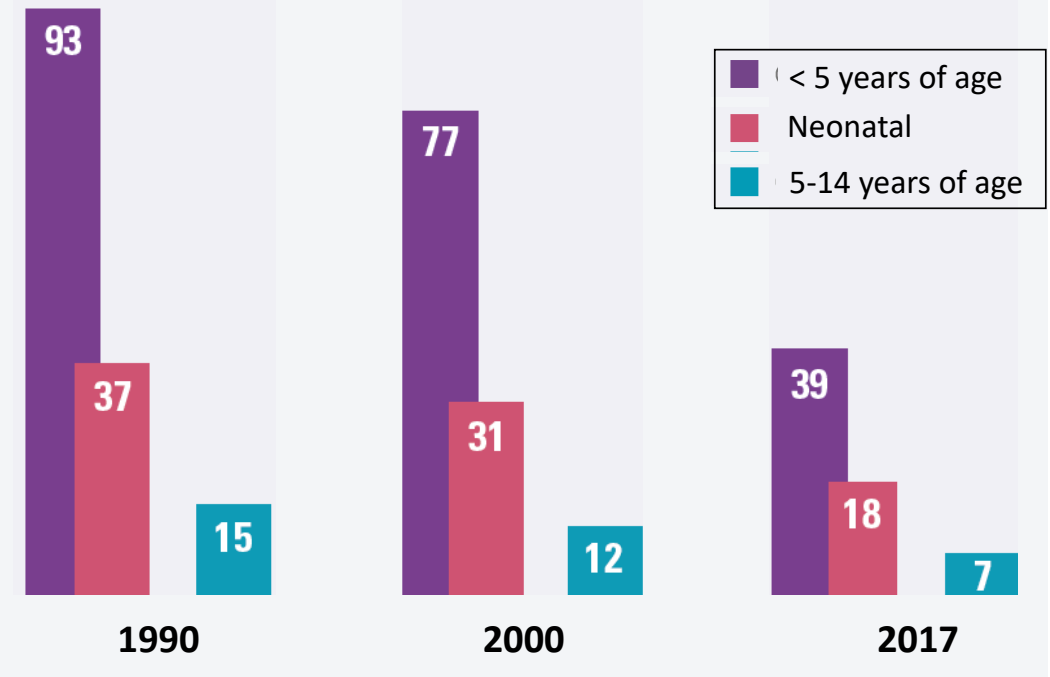

Figure 1-1: Global levels and trends in child mortality rates (per 1, 000 livebirths) by ages in the last three decades.

Adapted from the UNICEF-WHO 2018 Levels and Trends in Child Mortality rates report (United Nations Inter-Agency Group for Child Mortality Estimation (UN IGME) 2018).

In many of the LMICs like PNG, U5MR remain unacceptably high and reducing under-5 mortality remains an unfinished business. PNG reports among the highest U5MR in the Oceania region seeing 40 or more deaths per 1, 000 live births (WHO , United Nations Inter-Agency Group for Child Mortality Estimation (UN IGME) 2019); missing the country-tailored Millennium Development Goal 4 (MDG-4) target of reducing under-5 mortality from 90 (in 2000) to 32 deaths per 1, 000 livebirths by 2015 (PNG National Department of Health and Paediatric Society of Papua New Guinea 2015). The modified MDG-4 target now includes an extension of the target period to 2020, to reduce the U5MR to 32 per 1, 000 livebirths by 2020 (PNG National Department of Health and Paediatric Society of Papua New Guinea 2015). Understanding how to improve the chances of survival among young children is thus an important area of research in PNG as well as globally.

The current sustainable development goal (SDGs) included a specific target (3.2) which aims to reduce neonatal mortality rate and U5MR as low as 12 and 25 deaths per 1, 000 live births respectively by 2030 (United Nations). Target 3.2 is one of the 9 targets that are included in SDG 3 with the overall goal to ensure healthy lives and promote well-being at all ages (United Nations). Finding appropriate programs and approaches to address this target is becoming increasingly urgent. Recent evidence suggests that based on the current trends, 53 countries will not meet the target unless they accelerate their progress; 28 of these countries will need to double or even triple their rate of reduction in order to achieve this target on time (United Nations I.G.M.E 2015). Identifying the reasons underlying the poor progress by investigating the causes will be key if we are to support all countries to achieve these SDGs. Research therefore needs to focus on gaining a better understanding of how to best improve child survival and health in resource limited countries with fragile constrained health systems and how this knowledge can inform strategies and interventions to address the immediate causes that are major contributors of child mortality and morbidity.

1.1 Early child health – issues and priorities

Under-5 mortality is only the "tip of the iceberg", with more than 200 million children living in resource-constrained countries at risk of failing to thrive and unable to reach their full growth and developmental potential (Grantham-McGregor, Cheung et al. 2007, Black, Walker et al. 2016). It has long been recognised that the earliest years of life, especially the first 1, 000 days from conception to 2 years of age, are crucial for development and growth (Shrimpton, Victora

et al. 2001, Victora, de Onis et al. 2010). Children most rapidly develop and grow both physiologically and structurally during these years. Critical stages of development in all domains of children's growth including sensory-motor, cognitive-language and social-emotional as well as linear growth occur predominantly during this period (Institute of Medicine 2000, Thompson and Nelson 2001, Shonkoff, Garner et al. 2012). These first years of life are very important as they set the stage for all the future development and growth for the child.

Evidence linking adversities during this period to impairment in growth, development and health during later childhood and in adult life have been previously demonstrated (Barker and Osmond 1986, Barker, Osmond et al. 1989, Grantham-McGregor, Cheung et al. 2007, Black, Allen et al. 2008, Victora, Adair et al. 2008, Hoddinott, Behrman et al. 2013, Prendergast and Humphrey 2014). This first gained recognition in the early 80s from the research led by David Barker; he established epidemiological links between insults (e.g. undernutrition) during intrauterine life and infancy and adult diseases and proposed the "Barker Hypothesis" (currently known as "foetal origins of adult diseases") suggesting that insults during the intrauterine life and early infancy can lead to 'programming' of one's risk for development of diseases in adulthood (Barker and Osmond 1986, Barker, Osmond et al. 1989, Barker, Winter et al. 1989, Barker, Gluckman et al. 1993). Since then, there has been increasing evidence linking adversities during the earliest years with various poor outcomes including poor motor, social and emotional development in children, impaired cognition, poor academic performance, lower economic productivity later in life, poor physical and mental well-being and various adult onset diseases such as hypertension, diabetes mellitus and heart diseases (Grantham-McGregor, Cheung et al. 2007, Black, Allen et al. 2008, Victora, Adair et al. 2008, Dewey and Begum 2011, Hoddinott, Behrman et al. 2013). Of critical concern is that this becomes a vicious cycle of intergenerational transmission of poverty (i.e. deficits in resources and assets) and long-term consequences (Ramakrishnan, Martorell et al. 1999, Grantham-McGregor, Cheung et al. 2007, Martorell and Zongrone 2012, Walker, Chang et al. 2015).

Although, a multitude of factors have been implicated, poverty related factors including LBW (defined as birthweight <2500 g), childhood undernutrition and infectious diseases have consistently been the leading causes of poor child health and growth; subsequently contributing to the high burden of mortality and morbidity as well as the long-term adverse consequences observed in the resource-constrained countries (Grantham-McGregor, Cheung et al. 2007,

Black, Allen et al. 2008, Black, Cousens et al. 2010, Liu, Johnson et al. 2012, Black, Victora et al. 2013, G. B. D. Mortality and Causes of Death Collaborators 2016, United Nations Inter-Agency Group for Child Mortality Estimation (UN IGME) 2019). Child undernutrition is an aggregate of wasting (too thin for one's height), stunting (too short for one's age), underweight (too light for one's age), intrauterine growth restriction (IUGR) and deficiencies of essential vitamins, that are collectively responsible for about 45% of under-5 child deaths seen in LMICs (Black, Victora et al. 2013). Importantly, they have been linked to life-long consequences including delayed childhood growth and development (e.g. poor cognition) and various adult onset diseases such as hypertension and diabetes mellitus (Grantham-McGregor, Cheung et al. 2007, Victora, Adair et al. 2008, Dewey and Begum 2011, Upadhyay, Naik et al. 2019). This is of a particular concern for PNG where about 11% - 15% of neonates are born low birthweight each year (Klufio, Amoa et al. 1997, Unger, Ome-Kaius et al. 2015) and 40% or more of children aged under 5 years are stunted. Notably, this indicator has remained unchanged for the past three decades (Figure 1.2). Various individual studies have shown similarly high rates and the next census, planned for 2021 will provide updated estimates (Goris, Zomerdijk et al. 2017, Samiak and Emeto 2017). More recently, severe malnutrition is becoming an increasingly common presentation in hospitals across PNG constituting 10.2% of hospital admissions in 2018 making it one of the top 5 common problems seen (PNG Paediatric Society and PNG Department of Health 2018).

Despite the significant reductions observed in their prevalence (Collaborators 2017, Troeger, Colombara et al. 2018, McAllister, Liu et al. 2019, WHO 2019), pneumonia, diarrhoea and malaria remain the leading infectious causes of under-5 mortality and morbidities globally; collectively accounted for about a third of global under-5 mortality in 2018 (United Nations Inter-Agency Group for Child Mortality Estimation (UN IGME) 2019). Besides causing ill-health and deaths in the short term, they have also been linked to childhood growth faltering (Checkley, Buckley et al. 2008, Kang, Kreuels et al. 2013, Khalil, Troeger et al. 2018, Troeger, Colombara et al. 2018). However evidence is not consistent across all the settings (Briend 1990, Briend, Hasan et al. 1990, Black 1991) and is very limited from settings such as PNG. Childhood growth faltering in itself is a key predictor of child morbidities and mortality and various adverse outcomes later in life (Grantham-McGregor, Cheung et al. 2007, Black, Allen et al. 2008, Victora, Adair et al. 2008, Dewey and Begum 2011). Both child undernutrition and infectious diseases are highly prevalent, particularly in resource-constrained settings and while the associated risks of each with child mortality and other poor outcomes are well established

in many settings (as aforementioned), the association between each other remains to be clarified in some settings like PNG where they continue to be major contributors of morbidities and deaths among young children. Of special interest is the lowlands of PNG where pneumonia, diarrhoea and malaria are highly prevalent, especially malaria given its hyperendemicity in these areas and yet little is known about their interaction.

Addressing these major health problems of LBW, child undernutrition and infectious diseases in settings like PNG will be extremely difficult because of the inequalities and disparities in maternal and child health among populations (e.g. between the rural and urban populations), the weak health systems and low human capacity for adequate health care delivery (PNG National Department of Health and Paediatric Society of Papua New Guinea 2015) and will require a better understanding of the underlying causes and the impact of the interventions implemented in the country to address health issues by addressing critical gaps in knowledge with research and evidence.

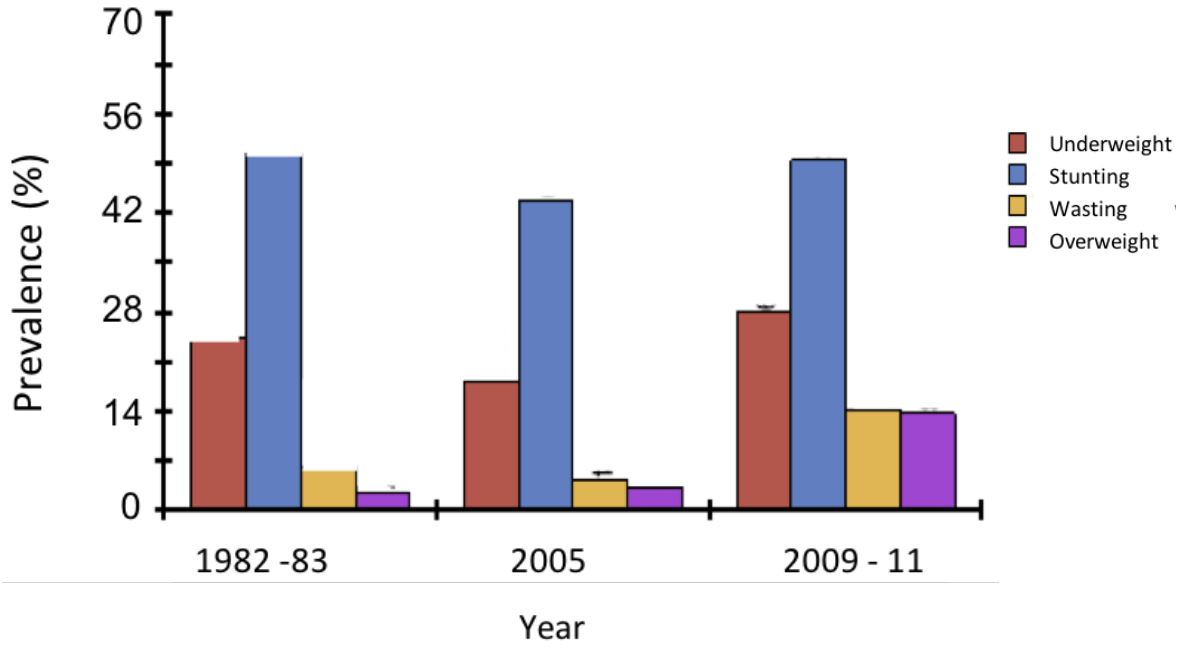

Figure 1-2: Burden and trends in childhood undernutrition in PNG from 1982 to 2011.

Source: (WHO).

1.2 Pneumonia and Diarrhoea

1.2.1 Pneumonia

Pneumonia is an acute respiratory infection of the lungs and is caused by viruses, bacteria and fungi. The most common causative agents however are *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, both of which are already targeted by licensed vaccines currently in use and the respiratory syncytial virus. For infants who are immune compromised as in those infected with human immunodeficiency virus (HIV), the most common causative agent is *Pneumocystis jiroveci* (Rudan, O'Brien et al. 2013, G. B. D. Mortality and Causes of Death Collaborators 2016).

As it is an infection of the lungs, pneumonia is characterised by cough, difficulty breathing (shortness of breath) and fever. In resource poor settings, confirmation of pneumonia by chest x-ray is of often not possible and relies on clinical presentation. Diagnosis is therefore based on the presence of fast breathing with respiratory rate higher than the expected rate for the child's age and chest indrawing, in addition to cough, difficulty breathing and fever. If correctly diagnosed, children suffering from pneumonia can be easily managed with low cost antibiotics and oxygen treatment among other care that can be provided. Vaccination against the common causative agents of pneumonia (e.g. 13-valent Pneumococcal conjugate for *Streptococcus pneumoniae*) have been shown to be an effective strategy to prevent high rates of pneumonia (Bhutta, Das et al. 2013, Berical, Harris et al. 2016). Yet, pneumonia continues to be the biggest infectious killer of young children claiming one child every 39 seconds worldwide (UNICEF , United Nations Inter-Agency Group for Child Mortality Estimation (UN IGME) 2019). This is largely because pneumonia is considered a disease of poverty as it is mostly concentrated in the poorest populations worldwide (UNICEF). Children living in these settings often have limited or no access to vaccines that are currently in use, antibiotics and oxygen treatments, reflecting the inequalities in access to available interventions. Efforts to increase access and availability of these interventions to children in the disadvantaged populations is critical to significantly reducing the global burden of pneumonia-related deaths in young children.

While it is important to continue focussing efforts on ending child deaths due to pneumonia, it is also important to be reminded that far more children will survive pneumonia but will fail to thrive (Grantham-McGregor, Cheung et al. 2007). Pneumonia not only causes death and acute illness in the short-term but children suffering from pneumonia in these early years have also

been associated with risk of respiratory sequelae such as restrictive lung disease, obstructive lung disease and bronchiectasis later in life (Edmond, Scott et al. 2012, Grimwood and Chang 2015). Additionally, pneumonia during early childhood has been associated with growth faltering during childhood (Rowland, Rowland et al. 1988, Black 1991). Suboptimal growth during early childhood have been linked to poor outcomes during later childhood (e.g. poor academic performance) and later in adulthood (e.g. low economic productivity) (Grantham-McGregor, Cheung et al. 2007, Dewey and Begum 2011, Hoddinott, Behrman et al. 2013). The reports of the association between pneumonia and growth faltering however are scanty and inconsistent (Black 1991). In a small study of 126 infants in an urban community in Gambia, Rowland and colleagues showed lower respiratory tract infections to be associated with decreased weight gain (Rowland, Rowland et al. 1988). Similar observations were made in a much larger study of 7131 under 5 year old children in Bangladesh, where higher rates of moderate and severe wasting were observed among children who suffered a recent respiratory illness (Rahman, Rahman et al. 2019). Although not specific for pneumonia, a small study conducted in the highlands of PNG showed a reduced weight gain during episodes of lower respiratory infections (Smith 1991). In contrast to these studies, a case-control study of 100 cases and 200 controls conducted in Ibadan in Nigeria revealed that a recent respiratory illness was not associated with undernutrition (Owoaje, Onifade et al. 2014). Lack of any association was also observed among another study in Bangladeshi children (Torres, Peterson et al. 2000). Further research is therefore needed to clarify this association.

1.2.2 Diarrhoea

Diarrhoea, defined as the passage of three or more loose or liquid stools per day is usually an indication of intestinal infections caused by bacteria, viruses and parasites. The two most common infecting agents, particularly in the resource-constraint settings are rotavirus and *Escherichia coli* (Kotloff, Nataro et al. 2013, Walker, Rudan et al. 2013, G. B. D. Mortality and Causes of Death Collaborators 2016). Diarrhoea is often a result of contaminated food or water sources or poor hygiene practices. Access to safe drinking water and adequate sanitation and hygiene facilities are globally recognised to be important to prevent diarrhoea which in many poor and disadvantaged settings are still lacking. In the 2017 report by WHO and United Nations International Children's Emergency Fund (UNICEF) on the progress on drinking water, sanitation and hygiene, 2.3 billion people worldwide still lack basic sanitation services and about 1 billion still did not have access to safe drinking water (WHO and UNICEF). Lack

of access to these basic essentials allows diarrhoea-causing pathogens to spread more easily and continue to put the majority of young children at risk of diarrhoea.

Diarrhoeal diseases have consistently been among the leading causes of child deaths and morbidities and been the common reasons for hospital visits and admissions. The children that bear the highest burden are those aged under 2 years and those from the resource poor countries (Walker, Rudan et al. 2013, United Nations Inter-Agency Group for Child Mortality Estimation (UN IGME) 2019). During the past decades, there have been substantial reductions in diarrhoea-related under-5 mortality, in part owing to the increasing availability of simple and cost-effective interventions such as oral rehydration salts solution (ORS), exclusive breastfeeding, zinc treatment and rotavirus vaccine among other treatment and/or preventive measures (Bhutta, Das et al. 2013, UNICEF 2015). Yet, in 2018, a period when simple yet effective and low-cost interventions are available, diarrhoea remains a major infectious cause of under-5 deaths accounting for 8% of global under-5 deaths (United Nations Inter-Agency Group for Child Mortality Estimation (UN IGME) 2019). This may largely be due to the persisting inequalities in the access to the interventions; only a minority of the children living in resource poor settings have access to these interventions (Gill, Young et al. 2013, Wilson, Morris et al. 2013). Ensuring universal access to these already existing effective interventions in resource poor settings is an important step forward in reducing the burden of diarrhoea-related deaths.

As equally important as preventing deaths from diarrhoea is preventing the long-term consequences of diarrhoea on child health, growth and development. Besides causing death, there have also been growing evidence indicating an association between diarrhoea and early child growth faltering (Checkley, Epstein et al. 2003, Checkley, Buckley et al. 2008, Richard, Black et al. 2013, Khalil, Troeger et al. 2018, Troeger, Colombara et al. 2018). In a study conducted among young Peruvian children, diarrhoea during the first 6 months of life was associated with permanent height deficits (Checkley, Epstein et al. 2003). Similar diarrhoea associated growth deficits were also observed in studies conducted in Brazil, Bangladesh and Guinea-Bissau (Torres, Peterson et al. 2000, Moore, Lima et al. 2001, Richard, Black et al. 2013). Early child growth faltering as it is well known is an important risk factor for poor health, social and economic outcomes among others later in life (Dewey and Begum 2011, Hoddinott, Behrman et al. 2013, Adair 2014). While these associations are established in some settings, such data is lacking in a PNG setting.

1.3 Malaria

Malaria is an infectious disease caused by *Plasmodium* spp. parasites. The six species known to infect humans include *P. falciparum, P. vivax, P. malariae, P. ovale curtisi, P. ovale wallikeri* and the zoonotic *P. knowlesi.* The latter is a monkey malaria which is mostly found in parts of Southeast Asia. *P. falciparum* and *P. vivax* are the two main species; the former is the predominant and highly prevalent species in the African continent while *P. vivax* is the most widespread species that is common in Southeast Asia, Western Pacific, South America and few areas in Africa. The most virulent species is *P. falciparum* which accounts for most of the infections and the associated disease and deaths. In 2018, *P. falciparum* accounted for almost all of the estimated cases of malaria in the African region, 50% in the Southeast Asia region, 71% in Eastern Mediterranean and 65% in Western Pacific (of which PNG is a part). *P. vivax* is increasingly the predominant species in settings outside of Africa; 53% of the global *P. vivax* burden in 2018 was observed in Southeast Asia with almost half of it in India. It is the predominant species in the Americas representing 75% of malaria cases in 2018 (WHO 2019). The other species are important but often occur at low prevalence where the two main species are endemic. Given this thesis is focussed mainly on *P. falciparum* and *P. vivax,* this review will only cover these two species.

1.3.1 Parasite life cycle

Infection with malaria in a human host begins with a mosquito bite when an infected female anopheles mosquito injects into the skin of the host (human) sporozoites during its blood meal. Once inside the host, the asexual phase of the mosquito's life cycle occurs (Mueller, Galinski et al. 2009). The injected sporozoites migrate through the circulatory system to the liver where they invade hepatocytes (Figure 1.3). At this liver stage, the growth and development differ for the two species; whereas *P. falciparum* parasites will replicate as schizonts until thousands of merozoites are developed and released into the bloodstream, *P. vivax* can either develop into an actively dividing schizont subsequently releasing merozoites or remain quiescent in the liver (hypnozoite), resuming replication and infection, clinical illness, the potential for transmission weeks to years after the first mosquito bite (Mueller, Galinski et al. 2009). This unique ability of *P. vivax* allows it to evade the commonly used blood-stage antimalarials and the dormant hypnozoites serve as reservoirs of relapsing *P. vivax* infections.

Upon release into the blood stream, the merozoites invade the erythrocytes; *P. falciparum* can invade both mature (normocytes) and young erythrocytes (reticulocytes) (Pasvol, Weatherall et al. 1980, Mitchell, Hadley et al. 1986) while *P. vivax* only invades reticulocytes or even younger immature cells (erythroblasts) (Cromer, Evans et al. 2006). During this blood-stage phase, the parasites asexually replicate forming mature schizonts which then rupture releasing new merozoites that invade new erythrocytes. Some parasites however will develop into male and female gametocytes which if ingested by a mosquito during a blood meal will initiate the sexual cycle (Figure 1.3). Another notable difference between the two species is that while gametocyte development takes longer for *P. falciparum* (10 -12 days) and is largely sequestered in the bone marrow, the gametocyte development for *P. vivax* is very rapid, often appearing in the bloodstream just three days after the first asexual parasites are observed or even before the first symptoms develops, making *P. vivax* very highly transmissible (Mueller, Galinski et al. 2009).

After ingestion by the mosquito during a blood meal, in the mosquito midgut, the gametocytes become activated to form gametes which then fuse to produce a zygote. The zygote develops into a motile ookinete which later transforms into an oocyst where thousands of sporozoites are produced. Upon the rupture of the oocyst, these thousands of sporozoites are released migrating to the mosquito salivary glands from where the parasite can be transmitted to the next host (Figure 1.3).

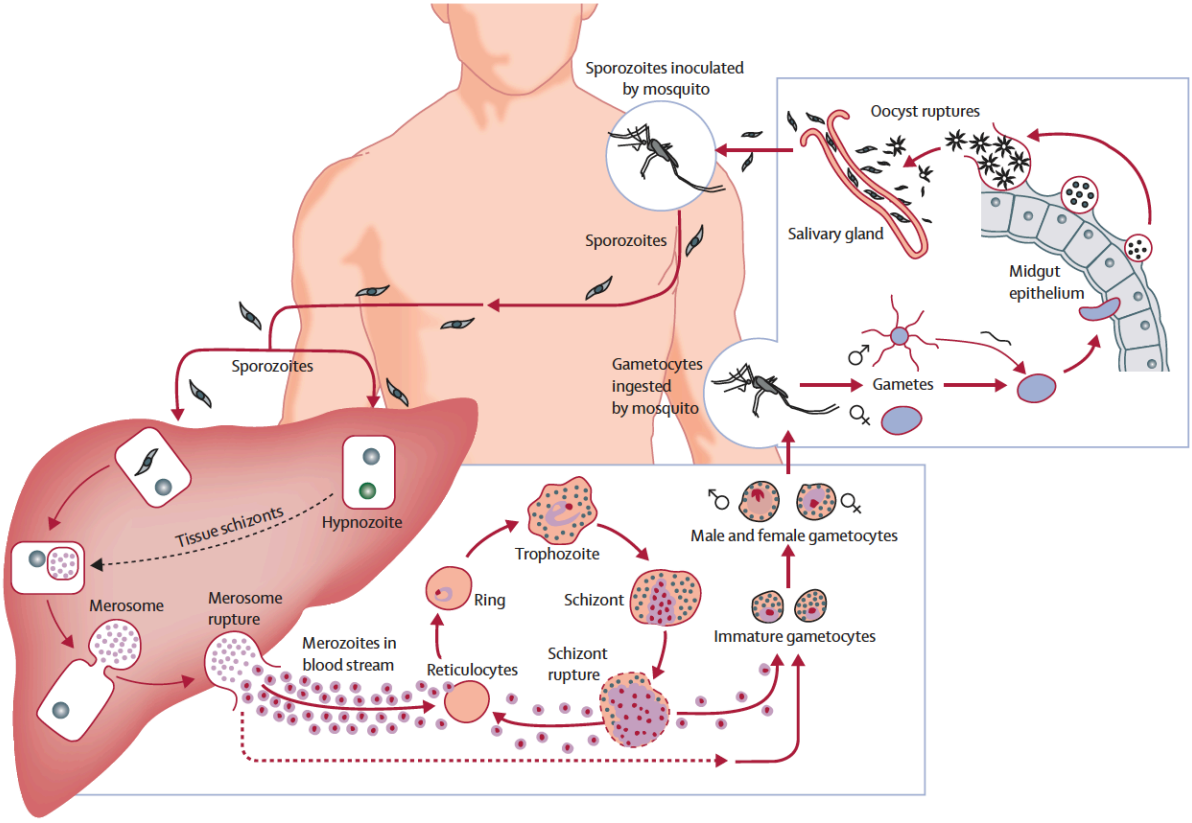

Figure 1-3: Life cycle of human malaria parasite *Plasmodium vivax*.

Once infective sporozoites are inoculated into the skin by female anopheles' mosquitoes, they reach the bloodstream and enter hepatocytes initiating the exoerythrocytic stage. Within the liver, *P vivax* can either differentiate into tissue schizonts, which after thousands of mitotic replications in individual hepatocytes release merozoites into the bloodstream or differentiate into a dormant stage called a hypnozoite that, upon activation after months or years, causes clinical relapse. The merosome featured here has, so far, only been described in malaria in rodents but is predicted to be present in late stage liver infections with *P vivax* and other species. During the erythrocytic stages, *P vivax* merozoites predominantly, if not exclusively, invade reticulocytes, and these cells become enlarged and more deformable. This cyclical developmental process takes about 48 h. In addition, *P vivax* produces specific proteins to create caveola–vesicle complexes that appear as profuse speckling in Giemsa-stained blood smears, known as Schüfnner's dots. Moreover, some *P vivax* parasites can differentiate into mature gametocytes before a clinical infection and illness develops, thus having the advantage of continued transmission to the insect vector before the appearance of clinical symptoms and subsequent treatment. Circulating gametocytes are a rounded shape and on uptake in the blood meal of anopheles mosquitoes begin the sexual cycle, which includes release of the male and female gametes, fertilisation, and formation of a motile ookinete that crosses the midgut epithelium. Differentiation into a new replicative form known as the oocyst, release of sporozoites, migration, and invasion of the salivary glands ends this complex life cycle in which the parasite undergoes more than ten stages of cellular differentiation and invades at least four types of cells within two different hosts.

Source (Mueller, Galinski et al. 2009)

1.3.2 The changing epidemiology

Concerted global efforts through large increases in funding and increased political commitment have supported the scale-up of core malaria interventions such as indoor residual spraying and insecticide treated nets as well as the significant improvements in malaria diagnosis and treatment strategies which have now reached many people living in malaria endemic areas (WHO 2019). As a result, there have been substantial reduction in the overall malaria transmission and the burden of malaria morbidities and mortalities; with 30% (228 million) fewer malaria cases and 60% (405 000) fewer deaths in 2018 as compared to that in 2000 (WHO 2019). Although impressive, there are still 272 000 young children dying from malaria every year, i.e. about 67% of all malaria deaths that could occur in a year.

Of great concern lately is that despite these gains, the rate of progress has been stalling since 2014 (WHO 2019). Globally, almost 85% of all malaria cases are concentrated in just 19 countries: India and 18 African countries; and while reductions were seen in some of these countries (India, Uganda and Zimbabwe), there were notable increases in others like Ghana and Nigeria while no progress was made in other 14 countries (WHO 2019). Additionally, malaria incidence was slightly increased in the Mediterranean and Western Pacific regions while a moderate increase was seen in the Americas. Although a widespread species, 85% of the estimated cases of *P. vivax* were concentrated in only 6 countries: India, Afghanistan, Pakistan, Ethiopia, PNG and Indonesia. Overall, some progress was made in Africa, however, most of the recent successes in the control of malaria was observed mostly in the Southeast Asian region, largely owing to the significant reductions made in India, Indonesia and the countries in the Greater Mekong subregion. Unless efforts are accelerated across all the regions to achieve similar gains, the observed gains may be short lived as has already been seen in some settings such as PNG (Hetzel, Saweri et al. 2018) and in the Americas such as Venezuela and Nicaragua (Espinoza 2019, WHO 2019).

Significant reductions in the overall malaria transmission have been associated with marked shifts to the predominance of *P. vivax* in some co-endemic settings such as Brazil and Thailand (Coura, Suarez-Mutis et al. 2006, Nguitragool, Karl et al. 2019, WHO 2019). Perhaps the major factor contributing to this change is the relatively poor uptake and/or adherence of anti-hypnozoite treatment (like primaquine) (Khantikul, Butraporn et al. 2009, Takeuchi, Lawpoolsri et al. 2010). Although, compared to *P. falciparum*, *P. vivax* possess unique biological abilities and qualities such as: the ability to lie dormant in the hepatocytes as

hypnozoites and serve as reservoir of relapsing infections; its preference for reticulocytes allowing it to avoid hyperparasitaemia hence evade detection and treatment; its ability to form gametocytes rapidly hence early presentation in the bloodstream increasing its transmissibility; faster development in its mosquito host; which taken together gives the ability of *P. vivax* to circumvent standard interventions and sustain transmission (Figure 1.3) (Mueller, Galinski et al. 2009).

Additionally, heterogeneity in transmission is becoming more pronounced (Ernst, Adoka et al. 2006, Bousema, Drakeley et al. 2010, Bousema, Griffin et al. 2012). The heterogenous nature of malaria transmission has long been recognized, although it is often obscured in areas of high transmission, as in these settings the majority of the population are often infected (Strickland, Zafar-Latif et al. 1987, Burkot, Graves et al. 1988, Greenwood 1989). However, as transmission declines to lower levels, there is a tendency for remaining infections to become highly clustered in high-risk populations and high-risk areas, such that a considerable proportion of the population may remain free of malaria while others experience multiple infections and clinical episodes (Woolhouse, Dye et al. 1997, Bejon, Williams et al. 2010, Bousema, Drakeley et al. 2010, Bousema, Griffin et al. 2012). Although not fully understood, an interplay of host (age, level of exposure), environmental (e.g. mosquito breeding sites and presence of water) and vector factors may play a part in this (Bousema, Griffin et al. 2012).

There has also been an increase in the burden of low-density asymptomatic infections following upscale of control interventions (Harris, Sharrock et al. 2010, Waltmann, Darcy et al. 2015, Koepfli, Ome-Kaius et al. 2017, Sattabongkot, Suansomjit et al. 2018). Due to cumulative exposure, natural acquired immunity in individuals, particularly those living in traditionally high transmission areas may remain high for longer period (Galatas, Bassat et al. 2016); parasite densities are thus likely to remain low and very few people may experience a clinical episode. Asymptomatic and submicroscopic infections are more common in adults than children and are largely influenced by transmission intensity in a setting. When transmission is significantly reduced to very low levels, the burden of asymptomatic and submicroscopic infections may be as high as 80% (Waltmann, Darcy et al. 2015, Koepfli, Ome-Kaius et al. 2017).

1.3.3 Malaria in PNG

Burden of malaria in PNG is among the highest in the Asia Pacific region with over 90% of the population considered at risk (Muller, Bockarie et al. 2003, Kazura, Siba et al. 2012). The country is home to five out of the six *Plasmodium* species known to infect humans: *Plasmodium falciparum*, *P. vivax*, *P. malariae, P. ovale curtisi* and *P. ovale wallikeri*; with intensity of transmission geographically variable across the country (Mehlotra, Lorry et al. 2000, Muller, Bockarie et al. 2003, Hetzel, Morris et al. 2015). *P. falciparum* and *P. vivax* are the two main species with *P. falciparum* reaching hyper-endemic levels in most parts of the coastal and lowlands, particularly along the north coast of PNG (Genton, al-Yaman et al. 1995, Muller, Bockarie et al. 2003, Kazura, Siba et al. 2012). The transmission is less intense along the south coast of PNG and on the islands of PNG while unstable transmission occurs in areas at higher altitude (Muller, Bockarie et al. 2003). Furthermore, fine-scale spatial heterogeneity of malaria endemicity occurs at the village or household cluster level (Cattani, Tulloch et al. 1986, Mueller, Widmer et al. 2009).

Malaria in PNG is largely transmitted by mosquitos that belong to the *Anopheles (An.) punctulatus* group (Burkot, Graves et al. 1988). This group comprises at least 13 species (Beebe, Russell et al. 2015) and are predominantly exophilic and exophagic with differing choice of host (Burkot, Graves et al. 1988, Kazura, Siba et al. 2012). Five major vectors widely distributed and highly abundant in PNG include *Anopheles farauti, Anopheles hinesoru, Anopheles farauti 4, Anopheles koliensis and Anopheles punctulatus* (Cooper, Waterson et al. 2009). All species exhibit differing feeding behaviours, spatial distribution and habitat preferences giving rise to marked heterogeneities in transmission patterns across the country.

As in highly endemic areas elsewhere (Marsh 1992, Marsh and Kinyanjui 2006, WHO 2018), children under 5 years of age bear the brunt of malarial morbidity and mortality (Genton, D'Acremont et al. 2008, Lin, Kiniboro et al. 2010). Pregnant women are also at high risk of malaria infection and illness (Allen, Raiko et al. 1998, Mueller, Rogerson et al. 2008). *P. falciparum* is responsible for majority of the infections followed by *P. vivax* while infection with *P. malariae and P. ovale* are rare (Mueller, Widmer et al. 2009, Hetzel, Morris et al. 2015). The burden of infections and clinical episodes however are significantly age-dependent; whereas, infection and clinical episodes due *P. vivax* is most common among the younger age groups, peaking at 2-3 years of age (Genton, D'Acremont et al. 2008, Lin, Kiniboro et al. 2010), *P. falciparum* infections occur frequently among older children, peaking at 5-7 years of age

(Genton, al-Yaman et al. 1995, Michon, Cole-Tobian et al. 2007) (Mueller, Widmer et al. 2009, Hetzel, Morris et al. 2015). About 50-80% of *P. vivax* infections seen in children are likely to be due to relapses (Betuela, Rosanas-Urgell et al. 2012, Robinson, Wampfler et al. 2015). Overall, the bulk of the infections and clinical episodes observed in the country are contributed by children under 9 years of age.

In high malaria transmission settings such as PNG, children are believed to acquire clinical immunity to severe malarial disease relatively quickly after a few episodes of infection (Gupta, Snow et al. 1999). However, the speed of acquisition of clinical immunity varies for the different species with children acquiring clinical immunity to *P. vivax* more rapidly than to *P. falciparum* (Michon, Cole-Tobian et al. 2007, Lin, Kiniboro et al. 2010). This may be explained by the high rates of infection and clinical disease due to *P. vivax* in early childhood (Genton, D'Acremont et al. 2008, Lin, Kiniboro et al. 2010, Manning, Laman et al. 2011). A cohort study conducted in 1 - 3-year-old children in 2006 in a moderate-to-high transmission setting in PNG showed that children were experiencing 1 - 2 *P. falciparum* clinical episodes per child per-year-at-risk (PYAR) and 3 *P. vivax* clinical episodes per child PYAR (Lin, Kiniboro et al. 2010). In these same children, the authors showed that the rate of acquiring new genetically unique blood-stage infections was 6 new *P. falciparum* infections per child PYAR (Mueller, Schoepflin et al. 2012), while a much higher rate was observed for *P. vivax* at 14 new infections per child PYAR (Koepfli, Colborn et al. 2013). The rate of clinical disease due to *P. vivax* diminishes after 5 years of age even though the infections remain common throughout the childhood (Michon, Cole-Tobian et al. 2007, Lin, Kiniboro et al. 2010). On the other hand, risks of clinical disease due to *P. falciparum* continue throughout childhood (Michon, Cole-Tobian et al. 2007) suggesting that clinical immunity to *P. falciparum* is acquired much later on in adolescence to early adulthood. This clinical immunity has been linked to the children's increasing ability to control the parasite density at levels well below the pyrogenic threshold of 2500 and 500 parasites per microliter of blood for *P. falciparum* and non *P. falciparum* respectively (Michon, Cole-Tobian et al. 2007, Lin, Kiniboro et al. 2010).

Beginning in 2004, with the financial support from the Global Fund to Fight AIDS, Tuberculosis and Malaria, PNG scaled up its malaria control program. This included scheduled 3-yearly nationwide distribution of LLINs which commenced in 2004. Later in 2011, the presumptive treatment approach, i.e., administration of antimalarial for all febrile illness episodes based on clinical diagnosis was replaced with the test before treat approach. The latter

included parasitological confirmation of malaria by either malaria RDT or by light microscopy (LM) before antimalarial treatment could be administered. Artemether-lumefantrine (AL) or mala-1 as it is known in PNG was introduced as the first-line treatment for uncomplicated malaria replacing chloroquine (for adults) and amodiaquine (for children). At that time, primaquine was also formally adopted as a treatment for *P. vivax* malaria. Additionally, IPTp with monthly SP was introduced for pregnant women.

In the 10 years that followed (2004 -2014), the country has made significant progress in the control of malaria; an unprecedented decline in the overall prevalence of infection (by LM) from 11% in 2008/09 to < 1% in 2013/14 (Hetzel, Pulford et al. 2017). Both *P. falciparum* and *P. vivax* dropped to < 1% from 6.6% and 3.1%, respectively. The reduction was seen across all age groups (Figure 1.4). Entomological studies also revealed huge declines in human biting rates (Reimer, Thomsen et al. 2016). The significant reduction in the burden of malaria was deemed a historical success by many (Hetzel, Saweri et al. 2018) given the complexity of the malaria epidemiology and operational challenges in the PNG setting (Mueller, Tulloch et al. 2005, Hofmann, Karl et al. 2017). In that period, particularly between 2004 and 2012, distribution of LLINs was the only large-scale malaria intervention implemented in the country (Hetzel, Choudhury et al. 2014, Hetzel, Pulford et al. 2017). More than half (53.9%) of the people were using LLINs by 2013/2014, an increase from 32.5% in 2008/09 (Hetzel, Pulford et al. 2017). It is therefore plausible that the significant drop in the burden of malaria was due largely to the increase in bed net use.

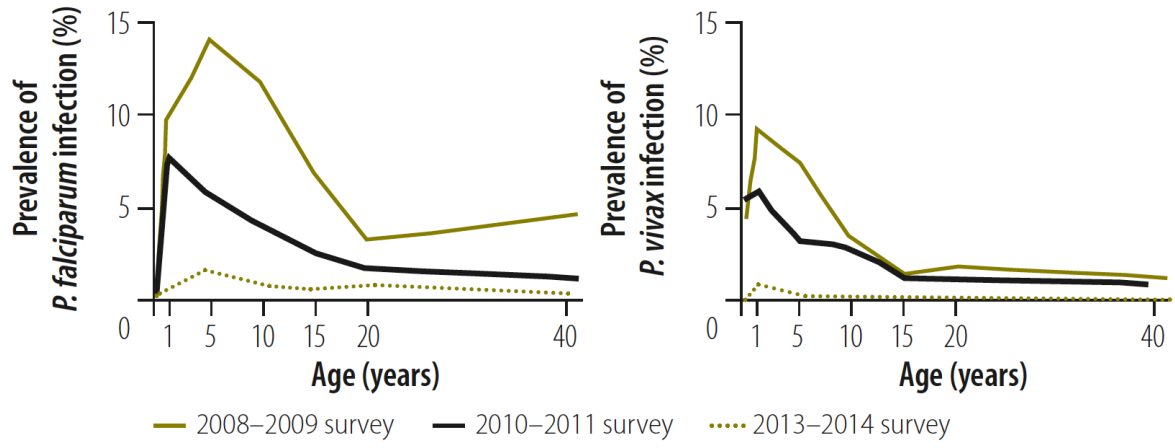

Figure 1-4: Prevalence of *P. falciparum* and *P. vivax* infection below 1, 600m by age and survey date. Source (Hetzel, Pulford et al. 2017)

Unfortunately the significant gains made in the 10 years was short-lived with a dramatic resurgence of malaria observed across the country after 2013/2014 (Figure 1.5) (Hetzel, Saweri et al. 2018). Several underlying factors have been suggested including a reduction in the financial support from the Global Fund, decline in PNG public expenditure in the health sector and prolonged stock-outs of RDTs and drugs among others (Hetzel, Saweri et al. 2018). Of concern is the shift in the peak mosquito biting times to earlier in the evening following the mass distribution of LLINs across the country (Thomsen, Koimbu et al. 2017) and recent evidence of reduced bio-efficacy of the LLINs that were distributed in PNG since 2013 (Vinit, Timinao et al. 2020). However, it is encouraging that insecticide resistance has not yet been observed in local mosquito species (Keven, Henry-Halldin et al. 2010, Hetzel, Saweri et al. 2018, Koimbu, Czeher et al. 2018).

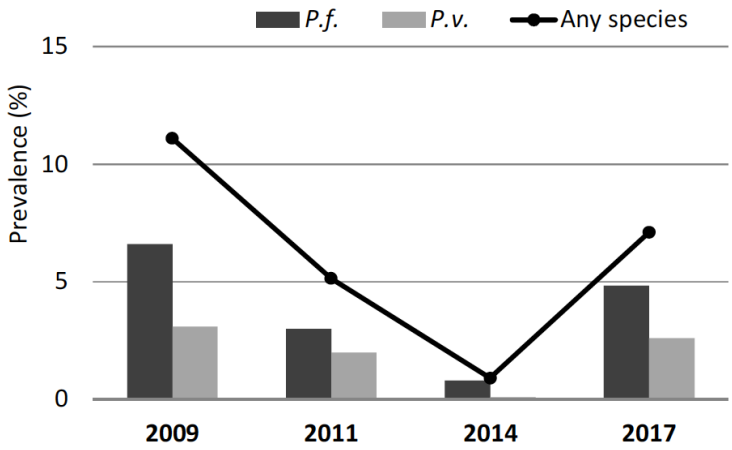

Figure 1-5: Prevalence of *P. falciparum* and *P. vivax* infection in the general population from 2008 to 2017 highlighting the resurgence of malaria observed in the 2016-2017 national survey.

Source (Hetzel, Saweri et al. 2018).

1.3.4 Clinical manifestations

Infection with malaria parasites does not always result in ill-health or lead to symptoms. Age and immune status (generally as well as specific immunity against malaria) are among many other factors that influence this (Gupta, Snow et al. 1999, Michon, Cole-Tobian et al. 2007, Lin, Kiniboro et al. 2010, Griffin, Hollingsworth et al. 2015). In any case, there are a variety of symptoms and signs that have been associated with malaria including nonspecific symptoms such as fever, chills, fatigue, nausea, muscle and joint pain to serious life threatening complications such as convulsions and coma as in cerebral malaria, severe respiratory distress

and severe anaemia among others (Allen, O'Donnell et al. 1996, Mockenhaupt, Ehrhardt et al. 2004, Manning, Laman et al. 2011, Manning, Laman et al. 2012). Most of these symptoms may in part be a result of the body's immune response to the constant haemolysis of the infected red blood cells (iRBC), the release of parasites and host cellular materials into the bloodstream; monocytes and macrophages are activated which in turn induces the release of the proinflammatory cytokines such as tumour necrosis factor (TNF) causing fever, nausea and vomiting among others (Karunaweera, Grau et al. 1992, Clark and Schofield 2000, Clark, Budd et al. 2006).

1.3.5 Maternal and child consequences of malaria

Malaria can affect individuals of all age groups, but in malaria endemic areas where adult populations are relatively immune, the two groups that are most vulnerable are young children under 5 years of age and pregnant women (WHO 2019). For young children, it is simply because they have yet to develop sufficient immunity against infection due to their lack of previous exposure to the parasites. They therefore often bear the brunt of malaria mortalities, morbidities and other malaria-related consequences (e.g. anaemia) in the population (Marsh 1992, Marsh and Kinyanjui 2006, Lin, Kiniboro et al. 2010, Manning, Laman et al. 2011). In 2018, young children accounted for 67% (272, 000) of all the malaria-related deaths that occurred worldwide (WHO 2019). However, out of all infectious diseases that affect young children, malaria is the third leading cause of under-5 mortality and is among the top reasons for health facility visits and hospital admissions in malaria endemic regions (G. B. D. Mortality and Causes of Death Collaborators 2016, Paediatric Society of Papua New Guinea 2017, WHO , United Nations Inter-Agency Group for Child Mortality Estimation (UN IGME) 2019).

One of the two common mechanisms through which malaria may be causing severe morbidity and mortality is severe anaemia (defined as haemoglobin <50 g/L in children and < 70 g/L in adults (WHO Vitamin and Mineral Nutrition Information System (VMNIS))) (Phillips and Pasvol 1992, Menendez, Fleming et al. 2000, Manning, Laman et al. 2011). Mild (haemoglobin 100 - 109 g/L) to moderate (70 - 99 g/L) anaemia however is the common consequences of malaria infection in young children; in 2018 25% and 33% of people living in the moderate and high transmission sub-Saharan African region in 2018 had mild and moderate anaemia, respectively (WHO 2019). Malaria causes anaemia primarily through the increased and often premature destruction of red blood cells (RBC) and the decreased erythrocyte production

(Menendez, Fleming et al. 2000). Destruction of erythrocytes occurs through constant rupture of iRBC and the clearance of the infected as well as the uninfected RBC by phagocytosis and hypersplenism, the latter occurs by filtering iRBC from the circulation or ingesting RBC without lysis. Decreased RBC production may occur through inflammation-induced erythrocyte hypoplasia, dyerythopoiesis and the imbalance in the level of cytokines. Consequently, the oxygen carrying capacity of the blood is significantly reduced and therefore unable to meet the body's physiological demands. Hypoxia and/or congestive heart failure may ensue with eventual death (Menendez, Fleming et al. 2000). Another equally important mechanism for development of severe malaria is cerebral malaria; a multifactorial process involving parasite sequestration, inflammation, endothelial dysfunction in the brain microvasculature leading to vascular leakage with eventual coma and death (Gillrie, Lee et al. 2012, Storm and Craig 2014, Luzolo and Ngoyi 2019).

Malaria has also been associated with childhood undernutrition (Sharp and Harvey 1980, Nyakeriga, Troye-Blomberg et al. 2004, Gari, Loha et al. 2018) although, the reports have been inconsistent and less frequent (Black 1991). In PNG, a setting this thesis is focussed on, there is limited data on this association, particularly in the lowlands of PNG where malaria is highly endemic. A study by Sharp *et al* in early 1980s showed an association between malaria and stunting (Sharp and Harvey 1980), although the study was conducted in children living in the highlands of PNG where malaria transmission is usually low and unstable and prone to epidemics (Muller, Bockarie et al. 2003, Betuela, Maraga et al. 2012). Moreover, the children in the highlands as compared to the coastal and lowlands of PNG are generally of short stature (Mueller and Smith 1999, Mueller, Vounatsou et al. 2001) which, altogether may bias this observation. There have also been other reports (Hou 2016). A major limitation of these studies in PNG and elsewhere (Bradley-Moore, Greenwood et al. 1985, Deen, Walraven et al. 2002, ter Kuile, Terlouw et al. 2003) is the inability to fully control for confounding by factors that may affect both malaria and stunting such as nutritional deficiencies, socioeconomic status and other infections. However, a study by Kang and colleagues using Mendelian randomization and matching to control for potential confounding showed that the risk of stunting was significantly increased by 0.32 for every malaria episode (Kang, Kreuels et al. 2013). There is some evidence of a pathophysiological link between malaria and stunting. Malaria may cause stunting through its effect on bone integrity and growth. Murine malaria models investigating the effect of infection on bone tissue demonstrate chronic bone inflammation leading to bone loss post-infection (Lee, Maruyama et al. 2017). While associations are mostly reported for

stunting, other forms of childhood undernutrition including wasting which is the most dangerous form of undernutrition and underweight have not been thoroughly investigated in PNG setting.

The relationship between malaria and child growth as well as the child's vulnerability to malaria infection during infancy however begins long before the child is born, i.e. during peri-conception and pregnancy. Compared to their non-pregnant counterparts, pregnant women are more susceptible to malaria and this susceptibility is greatest for the first-time mothers (primigravidae). Perhaps the most documented explanation for this is the immunological change that occurs to prevent the mother's body from rejecting the foetus. This involves a transient suppression of the cell-mediated immunity during the pregnancy. The cell-mediated immunity however is important in the development and maintenance of immunity against malaria (and other intracellular parasites) and as such, suppression of it during pregnancy increases the mother's vulnerability to malaria (Raghupathy 1997, Rogerson, Hviid et al. 2007, Abrams and Meshnick 2009). Also of importance is the pregnancy-specific immunity against malaria; the parasite antigens expressed on placental iRBC differ to those normally expressed on iRBC of non-pregnant individuals nor do they bind to receptors commonly used for sequestration (Fried and Duffy 1996, Beeson, Brown et al. 1999). Chondroitin sulfate A, an epithelial receptor that becomes available only during pregnancy is used predominantly for parasite binding and sequestration in the placenta. Pregnancy-specific immunity against placental-binding iRBC is generally absent in first-time mothers and will develop with exposure to these parasites over the successive pregnancies. The first-time mothers are therefore often the ones suffering the severe effects of malaria, however, this decreases with subsequent pregnancies (Rogerson, Hviid et al. 2007, Abrams and Meshnick 2009).

Other possible risk factors for the increased risk of malaria in pregnant women include behavioural changes such as the pregnancy-related frequent urination, which at night may result in women going outside of their houses where they have increased exposure to night-biting mosquitoes; hence more likely to be bitten with higher likelihood of being infected (Lindsay, Ansell et al. 2000). Hormonal changes may include increased body warmth and chemoattractant release, signatures that mosquitoes use to locate pregnant women more quickly and easily (if in close range) (Ansell, Hamilton et al. 2002).

Malaria in pregnancy can have devastating effects on the mother, her foetus and the newborn child. There is now an accumulation of evidence that all malaria infections are harmful but infection with *P. falciparum* leads to more severe deleterious outcomes, being associated with increased risk of miscarriage, stillbirth, low birthweight and maternal mortality in malaria endemic countries (McGready, Lee et al. 2012, Moore, Simpson et al. 2017, WHO 2019, Saito, Briand et al. 2020). Adverse effects caused by *P. vivax*, the second most common species are similar to that of *P. falciparum* but are less severe (Nosten, McGready et al. 1999, Singh, Shukla et al. 1999, McGready, Lee et al. 2012). This may be explained by key biological differences between the two species. *P. vivax* preferentially invades reticulocytes, which may limit hyper-parasitaemia reducing negative impact on birth outcomes. Additionally, *P.vivax*-infected erythrocytes do not express erythrocyte proteins that allow them to sequester in blood vessels including the placenta. In *P. falciparum* infections, this sequestration is observed to lead to high parasite load and associated increased inflammatory response, an implication in the pathogenesis of LBW (McGready, Davison et al. 2004, Rogerson, Hviid et al. 2007, ter Kuile and Rogerson 2008, Mueller, Galinski et al. 2009). There is growing evidence that *P. vivax* parasites sequester in other organs and tissues such as the spleen and the bone marrow. The implication of this for *P. vivax* in pregnancy remains an area of interest. Adequate treatment and control of *P. vivax* malaria during pregnancy is a critical gap due to high likelihood of asymptomatic relapsing infections, concerns of antimalarial treatment during first trimester of pregnancy and inability for pregnant women to receive primaquine or tafenoquine due to the unknown G6PD status of the foetus (Poespoprodjo, Fobia et al. 2008, ter Kuile and Rogerson 2008, Saito, Briand et al. 2020).

Effects may also vary by malaria endemicity, including by the mother's level of acquired immunity pre-pregnancy. In moderate and high transmission settings, where the women are likely to have acquired sufficient immunity before becoming pregnant, the impact of malaria can be minimal, often limited to anaemia (defined as haemoglobin < 110 g/L (WHO Vitamin and Mineral Nutrition Information System (VMNIS))), although, parasites may be present in the placenta hence the foetus often suffers the consequences (Desai, ter Kuile et al. 2007, WHO 2017, WHO 2019). Conversely, in areas of low and unstable transmissions, where level of acquired immunity tend to be low to none, impact of malaria on pregnancy is likely to result in severe symptoms and consequences including maternal and foetal death (Nosten, ter Kuile et al. 1991, Desai, ter Kuile et al. 2007).

The main outcome of malaria in pregnancy that has been consistent is LBW, which is a result of preterm delivery (PTD, birth < 37 weeks' gestation) and/or IUGR (Brabin and Piper 1997, Kalanda, Verhoeff et al. 2006, Desai, ter Kuile et al. 2007, Briand, Saal et al. 2016, Kapisi, Kakuru et al. 2017), with IUGR considered perhaps the major contributor to early child deaths, morbidities, delayed childhood growth and development with lifelong consequences (Christian, Lee et al. 2013, Katz, Lee et al. 2013, Berglund, Kristrom et al. 2016, Watkins, Kotecha et al. 2016). However, it was also shown that malaria in pregnancy may adversely influence childhood growth independent of LBW (Kalanda, van Buuren et al. 2005, Walther, Miles et al. 2010). Moreover, children born to women exposed to malaria infection during pregnancy have been shown to have an increased risk of malaria-related morbidities (Schwarz, Adegnika et al. 2008, Malhotra, Dent et al. 2009, Bardaji, Sigauque et al. 2011, Asante, Owusu-Agyei et al. 2013, Borgella, Fievet et al. 2013, Sylvester, Gasarasi et al. 2016) and anaemia (Desai, ter Kuile et al. 2007, Boel, Rijken et al. 2012) during infancy. The other major consequences of malaria in pregnancy include miscarriage, stillbirth and maternal anaemia (Desai, ter Kuile et al. 2007, Brabin, Tinto et al. 2019), the latter being an important risk factor for maternal mortality, PTD, IUGR, LBW (Malhotra, Sharma et al. 2002, Levy, Fraser et al. 2005, Figueiredo, Gomes-Filho et al. 2018).

The causes of LBW are however multifactorial (Kramer 1987). There are two main mechanisms through which malaria is thought to cause LBW: maternal anaemia and placental malaria. Maternal anaemia is a common consequence of malaria during pregnancy (Allen, Raiko et al. 1998, Desai, ter Kuile et al. 2007, Huynh, Fievet et al. 2011, WHO 2017, WHO 2019) and has been associated with LBW and preterm delivery and has been linked to maternal mortality (Allen, Raiko et al. 1998, Levy, Fraser et al. 2005, Rahman, Abe et al. 2016, Figueiredo, Gomes-Filho et al. 2018, WHO 2019). Suboptimal supply of oxygen and nutrients to the placenta and the foetus may adversely affect the growth rate of the foetus resulting in IUGR with subsequent LBW.

While the mechanism through which anaemia may cause LBW is clear from this perspective, it is noteworthy that the reports of the association of maternal anaemia (or haemoglobin in general) with the risk of LBW as well as other adverse birth outcomes have been mixed; it is thus a controversial subject in literature. There have been studies that did not show any association between maternal anaemia and adverse birth outcomes (Lao and Pun 1996, Xiong, Buekens et al. 2000, Abeysena, Jayawardana et al. 2010) while in some, the authors indicated

that anaemia may have a positive impact on birth outcomes (Steer, Alam et al. 1995, Steer 2000, Xiong, Buekens et al. 2003, Dewey and Oaks 2017). Moreover, some studies demonstrated an association between higher haemoglobin levels, particularly during the end of pregnancy and unfavourable birth outcomes (Garn, Ridella et al. 1981, Murphy, O'Riordan et al. 1986, Abeysena, Jayawardana et al. 2010, Dewey and Oaks 2017). Like anaemia, the latter association seems biologically plausible; higher haemoglobin levels may increase the viscosity of the blood which may result in increased resistance in uteroplacental blood flow causing poor placental perfusion and consequently, the adverse birth outcomes (Klebanoff, Shiono et al. 1989).

In light of the mechanisms by which LBW may occur, it is clear that both low and high haemoglobin levels may result in adverse birth outcomes. This is clearly demonstrated in several studies showing a U-shaped relationship between haemoglobin levels and the risks of adverse birth outcomes, with risks highest at both ends of the haemoglobin range (Garn, Ridella et al. 1981, Steer, Alam et al. 1995, Dewey and Oaks 2017). However, the relationship is said to vary by gestational age (or trimesters), some of which is owing to the pregnancy-induced haemodilution (due to plasma volume expansion). Plasma volume expansion and the increase in total red cell mass are physiologically normal haematological changes that occur to accommodate for the additional demands of pregnancy. The plasma volume increases progressively throughout pregnancy, mostly during the second and third trimesters and is believed to plateau at about 32 weeks of gestation. Red cell mass also increases during pregnancy but to a much lesser extent and therefore, haemoglobin levels as well RBC count and haematocrit are often low (haemodilution) (Hytten 1985, Carlin and Alfirevic 2008, Soma-Pillay, Nelson-Piercy et al. 2016). The timing or the gestational age at the moment of haemoglobin measurement is therefore very important when determining associations between haemoglobin levels and risks of adverse birth outcomes. For the studies reporting adverse associations between anaemia and birth outcomes, effects of anaemia on LBW appeared to be restricted to first trimester of pregnancy while there were mixed reports of such associations in the second and third trimesters of pregnancy (Rasmussen and Oian 1993, Hamalainen, Hakkarainen et al. 2003, Dewey and Oaks 2017, Rahmati, Delpishe et al. 2017, Badfar, Shohani et al. 2019). There was mixed evidence of associations between high haemoglobin levels and adverse birth outcomes by trimesters {Dewey & Oaks, 2017}.

What remains to be made clear from among these controversies is at what level, i.e. what cut-off is appropriate to define low and high haemoglobin levels. Moreover, what range of haemoglobin levels can one consider appropriate for ensuring optimal birth outcomes (Dewey and Oaks 2017). These are needed to clearly identify women that may be at risk of anaemia-related consequences and may therefore require treatment. So far, it is being shown in some studies that only the extreme levels of haemoglobin (e.g. moderate to severe anaemia) were associated with adverse birth outcomes (Steer, Alam et al. 1995, Chang, O'Brien et al. 2003, Geelhoed, Agadzi et al. 2006, Zhang, Ananth et al. 2009, Kozuki, Lee et al. 2012). In a large study conducted in United Kingdom that included women from all ethnicities (Steer, Alam et al. 1995), the authors showed haemoglobin of more than 105 g/L to be associated with increased risk of adverse outcomes while haemoglobin levels of 95 - 105 g/L were indicated to be associated with the lowest incidence of LBW and PTD. As it can be seen, these values fall within the range currently defined by WHO as anaemia (haemoglobin <110 g/L); which may explain the lack of associations seen in some of the studies as well as the mixed reports of associations. It may therefore be useful to re-evaluate the current cut-offs to identify appropriate cut-offs that may be of clinical significance as well to reflect the normal haematological changes (haemodilution) and the differences across geographical areas.

Besides maternal anaemia, malaria may cause LBW through malaria-induced changes that occur at the placental level. Placenta, as perfectly described by Wang and Zhao is a "tree of life" that functions like: (1) a lung in oxygen and carbon dioxide exchange, (2) a digestive system to absorb nutrients for foetal growth and development, (3) a kidney to remove waste, (4) a protective immune barrier to protect the foetus from infections from maternal system and (5) an endocrine organ producing hormones and growth factors pivotal to pregnancy progression and maintenance as well as supporting and promoting the growth and development of the foetus (Wang and Zhao 2010). During normal placental development, extravillous trophoblasts (EVT) invade endometrium and remodel the tightly coiled maternal spiral arteries into low-resistance high-flow blood vessels to increase placental perfusion. This establishes the circulation between the mother and the placenta (uteroplacental circulation) which facilitates nutrient and gas exchanges between the mother and the foetus. The development of placental blood vessels on the foetal side of the placenta (fetoplacental circulation) determines the level of blood flow between the placenta and the foetus. Proper placental vascular development is dependent on normal placental vasculogenesis and angiogenesis (Wang and

Zhao 2010, Ngai, Weckman et al. 2020). Normal uteroplacental and fetoplacental circulations are essential for normal placental function.

Malaria infection in the placenta has been associated with IUGR and LBW (Nosten, McGready et al. 1999, Brabin, Romagosa et al. 2004, Umbers, Aitken et al. 2011). In a malaria-infected pregnancy, the parasite-infected erythrocytes sequester in the placental intervillous space, a key feature of malaria in pregnancy, inducing both localised and systemic immune response (Fried, Muga et al. 1998, Fried, Kurtis et al. 2017, Ngai, Weckman et al. 2020). The infections are often of high density in the placenta as compared to that in peripheral circulation and the trophozoite and schizont stages of parasites which are often absent in the peripheral blood are observed to sequester in the placenta (Beeson, Amin et al. 2002, Rogerson, Hviid et al. 2007). Placental sequestration is common for *P. falciparum* while there is limited data on *P. vivax*, although there have been reports of placental changes in some studies of *P. vivax* (Mayor, Bardaji et al. 2012, Souza, Ataide et al. 2013). The link between these changes to placenta sequestration and adverse birth outcomes remains to be elucidated (Rogerson, Desai et al. 2018).

The two notable immunological changes that occur in response to malaria infection in the placenta that have been associated with poor birth outcomes are the cytokine imbalance due to high production of proinflammatory cytokines including TNF-α, interleukin-(IL)-1β, IL-2, and IL-8 (Fievet, Moussa et al. 2001, Rogerson, Brown et al. 2003, Rogerson, Hviid et al. 2007, Abrams and Meshnick 2009) and the accumulation of monocytes in the maternal placental blood (Ordi, Ismail et al. 1998, Rogerson, Pollina et al. 2003, Rogerson, Hviid et al. 2007, Abrams and Meshnick 2009). High levels of TNF-α levels have been associated with LBW (Fried, Muga et al. 1998, Rogerson, Brown et al. 2003), IUGR (Moormann, Sullivan et al. 1999) and PTD (Suguitan, Cadigan et al. 2003), In one study, increased levels of interferon γ were associated with low birthweight (Fried, Muga et al. 1998) while in one other study, IL-1β was associated with pregnancy loss and PTD (Fried, Kurtis et al. 2017).

The increased inflammatory response at the placental level may lead to placental insufficiency, i.e. when the placenta fails to deliver adequate oxygen and nutrition to the growing foetus, hence resulting in adverse birth outcomes including IUGR (Rogerson and Boeuf 2007), LBW (Stanisic, Moore et al. 2015) as well as disorders of amino acid and glucose transport (Boeuf, Aitken et al. 2013, Chandrasiri, Chua et al. 2014) which may greatly influence the nutrient

supply to the foetus; and the dysregulation of the insulin-like growth hormone axis (Umbers, Boeuf et al. 2011) which may impair the growth of foetus. Poor placental development during early pregnancy has also been postulated to be the mechanism through which malaria may lead to poor adverse birth outcomes, especially IUGR as it is often considered a result of chronic malaria. Malaria is said to impair EVT invasion resulting in abnormal placental function (Brabin and Johnson 2005, Rogerson, Hviid et al. 2007, Umbers, Aitken et al. 2011). A study showed reduced levels of EVT invasion promoting hormones and factors and higher levels of invasion-inhibitory inflammatory factors in women who had malaria infection (Umbers, Stanisic et al. 2013). Impaired placental vascular development, due to dysregulation of inflammatory and angiogenic pathways possibly through activation of the complement cascade, alterations in the L-arginine-nitric oxide biogenesis and heme axis have also been suggested (Conroy, Silver et al. 2013, Rogerson, Desai et al. 2018, Ngai, Weckman et al. 2020). This with impaired trophoblast invasion may therefore result in poor development of the placenta and subsequent placental insufficiency leading to poor birth outcomes. The precise mechanisms however remain less clear and in particular, there is a lot more that remains to know about *P. vivax*, its role in placental sequestration and that link to adverse outcomes in pregnancy.

Effective prevention and treatment (early detection and prompt treatment) of malaria during pregnancy is required to protect women and their children from these deleterious consequences. WHO recommends a combination LLINs for vector control and IPTp with monthly SP for HIV-negative women (WHO , Desai, Hill et al. 2018) or daily co-trimoxazole prophylaxis for HIV-positive women (Gonzalez, Sevene et al. 2016, Desai, Hill et al. 2018). In low transmission settings of Asia and Latin America, the control of malaria involves active screening of infections at the first antenatal visit, passive case detection of symptomatic cases and LLINs given as part of antenatal care (Desai, Hill et al. 2018), In PNG, a combination of LLINs and monthly SP starting in second trimester is recommended as part of antenatal care. Artemisinin combination regimens (e.g. AL) are employed as treatments for clinical episodes of malaria in pregnancy.

The protective effect of these interventions against placental malaria and adverse birth outcomes are well documented (Dolan, ter Kuile et al. 1993, Shulman, Dorman et al. 1998, Browne, Maude et al. 2001, Gamble, Ekwaru et al. 2006, Eisele, Larsen et al. 2010, Radeva-Petrova, Kayentao et al. 2014). In a review by Gamble *et al*, the authors showed that treated

nets were associated with a reduction in placental malaria in all pregnancies and LBW and foetal loss in first to fourth pregnancy (Gamble, Ekwaru et al. 2006). While there is clearly an urgent need for alternative drugs to SP, due to the increasing parasite resistance, it is still an effective preventive treatment against parasitaemia as well as birth outcomes, at least in settings where the severe mutations *Pfdhfr* I164L and *Pfdhps*-K540E and are not prevalent (Desai, Gutman et al. 2016, Desai, Hill et al. 2018). In a review by Radeva-Petrova *et al*, IPTp with SP was associated with a reduction in the risk of moderate-severe anaemia (by 40%), antenatal parasitaemia (by 62%), placental parasitaemia (55%), miscarriage (39%) and LBW (19%) (Radeva-Petrova, Kayentao et al. 2014). In PNG, IPTp with SPAZ combination regimen was associated with increased mean birthweight (by 41.9g), reduction in LBW (26%), preterm delivery and maternal parasitaemia (Unger, Ome-Kaius et al. 2015). However, the impact of the currently implemented IPTp regimen of SP on these pregnancy outcomes in PNG remains to be evaluated in the context of a routine programmatic intervention.

Unfortunately, the recommended interventions are not reaching everyone at risk. In the moderate and high transmission sub-Saharan African region, nearly 40% of pregnant women and young children did not sleep under a treated mosquito net and two thirds of pregnant women did not receive the recommended three or more doses of IPTp in 2018 (WHO 2019). These are reflected in countries like PNG where in 2017, only about 60% young children and also 60% of pregnant women slept under a LLIN (Hetzel, Saweri et al. 2018), whilst data on IPTp coverage (2009-2014) reveal low coverage with less than 2% of pregnant women receiving IPTp (Hetzel, Pulford et al. 2014). There are critical gaps in empowering women, communities, and health facilities to increase antenatal care attendance, promote uptake of IPTp and addressing health system barriers such as quality of care and medical supply chain. Similar lags have been seen for other interventions (van Eijk, Hill et al. 2013, Desai, Hill et al. 2018, WHO 2019). Apart from the bigger issues such as the lack of political financial commitments, the observations that women particularly in the LMICs continue to present late for their first antenatal care visits (Simkhada, Teijlingen et al. 2008, Kisuule, Kaye et al. 2013) and the issues associated of the supply and uptake of these interventions (van Eijk, Hill et al. 2013, Hill, Hoyt et al. 2016) may be contributing factors. Unless such factors are addressed to achieve a universal coverage of these interventions, women and their children will continue to suffer the consequences of malaria.

1.4 Low birthweight

1.4.1 Overview

The size of a neonate at birth is a reflection of growth in utero and is determined by the duration of gestation and the intrauterine growth rate. LBW is therefore a result of either a short gestational duration, reduced foetal growth rate hence IUGR or from a combination of the two (WHO and UNICEF 2004). In 2015, approximately 15% of all births worldwide (> 20 million) were born LBW, that is, one in seven live births (Blencowe, Krasevec et al. 2019, UNICEF and WHO 2019). A great majority of these births occur in LMICs but LBW is also fairly common among the most disadvantaged populations in the high-income countries (WHO and UNICEF 2004, Kim and Saada 2013, Blencowe, Krasevec et al. 2019, UNICEF and WHO 2019). Considering PTD alone, an estimated 15 million babies are born preterm every year (Blencowe, Cousens et al. 2012, March of Dimes 2012, Blencowe, Cousens et al. 2013).

LBW can be measured with validity and precision due to the standard cut-off as defined by WHO, i.e., birthweight <2, 500 g, while measuring PTD and IUGR requires an accurate estimation of gestational age (Kramer 2003). Measuring PTD and IUGR is often difficult in resource poor countries like PNG because of the late and infrequent access to prenatal care (Simkhada, Teijlingen et al. 2008, Kisuule, Kaye et al. 2013), poor recollection of last menstruation dates and unavailability of ultrasound for early and routine ultrasound examinations (Amos, Wapi et al. 1993, Kramer 2003, Jehan, Zaidi et al. 2010). Accurate estimation of gestational age is important to health workers in knowing the approximate gestational age of a pregnancy which helps health workers to correctly diagnose preterm labour and post-dates pregnancies and subsequently manage these conditions appropriately.

Because of the ease and the convenience of measuring it, LBW is the indicator most widely used by health workers, particularly in the LMICs to identify newborns at risk. The issue with LBW however is that it does not reveal the underlying mechanisms leading to LBW, i.e., whether LBW is caused by preterm delivery or by IUGR as it does not adjust for gestational age. Moreover, it excludes children who may have been growth restricted or born preterm but not of LBW (Rijken, De Livera et al. 2014) and yet, these children are also at increased risk of adverse health outcomes. Nevertheless, LBW remains a widely used measure of adverse pregnancy outcome in research (Rijken, De Livera et al. 2014) and has been demonstrated repeatedly to be the most important determinant of childhood morbidities and mortality and a

significant contributor to growth faltering in early childhood and adverse health outcomes later in life (Bukenya, Barnes et al. 1991, Barros, Huttly et al. 1992, Arnold, Hoy et al. 2016, Berglund, Kristrom et al. 2016, Watkins, Kotecha et al. 2016). In recognition of these adverse effects, identifying ways to reduce LBW is an urgent global agenda and the global community pledged their commitment to achieve a 30% reduction in LBW by the year 2025 (WHO 2014). Achieving this target will require interventions and strategies that can prevent the causes of LBW.

1.4.2 Causes and mechanisms

1.4.2.1 Intrauterine growth restriction

Causes of LBW are multifactorial and often inter-related (Kramer 1987, Ashorn, Hallamaa et al. 2018). Whereas LBW due to PTD is generally common in high-income countries, majority of the LBW seen in LMICs are due to IUGR (Kramer 1987). Importance of risk factors causing LBW may therefore differ across settings. This can have significant implications for policy making and strategizing interventions; standardised strategies may not have the same impact in all the settings as importance of one risk factor in causing LBW in one setting may not be the same in the other setting (Kramer 1987). To effectively address LBW thus requires identifying causes that are important in that setting. In resource poor settings where almost all of the LBW occurs, LBW can be attributed to a wide range of factors, most of which are preventable including but not limited to: infections such as malaria, reproductive tract infections, HIV, inflammation and nutritional conditions such as maternal undernutrition, micronutrient deficiencies and anaemia (Kramer 1987, Brabin and Piper 1997, Levy, Fraser et al. 2005, Walker, ter Kuile et al. 2014, Zheng, Suzuki et al. 2016, Lufele, Umbers et al. 2017, Shankar, Kumar et al. 2019, Shava, Moyo et al. 2019, Unger, Hansa et al. 2019). Other key risk factors of LBW include maternal smoking, low socioeconomic status, primigravidae and female infant (Kogan 1995, Okah, Cai et al. 2005, Watson-Jones, Weiss et al. 2007, Bharati, Pal et al. 2011).

IUGR occurs when foetal growth rate is reduced resulting in the foetus failing to reach its expected biological growth potential. Normal foetal growth is regulated by 4 main processes. Expected foetal growth potential is genetically predetermined by the parents' characteristics which is further modulated by foetal health (i.e. foetal use of the supply), maternal health (i.e. nutrient availability from the mother) and placental function (i.e. ability of the placenta to

adequately transfer nutrients and oxygen to the foetus) (Lin and Santolaya-Forgas 1998, Baschat 2004, Brodsky and Christou 2004, Burton and Jauniaux 2018). Adequate placental function and healthy mother and foetus are therefore essential for normal healthy foetal growth. Causes of IUGR may therefore be considered under placental, maternal and foetal causes; the latter including familial, genetic and chromosomal abnormalities or congenital infections while the former two relate to insufficient delivery of oxygen and nutrients to the foetus (Lin and Santolaya-Forgas 1998, Baschat 2004, Brodsky and Christou 2004, Sayers, Lancaster et al. 2017, Burton and Jauniaux 2018).

At the foetal level, chromosomal abnormalities (e.g. trisomy 21 - Down syndrome, trisomy 13, 18, Turner's syndrome, uniparental disomy) have been associated with poor foetal growth (Bauld, Sutherland et al. 1974, Moore, Ali et al. 1997, Lin and Santolaya-Forgas 1998, Sayers, Lancaster et al. 2017). Congenital infections including syphilis, HIV, rubella, toxoplasmosis, cytomegalovirus are important foetal causes of IUGR (Stegmann and Carey 2002, Watson-Jones, Changalucha et al. 2002, Sayers, Lancaster et al. 2017). The resultant suboptimal foetal growth is postulated to be due to infections of the vascular endothelium and limitation of cell multiplication (Sayers, Lancaster et al. 2017).

Impaired placental function may be a result of compromised uteroplacental circulation due to abnormal placental development; or abnormal fetoplacental circulation due to impaired placental vasculoangiogenesis. Abnormal placental development and subsequent inadequate perfusion may result from deficiencies in EVT invasion and maternal arterial remodelling. Consequently, there is increased hypoxia, placenta becomes ischaemic and prone to chronic inflammation (Sayers, Lancaster et al. 2017, Burton and Jauniaux 2018) leading to placental insufficiency thus growth retardation. Increased hypoxia and inflammation may also adversely affect angiogenesis further contributing to placental insufficiency (Baschat 2004, Brosens, Pijnenborg et al. 2011, Conroy, Silver et al. 2013, Burton and Jauniaux 2018, Ngai, Weckman et al. 2020). Additionally, the transplacental nutrient transfer process may be impaired independent of placental vascular abnormalities as in cases of inflammation (Boeuf, Aitken et al. 2013). Malaria, preeclampsia and inflammation are among the common reported conditions that interfere with placental vascular development (Brabin and Johnson 2005, Rogerson, Hviid et al. 2007, Umbers, Aitken et al. 2011, Conroy, Silver et al. 2013, Umbers, Stanisic et al. 2013, Rogerson, Desai et al. 2018, Ngai, Weckman et al. 2020).

Maternal health is perhaps the major player in the development of growth restriction. A wide range of factors including infections (e.g. malaria, HIV), maternal undernutrition, smoking and other maternal diseases (e.g. hypertension) have been implicated (Kramer 1987, Brabin and Piper 1997, Brabin and Johnson 2005, Unger, Ashorn et al. 2016). In nutritional deficiencies, mother often lacks essential nutrients such as protein, iron and carbohydrates which can translate into lack of substrates required for foetal growth. Infections may cause IUGR through effects on placental function such as that seen in malaria (chapter 1.2.5). It may also lead to increased metabolism leading to undernutrition, increased inflammation, anaemia (e.g. malarial anaemia; chapter 1.2.5) and disturbances in growth hormone levels (Umbers, Boeuf et al. 2011)(Allen, Raiko et al. 1998, Desai, ter Kuile et al. 2007)(ter Kuile, Parise et al. 2004). Maternal habits such as cigarette smoking which can significantly affect the oxygen-carrying capacity have been associated with increased risks of poor birth outcomes. Maternal conditions that also affect oxygen-carrying capacity include haemoglobinopathies, cyanotic heart disease and severe maternal hypoxic lung diseases (Sayers, Lancaster et al. 2017).

1.4.2.2 Preterm delivery

The mechanisms through which PTD occurs is considered not different to the normal term delivery, it is only different because it occurs earlier (Romero, Dey et al. 2014). Understanding mechanisms of normal term delivery may therefore assist in understanding the pathology of PTD. Three broad processes form the 'common pathway' of labour (Romero, Dey et al. 2014). First, the increased expression of the microRNA-200 results in increased expression of contraction-associated proteins (oxytocin receptor, connexin 43, prostaglandin receptors) alongside pro-inflammatory chemokines (IL-8) and cytokines (IL-1, IL-6) with progesterone catabolism. Progesterone is key in repressing the expression of the mentioned pro-inflammatory and pro-contraction proteins until term. This first process is referred to as myometrial switch (Renthal, Williams et al. 2013, Romero, Dey et al. 2014). Second process involves cervical ripening to allow for dilatation; which involves alterations in the extracellular matrix proteins (loss in collagen cross-linking, an increase in glycosaminoglycans) and the epithelial barrier thus decreasing the tensile strength of the cervix (Mahendroo 2012). Third, the 'decidual-membrane activation' which involves changes occurring in the decidua itself leading to separation of the chorioamniotic membrane from the uterine wall and the eventual rupture of membrane. Inflammation, increased protease activity and breakdown of cellular proteins such as fibronectin as well as apoptosis may have been involved in this process

(Romero, Dey et al. 2014). It is postulated that activation of at least one of these 3 broad mechanisms could be leading to result in PTD.

PTD has been linked to a wide range of factors including infections (e.g. malaria), cervical pathology, uterine over-distension, progesterone deficiency, vascular alterations (e.g. uteroplacental ischaemia), maternal and foetal stress. Of these, intra-amniotic infection (mostly subclinical) is the only risk factor that has been causally linked to PTD (Romero, Gomez et al. 2001). Although it is possible that microorganisms may gain access to the amniotic cavity through other routes such as haematogenous (Madianos, Bobetsis et al. 2013), the most common route of infection is the ascending pathway (Romero and Mazor 1988). The presence of infections in the amniotic fluid are detected by pattern recognition receptors such as toll-like receptors which subsequently induce production of chemokines, cytokines, prostaglandins and proteases leading to activation of one or more of the 3 pathways to labour. The onset of preterm labour in the context of infection may also be thought of as an evolutionary mechanism of survival whereby the mother expels the infected tissue to maintain reproductive fitness (Romero, Dey et al. 2014).

While it is clear that ascending infections are the common cause of amnionitis and subsequent PTD, it is less clear as to why only some women are at risk of ascending infections whilst others are not. The association between bacterial vaginosis and the risk of PTD has demonstrated that the risk may be linked to the complex interactions between the changes in the mucosa of the lower genital tract (vagina and cervix) and the microbial ecosystem. Important questions remain as to whether there are differences in the microbiota and local immune responses of the vagina in women delivering preterm compared to those who deliver at term; furthermore the factors responsible for changes in the microbiota during pregnancy remain to be established (Hyman, Fukushima et al. 2014, Romero, Dey et al. 2014, Romero, Hassan et al. 2014). Although, viral infection has been shown to predispose women to increased risk of ascending infections through alteration of mucosal immunity in the cervix (Racicot, Cardenas et al. 2013). At a mechanistic level, there is evidence for amniotic fluid invasions; Group B Streptococcus, a commensal in genitourinary and gastrointestinal tract that has been associated with PTD, chorioamnionitis and neonatal sepsis was shown to breach the human amniotic epithelial cells and this process was mediated by its haemolytic pigment (Whidbey, Harrell et al. 2013). There are also speculations that variability in maternal immune responses to infections, due to genetic/epigenetic differences may play a role (Romero, Dey et

al. 2014). Malaria has been one infection apart from the intra-amniotic infections that has been strongly associated with risk of PTD (Suguitan, Cadigan et al. 2003, Rijken, De Livera et al. 2014, Fried, Kurtis et al. 2017). Malaria-induced systemic inflammation especially increased proinflammatory response with high levels of TNF-α and IL-10, fever and anaemia have been implicated (Poespoprodjo, Fobia et al. 2008, Fried, Kurtis et al. 2017).

Other possible pathological mechanisms that may lead to PTD include defective decidual haemostasis, where preterm labour occurs with intact membranes and preterm prelabour rupture of membranes; premature decidual senescence which has been implicated implantation failure, foetal death and PTD; a disruption of maternal-foetal tolerance where immune tolerance for successful pregnancy is impaired; and abnormal decline in the levels of progesterone hence its function in maintaining pregnancy until term (Romero, Dey et al. 2014).

1.4.2.3 Multifactorial causality of LBW presents a major challenge

Reduction of LBW has long been a global priority and yet 1 in 7 newborns are born LBW every year (UNICEF and WHO 2019). Additionally, the progress in the reduction of LBW has been stalling for the past 2 decades (WHO 2014). Consequently, there is now an urgent global call for action to reduce LBW by 30% by the year 2025 (WHO 2014). Achieving this target will require interventions and strategies that can prevent the causes of LBW.

Clearly, causes of LBW are multifactorial but these factors are also often inter-related; they can cause LBW independently or may act in combination with other factors (Kramer 1987, Moore, Fowkes et al. 2017, Ashorn, Hallamaa et al. 2018, Fowkes, Moore et al. 2018). The numerous interventions currently implemented are mostly targeted at one specific cause (e.g. iron supplementation for iron deficiency) (Bhutta, Das et al. 2013, Unger, Ome-Kaius et al. 2015, da Silva Lopes, Ota et al. 2017), generally assuming a single dominant cause. This clearly undermines the inter-relatedness of these factors and the possibility of joint effects in causing LBW. A study conducted in the rural Malawi community demonstrated a joint effect of several risk factors (nutrition, infection and other constitutional factors) in causing LBW (Ashorn, Hallamaa et al. 2018), providing evidence that interventions that can concurrently address multiple exposures may be more appropriate than those that target a single cause. Targeting a single dominant cause with an intervention obviously does not address the contribution of other key risk factors. To adequately address LBW, one needs to have a detailed insight into these

inter-relationships among factors in causing LBW whereby the direct independent effect of a factor can be differentiated from its effects that are indirect through one or a combination of several other factors in causing LBW. Distinguishing direct and indirect effects can inform strategies and interventions as to whether they are appropriate for targeting a single dominant cause or if interventions that can concurrently address multiple causes may indeed be more appropriate.

1.5 Childhood undernutrition

1.5.1 Growth faltering

Child growth reflects the health and nutritional status of the child. Weight, length and height in combination with age and sex serve as important anthropometric indices in evaluating and monitoring the growth of children (WHO 1995, WHO Multicentre Growth Reference Study Group 2006). Length-for-age and height-for-age reflect the linear growth in a child and are representative of the long-term nutritional and health status. Weight-for-length (WFL) and weight-for-height (WFH) reflect the proportionality of growth in terms of weight growth relative to the growth in height. Weight-for-age portrays the composition of the body and is influenced by the length-for-age or height-for-age and WFL or WFH (WHO 1995). The pattern of child growth is assessed by examining these relationships and significant deviations from World Health Organisation growth standards is indicative of impaired child growth (WHO 1995, WHO Multicentre Growth Reference Study Group 2006).

For the vast majority of children in LMICs, weight and length at birth are below the recommended growth standards (Victora, de Onis et al. 2010). Moreover, children experience significant growth faltering in the first 2-3 years of their lives (Shrimpton, Victora et al. 2001, Maleta, Virtanen et al. 2003, Victora, de Onis et al. 2010). Linear growth faltering commences immediately after birth and continues through to the second year of life with dramatic decline occurring in the first year of life (WHO multicentre Growth Reference Study Group 2006). Some improvement in height may occur after 2 years of age, however, it is often not sufficient to catch up on lost growth in height. Faltering in weight-for-age and WFL or WFH begins at 3 months of age with WFL or WFH growth faltering lasting until 9 months of age. The growth faltering in WFL or WFH is of much shorter duration and the compensatory growth is substantial with children exhibiting growth that is comparable to that of the reference group at

2 years of age. In contrast, weight-for-age falters until 2 years of age and remains relatively stable thereafter (Shrimpton, Victora et al. 2001, Victora, de Onis et al. 2010).

As in many developing countries elsewhere (Shrimpton, Victora et al. 2001, Victora, de Onis et al. 2010), children in PNG experience significant growth faltering in the first years of their lives (Mueller, Vounatsou et al. 2001). Growth patterns are geographically variable, however on average, compared to the global standard PNG children are significantly smaller and lighter for their age (Heywood 1983, Mueller, Vounatsou et al. 2001, Wand, Lote et al. 2012). Children are mostly stunted while wasting is rare (Gibson, Heywood et al. 1991, Genton, Al-Yaman et al. 1998, Keeble and Keeble 2006, Wand, Lote et al. 2012). Much of this may be explained by the diet, weaning and feeding practices and the high exposure to infections. A typical PNG diet is often made up of staple foods, mostly tuber crops such as sweet potatoes, taros, yams but there are others such as sago and bananas which are mostly low in protein and energy content (Gibson, Heywood et al. 1991, Mueller, Vounatsou et al. 2001). most of the growth faltering in PNG occurs during the first 2 years of life, when children switch from being exclusively breast fed to eat mostly basic staple food. The limited availability of complementary high protein and high energy weaning food is thus thought to be an important contributor to growth faltering in PNG.

1.5.2 Definitions and forms of undernutrition

Failing to meet the WHO growth standard indices is an indication of undernutrition, which is defined as Z-scores below -2 standard deviations irrespective of the indices used (Waterlow 1972) (Waterlow 1973). This is sometimes referred to as malnutrition, but one must note that the latter can also refer to overweight or obesity, which is defined by exceeding the standard indices (WHO 1995). Undernutrition denotes inadequate intake of energy and nutrients to meet an individual's body requirements. Child undernutrition is often presented in three forms: stunting, wasting and underweight (Table 1.1). The severe forms of undernutrition are defined by a deficit of 3 or more standard deviations below the WHO growth standard median (Waterlow 1972) (Waterlow 1973). When the prevalence of stunting, wasting and underweight among young children are higher than 40, 15 and 30% respectively, undernutrition becomes a very critical public health problem (WHO 2010).

Stunting is defined as the faltering or slowing of linear growth. As linear growth is a long-term process, stunting may generally be considered as a long-term process of height or length deficits thus reflecting the past nutritional and health status of the child (Waterlow 1972, Waterlow 1973, WHO 1995). It is therefore often referred to as chronic undernutrition, although, this terminology is discouraged on the basis that stunting could result not only from a long-term continuing process but also from a past event (WHO 1995). In many communities where short stature is common, stunting often goes unrecognised by families as it is often seen as a norm; even among clinicians especially when height is often not routinely measured as part of medical care in many settings (Dewey and Begum 2011). This presents a major challenge for programs designed to target stunting because unless stunting can be recognised as a problem, these interventions will not reach the children suffering from stunting. However, it is not only important that these children be identified, it is of utmost importance that they are identified early; as by age of 2 years, the chances of reversing stunting are very minimal (Martorell, Khan et al. 1994).

Table 1.1: Forms and definitions of child undernutrition

Stunting	- Being too short for one's age - Defined by a deficit of 2 or more standard deviations below WHO child growth standards median in length-for-age or height-for-age
Wasting	- Being too thin for one's height - defined by a deficit of 2 or more standard deviations below the WHO growth standard median in WFL or WFH
Underweight	- Being too small for one's age - defined by a deficit of 2 or more standard deviations below WHO child growth standards median in weight-for-age

Source: ((Waterlow 1972) (Waterlow 1973))

Wasting, on the other hand, is the reduction in weight or failing to gain weight relative to height, thus resulting in children being wasted or too thin for their height or length (Waterlow 1972, Waterlow 1973, WHO Multicentre Growth Reference Study Group). It is often considered an acute process resulting from a recent rapid loss of weight or failure to gain weight; that with appropriate short-term interventions, it is quickly reversible (Isanaka,

Roederer et al. 2010, Richard, Black et al. 2012). As such, it is often referred to as acute undernutrition, although, studies have shown that untreated severe wasting can last up to about 8 months (Garenne, Willie et al. 2009). Additionally, repeated episodes of wasting occurring year after year should probably not be viewed as a short-term issue (Emergency Nutrition Network (ENN) 2014). Hence, again, the terminology of acute undernutrition may be misleading. Clinically, severe acute malnutrition (SAM) is defined as severe wasting and/or mid-upper arm circumference (MUAC) <115 mm and/or bilateral pitting oedema while moderate acute malnutrition is defined as moderate wasting and/or MUAC ≥115 mm and <125 mm (WHO/UNICEF/WFP 2014). MUAC (which measures bone, availability of energy in the form of fat and muscle for protein reserves) serves as an important measure to identify these children to receive treatment for their SAM. It has been closely linked to mortality risks and wasting (Myatt, Khara et al. 2006) and has also been shown as a potentially useful indicator to identify children with stunting, although, the latter requires more investigation (Emergency Nutrition Network (ENN) 2014). Underweight is a composite indicator representing both stunting and wasting, i.e. an underweight child can be short, light or both. As it cannot distinguish between these two, it is a rarely used indicator to target programs at, although given weight is often routinely measured than height, it is still a widely used indicator to monitor growth (Emergency Nutrition Network (ENN) 2014).

1.5.3 Burden and consequences

Of the three forms of undernutrition, stunting is the most prevalent form, and in 2018 stunting affected over 21% (149 million) of children under 5 years of age worldwide (Figure 1.6). This was an improvement from 32.5% in 2000, although, the rate of decline had been slow and is far from reaching the global nutritional target of a 40% reduction set for 2025 (UNICEF, WHO et al. 2019). The vast majority of these children live in LMICs with Asian (81.7 million) and African (58.8 million) countries carrying the largest share worldwide (UNICEF, WHO et al. 2019). While Oceania which PNG is a part of (excluding Australia and New Zealand) as a region may contribute very little (0.5 million) to the global burden of stunting (UNICEF, WHO et al. 2019), individual countries relative to their total under-five populations observe some of the highest levels of stunting. For instance, in PNG, almost half of the children aged under 5 years experienced stunting (WHO) and in some parts of the country, the prevalence of stunting has been reported to be as high as 65% (Keeble and Keeble 2006). In the same year, wasting affected almost 50 million children globally, of which, about 17 million were severely wasted

(UNICEF, WHO et al. 2019). While the highest prevalence of wasting was observed in the Southern Asia (14.6%), countries in Oceania (excluding Australia and New Zealand), although with much smaller populations compared to other WHO regions reported the second highest prevalence (UNICEF, WHO et al. 2019). Underweight, as one would expect is highly prevalent in the LMICs but mostly concentrated in Asia (Stevens, Finucane et al. 2012).

The associated risk of death and disease with childhood undernutrition is widely documented (Black, Allen et al. 2008, Black, Victora et al. 2013, McDonald, Olofin et al. 2013, Olofin, McDonald et al. 2013). In LMICs, child undernutrition including stunting, wasting, IUGR and deficiencies in essential vitamins are collectively responsible for about 3 million child deaths annually (Black, Victora et al. 2013). Risks of death are significantly heightened even with mild deficits and increases progressively with the degree of deficit. Wasting in particular is associated with a much higher risk of mortality than stunting and underweight with a 11.6-fold risk of dying for severe wasting (Olofin, McDonald et al. 2013). However, it is noteworthy that stunting, especially severe stunting which often receives little attention in relation to mortality is associated with a much higher risk than moderate wasting (Olofin, McDonald et al. 2013, Emergency Nutrition Network (ENN) 2014). Risks of mortality is particularly significantly heightened for children with multiple deficits (Olofin, McDonald et al. 2013).

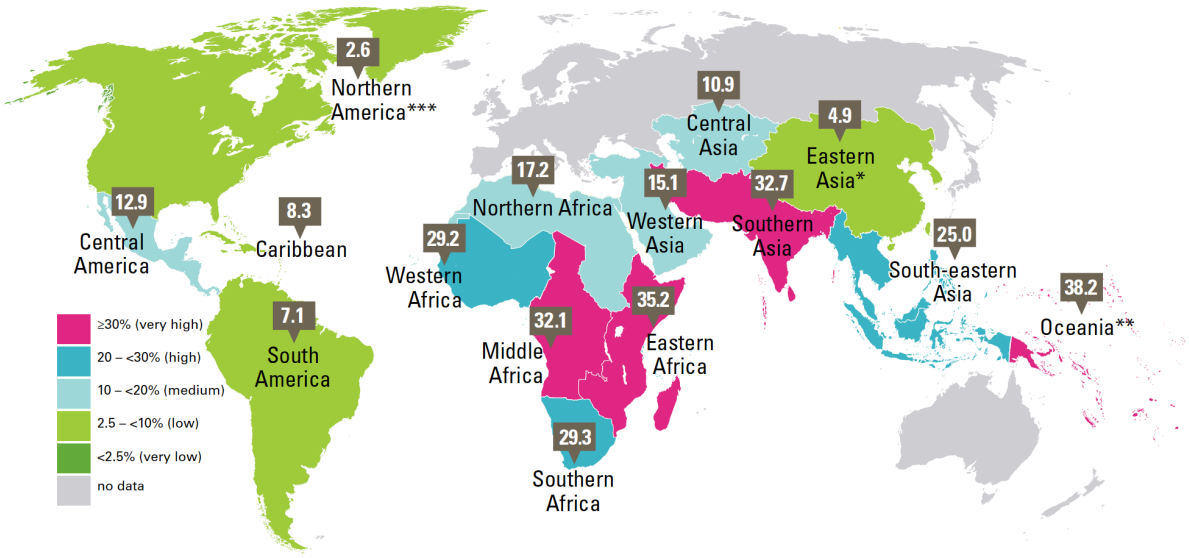

Figure 1-66: Global prevalence of stunting in 2019.

Note: *Eastern Asia excluding Japan; **Oceania excluding Australia and New Zealand; ***Northern America sub-regional average based on United States data. There is no estimate available for the sub-regions of Europe or Australia and New Zealand due to insufficient population coverage. These maps are stylized and not to scale and do not reflect a position by UNICEF, WHO or World Bank Group on the legal status of any country or territory or the delimitation of any frontiers. Source: (UNICEF, WHO et al. 2019).

Early childhood undernutrition has also been implicated in many adverse outcomes during later childhood as well as later in adulthood. It has been associated with poor cognitive function, attainment of low adult height (Emerson, Savage et al. 2020) (Grantham-McGregor, Cheung et al. 2007, Kar, Rao et al. 2008, Victora, Adair et al. 2008) and impaired behavioural development (Lasky, Klein et al. 1981). The World Bank estimates revealed that a loss of 1% in adult height due to childhood stunting can result in 1.4% loss in economic productivity (The World Bank 2006). Similarly, poor cognitive function can result in poor academic performance which can translate into reduced economic productivity later in adult life with lower earnings; unless, prevented by appropriate interventions (Hoddinott, Maluccio et al. 2008, Victora, Adair et al. 2008, Martorell, Melgar et al. 2010). In settings with the highest rates of undernutrition (especially stunting), such as PNG, this could translate into large proportion of unproductive human capacity and adversely affect societal and national development. Undernutrition has also been linked to various non-communicable diseases including hypertension, cardiovascular diseases and diabetes (Barker, Winter et al. 1989, Barker and Martyn 1992, Lucas 1998), conditions that are bound to affect the quality of life. Of critical concern is that there is evidence that these adverse consequences are transmitted through generations (Ramakrishnan, Martorell

et al. 1999, Grantham-McGregor, Cheung et al. 2007, Martorell and Zongrone 2012, Walker, Chang et al. 2015). Women born LBW or who experienced early childhood stunting tend to have children who are also small in size continuing the cycle of growth failure and their associated poor outcomes (Ramakrishnan, Martorell et al. 1999, Martorell and Zongrone 2012). Thus, the consequences of childhood undernutrition are pervasive, societal and potentially intergenerational.

1.5.4 Causes

The causes of childhood undernutrition are multifactorial and multisectoral including immediate (individual level) causes which are determined by the underlying (household or family level) causes which in turn are influenced by basic causes acting at the sociocultural, economic and political level (Figure 1.7) (UNICEF 1991). The immediate causes are inadequate nutrient intake and diseases which are determined by underlying factors such as children's access to food (quantity and quality), the psychosocial care environment, feeding practices (weaning, breastfeeding), the environment children live in (e.g. war, high infection burden) and the quality of health services accessible by the children (UNICEF 1991). Inadequate intake is not merely a result of too little food, but a combination of inconsistent food intake and insufficient intake of the essential nutrients such as proteins, energy and micronutrients (e.g. zinc) that are highly demanded by the body to support the rapid growth and development of the child. Acute deprivation of these nutrients may result in wasting while extended deprivation may lead to over utilisation of the body's nutritional reserves as a way of sparing the vital organs (especially brain) subsequently resulting in deficiencies in the body hence impacting the growth (Emergency Nutrition Network (ENN) 2014, Briend, Khara et al. 2015).

Infectious diseases have also been identified as important immediate causes of undernutrition among young children. The suggested mechanisms through which infections may result in undernutrition include reduced nutrient intake due to anorexia, increase nutrient losses due to intestinal malabsorption, internal diversion nutrients for metabolic and immune responses to infection, and increased basal metabolic rate in the presence of fever (Mata 1990, Bresnahan and Tanumihardjo 2014). Diarrhoeal disease (Moore, Lima et al. 2001, Checkley, Epstein et al. 2003, Checkley, Buckley et al. 2008), lower respiratory tract infections (Black 1991, Moffat 2003), malaria (Sharp and Harvey 1980, Kang, Kreuels et al. 2013) and measles (Black 1991)

are some of the infectious causes that have been implicated, although, the association with diarrhoea is perhaps the most substantiated (Black 1991). The associated risks of undernutrition with malaria (Sharp and Harvey 1980, Carswell, Hughes et al. 1981) and pneumonia (Moffat 2003, Assis, Barreto et al. 2005) are inconsistent (Black, Brown et al. 1982) and more evidence is needed to clarify these associations.

In the lowlands PNG, a high malaria transmission setting (Muller, Bockarie et al. 2003) as previously described, the contribution of malaria together with pneumonia and diarrhoea in these lowland settings have not been properly investigated. A study by Sharp et al (Sharp and Harvey 1980) showed that malaria was a significant contributor to stunting. This, however, was observed in a setting where malaria transmission is unstable. Understanding the relationships between these three diseases and undernutrition will help define the role of disease control in the prevention of childhood undernutrition.

Besides inadequate diet intake and infections, a wide range of other factors have been implicated in the literature as important risk factors of undernutrition. LBW is perhaps the leading risk factor associated with undernutrition, especially stunting (Black, Victora et al. 2013, Christian, Lee et al. 2013, Berglund, Kristrom et al. 2016, Danaei, Andrews et al. 2016). Evidence indicates that linear growth faltering may in fact begin during intrauterine life (Victora, de Onis et al. 2010, Christian, Lee et al. 2013) indicating the importance of intervening during pregnancy . Food insecurity (Ali, Saha et al. 2013, Moradi, Mirzababaei et al. 2019), suboptimal weaning practises (Black, Brown et al. 1982, Motarjemi, Kaferstein et al. 1993) and socioeconomic factors (Mohsena, Mascie-Taylor et al. 2010) have also been shown to be key to undernutrition.

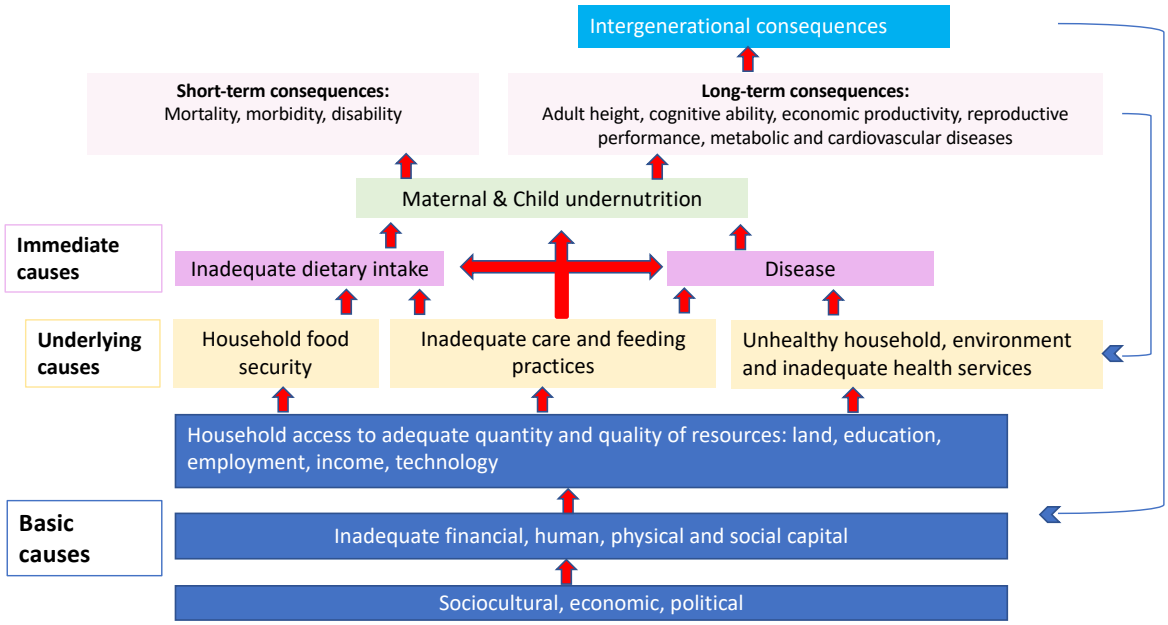

Figure 1-77: Determinants of child and maternal undernutrition by UNICEF framework.

The flowchart of the determinants of child and maternal undernutrition highlights the multitude of factors ranging from basic causes to immediate causes and how each level of causes is influenced by the other Adapted from the UNICEF's framework for determinants of maternal and childhood undernutrition. Source ((UNICEF 1991))

1.6 Aims and objectives of this thesis

Aim 1: Investigate the multivariate inter-relatedness among factors associated with low birthweight in PNG.

Specific Objectives

- Distinguish between the direct and indirect effects of risk factors associated with low birthweight;
- Quantify and test the magnitude of these relationships using structural equation modelling approach;
- Identify key intervenable factors and their pathways in a PNG setting.

Aim 2: Investigate the impact of acute illnesses on child growth in PNG lowlands setting.

Specific Objectives

- Develop longitudinal models of child growth to assess the proportion of stunting, wasting and underweight in a cohort study of 1-3-year-old children;
- Investigate the effect of acute illnesses on risk of wasting, stunting and underweight.

Aim 3: Evaluate epidemiology of malaria in relation to intensified malaria control in children 1-5 years of age.

Specific Objectives

- Determine the impact of increased control interventions on *P. falciparum* relative to *P. vivax;*
- Identify key risk factors of infection and clinical malaria during low transmission.

2 Chapter 2: General Methodology

2.1 Field studies and intervention trials

The analysis undertaken in this thesis included a primary analysis of one longitudinal child cohort that was conducted in 2013 and secondary analyses of several other studies including 3 child cohorts and a pregnancy intervention trial. These studies were conducted by PNG Institute of Medical Research in PNG. The studies were conducted in the villages and health facilities in Madang and East Sepik Provinces, both of which are situated in the northern part of PNG where malaria transmission is considered hyperendemic (Genton, al-Yaman et al. 1995) (Muller, Bockarie et al. 2003, Michon, Cole-Tobian et al. 2007, Lin, Kiniboro et al. 2010).

2.1.1 Pregnancy Intervention Trial

2.1.1.1 Study summary

To investigate the multivariate inter-relatedness among factors associated with low birthweight described in Chapter 3, data from a large pregnancy intervention trial (Unger, Ome-Kaius et al. 2015) was used. This was a single-blinded block-randomised controlled trial of Intermittent Preventive Treatment of malaria in pregnancy conducted in Madang and Sumkar Districts of Madang Province between November 2009 and February 2013 (Clinicaltrials.gov, NCT01136850). The study was designed to evaluate efficacy of IPTp using SPAZ combination regimen in preventing LBW in PNG pregnant women. It involved comparing the intervention group receiving AZ (1 g twice daily for 2 days) plus SP (1, 500/75 mg) monthly (over 3 months) starting from second trimester to the control group receiving a standard dose of SP and chloroquine (CQ, 450-600 mg daily for 3 days) (SPCQ) followed by SPCQ placebo. The study was conducted according to Good Clinical Practice (GCP) guidelines. Ethical approval for human participation in the study was granted by PNG Medical Research Advisory Committee (MRAC number 08.01) and women provided written informed consent to partake in the study.

A total of 2793 pregnant women aged between 16 and 49 years participated in the study. Of this, 18 women had incomplete consent forms and were therefore excluded post hoc leaving 2775 women to be considered for analysis. Almost two-thirds (62.2%) of these women resided in rural areas, 50.2% were primigravida and over 81% (81.4%) were anaemic. Although malaria was known to be hyperendemic in these study areas (Cattani, Tulloch et al. 1986, Brabin and Piper 1997, Allen, Raiko et al. 1998, Muller, Bockarie et al. 2003), the study observed a low prevalence of microscopically detected infections of 7.4% (Pf: n=177; Pv:

n=23) at enrolment, 83% of which were asymptomatic. It must be noted however that the overall burden of malaria across PNG was declining during that period due to the scale-up of malaria control interventions especially with the population-wide distributions of LLINs across PNG (Hetzel, Morris et al. 2015, Hetzel, Pulford et al. 2017). The baseline characteristics of the study participants are published elsewhere (Unger, Ome-Kaius et al. 2015).

Of the 2775 women considered for analysis, 2021 were eligible (i.e., they had birthweight data of congenitally normal live singletons and birthweights were measured within 7 days of delivery) and were therefore included in the analysis to determine the association between IPTp with SPAZ and LBW. Analysis was by intention-to-treat such that all the women randomized to a treatment group regardless of the number of doses received were included. A total of 1, 013 women were in the intervention arm (SPAZ arm) while 1, 008 were in the control arm (SPCQ arm). The overall prevalence of LBW was 15.1% and the mean birthweight was 2, 943 ± 479 g while among the women who had pregnancy dating by ultrasound (n=1, 320), 8.6% delivered preterm.

According to the main results of the study (Unger, Ome-Kaius et al. 2015), SPAZ reduced the risk associated with delivering LBW baby (12.8% vs 17.4%; risk ratio [RR], 0.74, CI_{95}: 0.60 - 0.91, p=0.005) and preterm delivery (6.6% vs 10.6; RR, 0.62, CI_{95}: 0.43 - 0.89, p=0.010). Mean birthweight of babies born to women who were randomised to SPAZ was 41.9 g (CI_{95}: 0.2 - 83.6, p=0.049) higher compared to that of babies born to women in the control group. Additionally, SPAZ reduced maternal parasitaemia (2.3% vs 3.9%; RR, 0.57, CI_{95}: 0.35 - 0.95, p=0.029) and active placental malaria (6.0% vs 8.8%; RR, 0.68, CI_{95}: 0.47 - 0.98, p=0.037) at delivery, and reduced carriage of gonorrhoea (0.66, 95% CI: 0.44– 0.99, P = 0.041) at second visit.

The impact of IPTp with SPAZ on association between plasma biomarkers of inflammation and angiogenesis and adverse birth outcomes was investigated in a subsequent analysis (Unger, Hansa et al. 2019). In this analysis, the stored samples collected from enrolment and delivery visit time points were re-analysed for levels of CRP, AGP, soluble endoglins (sEng), soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF) using commercially available enzyme-linked immunosorbent assays (ELISA) (Human Quantikine ELISA kits; R&D Systems, Minneapolis, MN, USA) according to manufacturer's instructions. As detailed in Unger *et al* (Unger, Hansa et al. 2019) "plasma samples were diluted with BSA-PBS (sEng:

1:25, PIGF and sFlt1: 1:5; CRP: 1:10,000; AGP: 1:1,000,000) and assayed in duplicates. Optical densities were read at 450 nm and 540 nm on a Fluostar Omega microplate reader (BMG Labtech, Ortenberg, Germany). Sample concentrations were interpolated from a standard curve and multiplied by the dilution factor. Samples above or below the standard curve, or with duplicates varying by >20%, were repeated, with dilution adjustments if required". Additionally, the ratio of sFlt-1 and PlGF (sFlt-1: PlGF ratio) was calculated. A raised CRP was defined as CRP ≥ 5mg/L and elevated levels of AGP, sFlt-1 and sEng were defined as readings in the 4th quartile, and a low PlGF as the first quartile. A high sFlt-1:PlGF ratio ≥ 38 was defined as elevated. The main findings of the analysis suggested that IPTp with SPAZ may protect against adverse birth outcomes by reducing inflammation and its associated adverse consequences including dysregulation of placental angiogenesis, in women with and without malarial infection (Unger, Hansa et al. 2019).

IPTp with SPAZ was also shown to be associated with a reduction in the nasopharyngeal carriage at delivery of *Streptococcus pneumoniae* and *Haemophilus influenzae* but not *Staphylococcus aureus* (Unger, Aho et al. 2015). In another (Unger, Wangnapi et al. 2016), the results suggested that IPTp with SPAZ positively impacted gestational weight gain which in turn may have positively influenced birthweight.

2.1.2 Infant Intervention Trial

To investigate the impact of acute malaria, pneumonia and diarrhoea on child growth in lowland settings of PNG described in Chapter 4, data from a large infant intervention trial (Senn, Rarau et al. 2012) was used. This was an individually randomised placebo-controlled trial of Intermittent Preventive Treatment for malaria in infants conducted in villages and health facilities in Madang and East Sepik Provinces of PNG between June 2006 and May 2010 (Clinicaltrials.gov, NCT00285662). The study was designed to evaluate the efficacy of IPTi using two different SP combination regimens, a single dose of SP (25 mg/1.25 mg per kg) combined with either 3-day course of amodiaquine (AQ, 10 mg/kg) or artesunate (AS, 4 mg/kg) with matching placebos in preventing malaria in infants (Senn, Rarau et al. 2012). The interventions were delivered as IPTi at routine vaccination time points at 3, 6, 9, and 12 months of age. The study was performed in accordance with GCP guidelines (ICH GCP E6). Ethical approval for the study was granted by PNG Medical Research Advisory Committee (MRAC number 05.20) and voluntary written informed consent was obtained from the

parents/guardians of the children following community awareness and individual study information sessions to allow participation in the study.

A total of 1, 125 infants from Madang and 480 infants from East Sepik were enrolled into the study at about 3 months of age. The main objectives of the study however were only tested using data from the infants enrolled in Madang. Originally, the trial started as a two-site study but due to finding the incidence of malaria in East Sepik to be significantly low compared to that in Madang in the mid-study interim analysis, enrolments in East Sepik were ceased. Sample size required to test the main study objectives was thus recalculated and enrolments were increased in Madang resulting in a total of 1, 125 enrolments. Of this 1, 125 infants, 4 were excluded retrospectively because they did not receive any assigned study treatment as they were already receiving antimalarial at the time of all the study treatment visits. With the remaining 1, 121 infants, 374 were in the SPAQ group, another 374 in the SPAS group and 373 were in the placebo group. The primary outcome was protective efficacy (PE) of the two regimens against all episodes of malaria from 3 to 15 months of age and the analysis was by intention-to-treat.

According to the main results of the trial, the incidence of all cases of malaria between the first dose at 3 months and 15 months of age was significantly reduced by 29% (CI_{95}: 10 - 43, $p \leq 0.001$) from 1.07 to 0.80 PYAR in children in the SPAQ group when compared to the control group while a 12% reduction from 1.07 to 0.97 PYAR was observed for the SPAS group (compared to control) but this was not statistically significant (CI_{95}: -11 - 30, p=0.12). In terms of the PE against *P. falciparum* and *P. vivax* specifically, the efficacy of SPAQ against *P. falciparum* was higher than *P. vivax* reducing the incidence of *P. falciparum* and *P. vivax* by 35% (CI_{95}: 9 - 54, p=0.012) from 0.28 to 0.18 PYAR and by 23% (CI_{95}: 0 - 41, p=0.048) from 0.82 to 0.66 PYAR respectively when compared to the placebo group while SPAS only protected against *P. falciparum* (PE, 31%, CI_{95}: 4 - 51, p=0.027) episodes and not *P. vivax* (PE, 6%, CI_{95}: -24 - 26, p=0.759) episodes.

2.1.3 Longitudinal Child Cohorts

Data from three consecutive longitudinal child cohorts conducted in 2006 (Lin, Kiniboro et al. 2010, Mueller, Schoepflin et al. 2012, Koepfli, Colborn et al. 2013), 2008 (Betuela, Rosanas-Urgell et al. 2012) and 2013 (unpublished) were used to evaluate the epidemiology of malaria

in relation to scale-up of malaria control interventions in children 1-5 years of age described in Chapter 5. The cohorts were conducted in the villages in the Ilahita area of Maprik District, East Sepik Province.

2.1.3.1 2006 cohort

The 2006 cohort (Lin, Kiniboro et al. 2010) was an observational study and the purpose of the study was to describe epidemiological patterns of infection and disease with *P. falciparum* and *P. vivax* as well as identify factors associated with either risk or protection from infection and disease. The study was conducted between March 2006 and August 2007 during the initial stages of the nationwide roll out of LLINs that commenced in 2004 but prior to the roll out in the study area which began in 2008. Ethical approval for the study was granted by PNG Medical Research Advisory Committee (MRAC number, 05.19). Voluntary written informed consent was obtained from the parents and guardians of the children to allow their participation in the study.

This study involved 264 children aged 1-3 years at enrolment who were followed up every 8 weeks for active detection of infection (ADI) for a total of 16 months. On each visit, clinical data was collected, and children provided blood samples in the form of venous sample at enrolment, a finger prick microtainer for all the other visits and blood slides on all visits for detection of infection. A fortnightly active morbidity surveillance as well as the passive case detection was maintained throughout the study to capture clinical episodes. Clinical data detailed the child's status of health and wellbeing at the time of the visit, their bednet use, their recent antimalarial use, malaria-related signs and symptoms, temperature and other standard clinical data. The collected samples were evaluated for presence of infection by both LM and molecular methods, namely semi-quantitative post-PCR (polymerase chain reaction), ligase detection reaction/fluorescent microsphere assay (LDR-FMA). This assay is described in detail in the publication for this study and others (McNamara, Thomson et al. 2004, McNamara, Kasehagen et al. 2006, Lin, Kiniboro et al. 2010) but in brief according to Lin *et al*, "this assay combines PCR amplification of the small subunit ribosomal RNA gene (491 - 500 bp fragments) using genus specific primers followed by a multiplex species-specific ligation detection reaction (LDR)" (Lin, Kiniboro et al. 2010). Confirmed infections with *P. falciparum* and *P. vivax* were further genotyped for *Pfmsp2*, *Pvmsp1F3* and *PvMS16* to identify individual parasite clones.

Collected blood films were read independently by two expert microscopists. Slides with discrepant results were re-read by a 3rd microscopist. Thick blood films were examined by LM for 100 thick-film fields (under 100X oil immersion lens) before being declared infection-negative. In the positive slides, parasite species were determined. The parasite densities were recorded as the number of parasites per 200 white blood cells (WBC) and converted to the number of parasites per microliter of blood, assuming 8,000 WBC per microliter of blood. Slides were declared LM-positive for an individual *Plasmodium* species, if the species was detected independently by at least 2 microscopists and/or subsequent PCR-based analysis confirmed the presence of the species. Densities were calculated as the geometric mean densities of all positive reads.

The study observed a high burden of infections and clinical episodes among these young children and *P. vivax* was the predominant species. At enrolment, the prevalence of infection (all species) was 63.6% (n=199) by LM and 75.4% (n=168) by PCR. The overall incidence of clinical malaria was comparable for both species at 2.56 episodes/child/year-at-risk for *P. falciparum* and 2.46 episodes/child/year-at-risk for *P. vivax* while the molecularly determined incidence of blood-stage infections ($_{mol}$FOB) was 5.9 clones/child/year-at-risk for *P. falciparum* and 14.0 clones/child/year-at-risk for *P. vivax*. The main finding of this study was that children living in co-endemic settings such as PNG are likely to acquire clinical immunity to *P. vivax* clinical malaria earlier than they do to *P. falciparum* clinical malaria. There was a strong age-dependent increase in the prevalence and incidence of *P. falciparum* infections as compared to *P. vivax* while a strong opposing age trends was observed in the incidence of clinical malaria episodes. *P. falciparum* incidence increased with age while the incidence of clinical *P. vivax* decreased with age. Moreover, the incidence of clinical *P. vivax* rapidly declined with age in children with high exposure to new infections than children with low exposure. Together, these observations indicated that children may not have acquired sufficient clinical immunity against *P. falciparum* and therefore continued to be at risk of clinical episodes while high exposure to new *P. vivax* infections during the early childhood may have contributed to children quickly acquiring sufficient immunity to protect against clinical *P. vivax* episodes.

2.1.3.2 2008 cohort

The 2008 cohort was a randomised placebo-controlled trial that was designed to assess the contribution of hypnozoites to the burden of *P. vivax* reinfection and disease in a cohort of young children by selectively treating pre-existing hypnozoites in a subset of them. It involved 433 children aged 1.1 - 5.6 years who were randomly allocated to receive 1 of the 3 treatment regimens: (1) artesunate (AS, 4mg/kg/day for 7 days) plus primaquine (PQ, 0.5 mg/kg/d for 14 days) (ASPQ), (2) artesunate alone (4mg/kg/day for 7 days) and (3) placebo group (control) (Betuela, Rosanas-Urgell et al. 2012). Following completion of the treatment, children were actively followed up fortnightly for detection of infection and febrile illness for the first 3 months and monthly thereafter for another 7 months. A passive case detection system was maintained throughout the study period to capture febrile illness episodes that occurred outside of scheduled study visit time points. For any febrile illness episode detected, malaria infection was investigated using RDT for malaria (ICT Diagnostics) and a 250 µL finger prick blood sample was collected for confirmation by LM and PCR. Febrile episodes with concurrent *plasmodium* infection confirmed by RDT and/or LM were treated with AL (Coartem®, Novartis) while all other illnesses were referred to local health centre and treated according to PNG standard treatment for common illness in children guidelines (PNG Paediatric Society 2006).

At the time this study started recruiting participants into the study, the national malaria program was nearing the end of its campaign in rolling out the population-wide distribution of LLINs in the study area. This was the first LLIN distribution into the study area. Access to LLINs in the community would have increased substantially during this period. Although the enrolments into the study began earlier, the treatment of the cohort however was delayed until 2-3 weeks following the completion of the campaign. Prior to treatment administration, baseline information detailing the child's use of bednet, their recent antimalarial use and malaria-related signs and symptoms was collected. The study treatment was administered as direct observed therapy.

Over the 40 weeks of follow-up, the overall incidence of clinical *P. vivax* (any density) was 0.89 episodes/child/year-at-risk while the incidence of episodes with *P. vivax* parasite density >500/µL was 0.37 episodes/child/year-at-risk. The incidence of *P. vivax* malaria episodes decreased strongly with age (incidence rate ratio [IRR] any density: 0.81, CI_{95}: 0.73 - 0.91, $p<0.001$; density >500/µL: 0.60, CI_{95}: 0.50 - 0.72, $p<0.001$) and varied significantly between

villages. The main observations of the study were that the treatment with artesunate and primaquine with the latter being the anti-hypnozoite markedly reduced the risk of *P. vivax* clinical episodes of any density as well as the risk of *P. vivax* reinfection. Risk of clinical *P. vivax* episodes was reduced by 28% in children in the ASPQ arm when compared to children in AS only treatment arm and by 33% when compared to children in the control arm. No such significant reductions were observed for episodes with density >500/µL. The observed differences were particularly more pronounced in the first 3 months of follow-up than during the rest of the follow-up (ASPQ vs placebo: IRR 0.42, CI_{95}: 0.23 - 0.76, p=0.004; ASPQ vs AS: IRR 0.51, CI_{95}: 0.27 - 0.94, p=0.031). Treatment with ASPQ also reduced the risk of PCR- and LM-positive recurrent blood-stage infections by 44% (CI_{95}: 28% - 57%, p<0.001) and 67% (CI_{95}: 55% - 75%, p<0.001), respectively. Such differences did not exist for *P. falciparum* infections.

The study was conducted according to GCP guidelines. Ethical approval for human participation in the study was granted by PNG Medical Advisory Committee (MRAC number, 07.34). Informed written consent for the child's participation was obtained from the child's parents and guardians.

2.1.3.3 2013 cohort

This cohort was conducted from 2013 through to 2014, 5-6 years following the inception of the malaria control interventions scale-up in the study area. Prior to this study, there had been 2 rounds of population-wide distributions of LLINs in the study area, the test-and-treat approach plus the use of AL and primaquine had been implemented for at least over a year or so (see Figure 5.1 of chapter 5). This cohort was described in detail in chapter 5.

2.2 Statistical Methods

2.2.1 Analysis of indirect and direct associations with birthweight and LBW

Using SEM approach for data analysis, we examined the relationships among selected factors and their association with birthweight and LBW in chapter 3. SEM utilizes various types of models including regression, path and factor analysis to model relationships among variables, allows multiple dependent variables to be simultaneously included in the same model, variables to be included as both outcome and predictor in the same model and can distinguish between

direct and indirect effects of variables (Schumacker and Lomax 2010, Wang and Wang 2012, Kline 2015), the latter being the focus of chapter 3.

The first step in any typical SEM analysis is specifying a path diagram to depict the variables of interest and the hypothesised relations among them that are to be translated into equations for modelling. Illustrated in Figure 2.1 is an example of a simple path diagram of a hypothetical model to examine the relationships between some variables designated here as x1, y1, y2 and y3. In this example, all the variables are observed, reflective of our fitted models. The relationships between these variables are indicated by black lines with the head of the arrow indicating the direction of the influences pointing to the dependent variable, which in this case are variables y1-y3. In SEM, these variables are referred to as endogenous variables, i.e., they were caused by at least one variable in the model. They can be both dependent as well as independent variables (e.g. y1 & y2). Opposite of these are called exogenous variables. These are variables that are not caused by a variable that is in the model (e.g. x1). Every endogenous variable has a disturbance which represents residual (unexplained) variation, i.e., it represents the effect of all the unmeasured causes or variables. These are respectively denoted Fy1, Fy2 and Fy3 for variables y1-y3.

The assigned letters ***a - e*** on the lines represent the path coefficients with each of the letters representing an estimate of the direct effect or association between two variables. For instance, the path coefficient ***a*** estimates the direct effect of x1 on y3. The direct, indirect and total effects of variable x1 on y3 may be calculated as follows;

Direct effect of x1 on y3 = ***a***

Indirect effect x1 on y3 through variable y1 = ***b * c***

Indirect effect of x1 on y3 through variable y2 = ***d * e***

Total indirect effect of x1 on y3 = ***(b*c)*** + ***(d*e)***

Total effect of x1 on y3 = ***a*** + ***(b*c)*** + ***(d*e)***

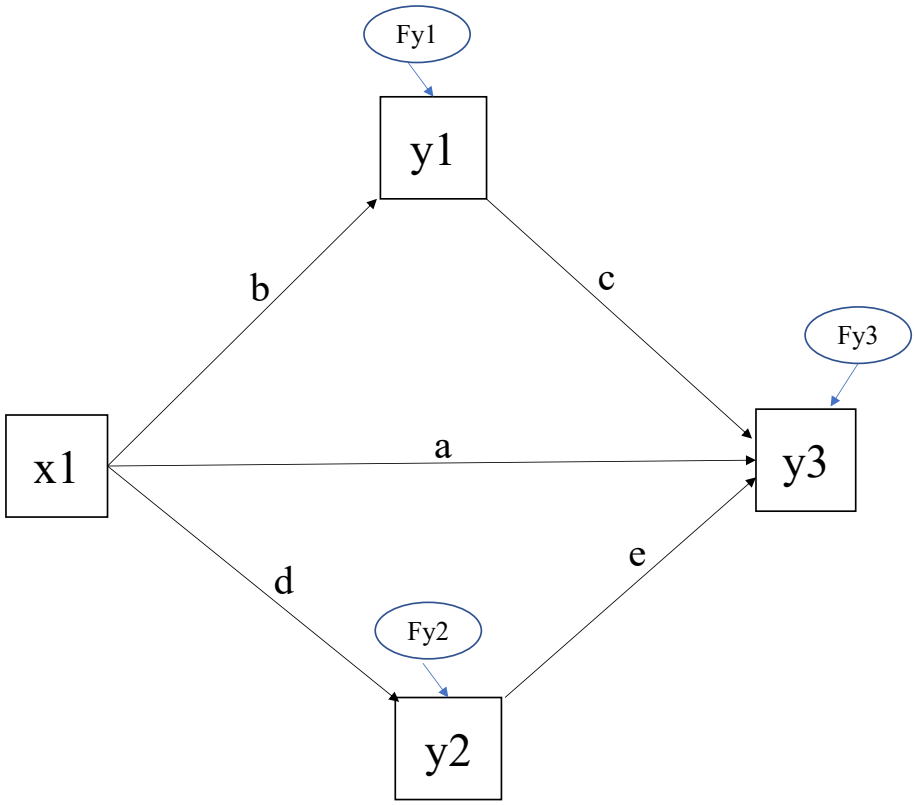

Figure 2-1: An illustration of a simple path diagram.

Shown in Figure 2.2 are the SEM conceptual models depicting the factors that were of interest and the expected relations among them and the role they played in influencing birthweight and LBW as the two main outcomes of interest. Figure 2.2a depicts the model we fitted to our data to examine the direct and the indirect effects (through anaemia, inflammation and angiogenesis at enrolment) of peripheral malaria infection at enrolment on birthweight and LBW. Relationship between iron deficiency at enrolment and birthweight and LBW were examined as per the specified model in Figure 2.2b. Here we examined the indirect effects of iron deficiency on birthweight and LBW through its influence on the risk of contracting peripheral malaria infection, anaemia and angiogenesis at enrolment. In Figure 2.2c, we examined the role IPTp played in influencing birthweight/LBW through peripheral malaria infection (detected in samples before delivery) and anaemia. Figure 2.2d - 2.2f show respectively the models for anaemia, inflammation and maternal undernutrition at enrolment. The SEM analysis was adjusted for potential confounders.

Only observed variables were included in the analysis, which results in a special case of SEM known as path analysis. Throughout the remainder of this chapter and thesis the terms SEM

and path analysis will be used interchangeably. SEM was implemented in the software package Mplus (Muthen and Muthen 1998-2017) guided by the defined conceptual models. Mplus estimates the direct and indirect effects of the risk factors (see Figures 2.1 and 2.2) by fitting linear or probit regression models to the continuous or categorical endogenous variables, respectively, in the conceptual models. Variables that have arrows pointing to an endogenous variable in the conceptual models are included as independent variables in each regression model. To ensure the parameters of the probit regression models are identifiable (i.e. unique parameter estimates can be derived from the data) the theta parameterisation was selected in Mplus and the weighted least square mean and variance adjusted (WLSMV) estimator was used to allow computationally faster estimation of models including categorical endogenous variables. Mplus uses all the observed data for each participant (also referred to as pairwise deletion) to estimate the model parameters. The WLSMV estimator of a parameter will converge to the true population value if missing data occur completely at random or at random with respect to the independent variables (Muthen and Muthen 1998-2017, Asparouhov and Muthen 2010).

2.2.2 Analysis of association with stunting, wasting and underweight

In chapter 4, we investigated the association between acute episodes of malaria, pneumonia and diarrhoea and the outcomes stunting, wasting and underweight. Generalised estimating equations (GEE) are an extension of generalised linear models developed by Liang and Zeger (Liang and Zeger 1986, Zeger and Liang 1986) that account for the correlation between repeated observations from an individual through specification of a working correlation matrix. As repeated outcome measures were observed for children in the study, GEE was used to estimate the association each morbidity has with stunting, wasting and underweight. For the binary outcome wasting a logistic link function was used, while identity link functions were used for the continuous outcomes stunting and underweight. An exchangeable correlation structure was selected for the working correlation matrix and robust variance estimator used to ensure the parameter estimates are consistent and asymptotically normally distributed even when the "working" correlation structure is misspecified (White 1980).

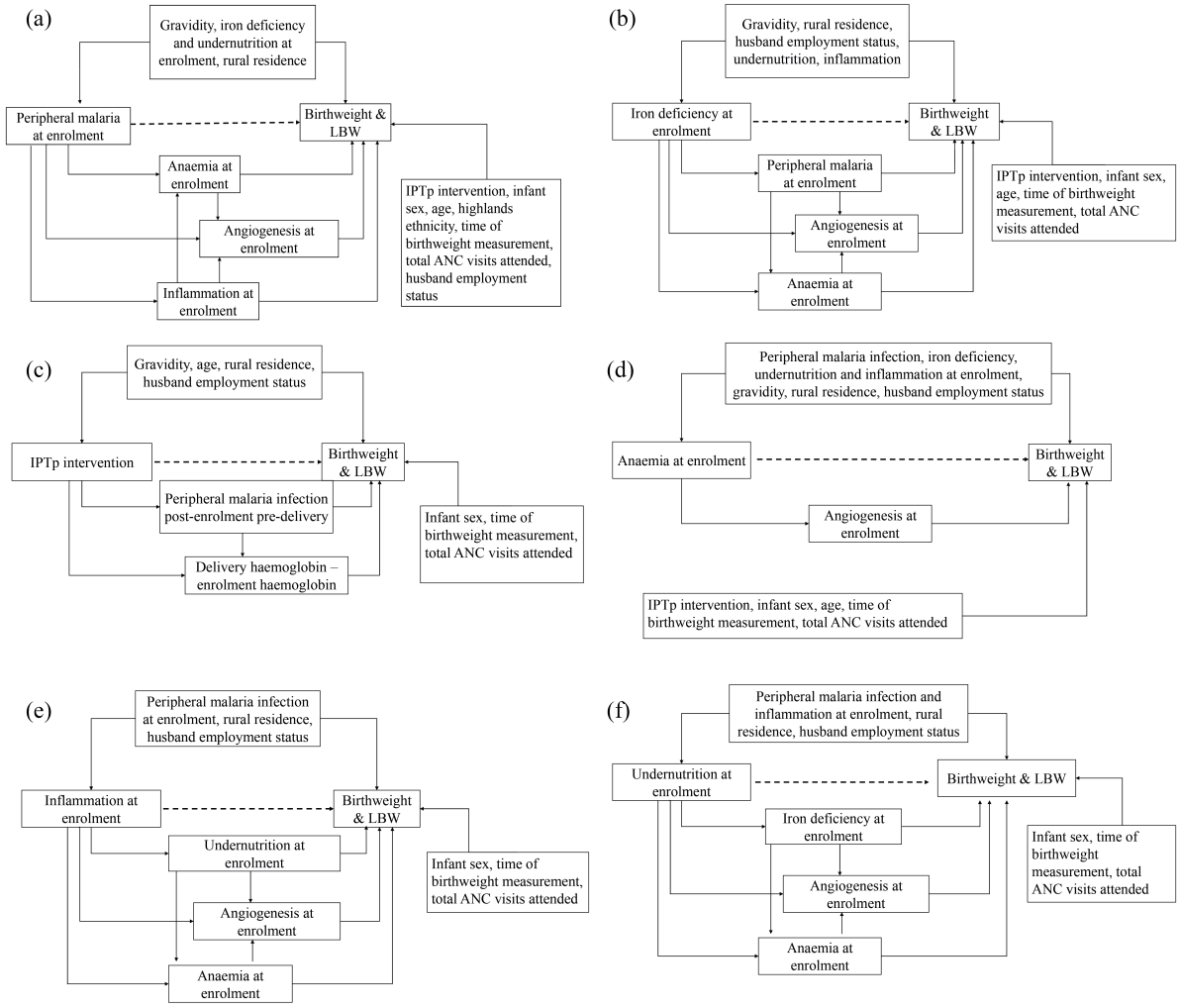

Figure 2-2: Illustration of SEM path diagrams of fitted models.

The fitted models examined the direct and indirect associations of **(a)** peripheral malaria infection at enrolment, **(b)** iron deficiency at enrolment, **(c)** IPTp intervention, **(d)** anaemia at enrolment, (**e)** inflammation at enrolment, **(f)** maternal undernutrition at enrolment with birthweight/LBW.

2.2.3 Analysis of lagged effects of childhood morbidities

Acute episodes of malaria, pneumonia and diarrhoea have immediate short-term effects on health, wellbeing and growth but they also show effects that are delayed in time, manifesting days to months and years after their occurrence (Checkley, Epstein et al. 2003, Checkley, Buckley et al. 2008, Lee, Yori et al. 2012, Richard, Black et al. 2012, Richard, Black et al. 2013). In chapter 4, we investigated delayed effects of acute malaria, pneumonia and diarrhoea on weight-for-age using distributed lag models (DLMs). (Armstrong 2006, Gasparrini, Armstrong et al. 2010, Gasparrini 2011).

A DLM for the longitudinal weight-for-age data can be expressed as follows:

$$\mathcal{Y}_{it} = \alpha_i + \Upsilon_i \times t + \mathcal{S}(x_{it};\beta) + \in_{it} \qquad (1)$$

In (1), $i = 1, \ldots . N$ indexes each child in the study and $t = t_1, \ldots\ t_{n_i}$ are the times at which weight was measured for each child, n_i is the number of observations for the i^{th} child. $\mathcal{Y}_{it}$ is the weight-for-age measurement for each child i at time point *t*. The relationship between weight-for-age ($\mathcal{Y}_{it}$) and time in the study (t) was modelled by a linear mixed effects model, where: α_i is the mean weight-for-age for the i^{th} child at baseline and is assumed to be normally distributed with population mean α and population standard deviation σ_α; and Υ_i is the slope of the outcome-time scale relationship for each child and is assumed to be normally distributed with population mean Υ and population standard deviation σ_γ. Υ_i is the average change in weight-for-age for a unit increase in time in study (1 day) for the i^{th} child. The relationship between weight-for-age and a morbidity was modelled by the cross-basis function $\mathcal{S}(x_{it};\beta)$, which allows $\mathcal{Y}_{it}$ to be described in terms of delayed morbidity effects (x_{it}).

The calculation of the cross-basis function first requires the following exposure history matrix ($\mathbf{Q}_i$) containing the morbidity history for a child to be derived

$$\mathbf{Q}_i = \begin{bmatrix} x_{i,t_1-l_1} & \cdots & x_{i,t_1-l_L} \\ \vdots & \ddots & \vdots \\ x_{i,t_{n_1}-l_1} & \cdots & x_{i,t_{ni}-l_L} \end{bmatrix} \qquad (2)$$

In (2), ($x_{1,t-l}$) denotes a morbidity measured at a lag $l = l_1, l_2, \ldots\ldots, l_L$ prior to the measurement of a child's weight-for-age at a given time *t* ($\mathcal{Y}_{it}$).

The general form of cross-basis in (1) may be expressed as

$$\mathcal{S}(x_{it};\boldsymbol{\eta}) = \boldsymbol{q}_{it.}^{\mathrm{T}}\boldsymbol{\mathcal{C}\eta} \qquad (3)$$

where $\boldsymbol{q}_{it.} = \left[x_{t-l_1}, x_{t-l_2}, \ldots\ldots, x_{t-l_L}\right]$ is the t^{th} row of the exposure matrix ($\mathbf{Q}_i$) for the i^{th} child; C is a matrix whose columns consist of functions of the lag variable *l* (i.e. the design matrix for the lag variable); $\boldsymbol{\eta}$ is an unknown vector of regression parameters to be estimated.

A natural cubic spline basis with 3 knots placed at the quartiles of the discrete lag variable (3.75 (25^{th}), 6.50 (50^{th}) and 9.25 (75^{th}) months) was selected for the matrix C.

The interpretation of the estimated parameters $\hat{\boldsymbol{\eta}}$ in equation (2) is aided by constructing linear effects $\hat{\boldsymbol{\beta}}$ at each lag from $\hat{\boldsymbol{\eta}}$ by evaluating the following expression

$$\hat{\boldsymbol{\beta}} = \hat{\boldsymbol{\beta}}(\boldsymbol{l}) = \boldsymbol{C}\hat{\boldsymbol{\eta}} \qquad (4)$$

The estimated parameters $\hat{\boldsymbol{\beta}}$ can be interpreted as the change in weight-for-age for those that experienced one or more morbidities compared to those that did not at different lags prior to the weight-for-age measurement.

3 Chapter 3: Structural equation modelling of direct and indirect effects of factors associated with birthweight and LBW in an intervention trial of malaria prevention in Papua New Guinean women.

3.1 Introduction

Birthweight is an important indicator of foetal health and is a key predictor of the newborn's subsequent health, growth and development. Neonates born with low birthweight (LBW, birthweight <2500g) due to either preterm delivery (<37 weeks' gestation) or IUGR are at increased risk of dying within the first month of their life (Katz, Lee et al. 2013, Lawn, Blencowe et al. 2014, G. B. D. Mortality and Causes of Death Collaborators 2016). Those that survive are at increased risk of childhood morbidities, have delayed childhood growth and development and experience various adverse health and socioeconomic consequences later in life with intergenerational influences (Christian, Lee et al. 2013, Negrato and Gomes 2013, Berglund, Kristrom et al. 2016, Skranes and Lohaugen 2016, Wang, Huang et al. 2016). LBW has therefore long been recognised as a major global health problem and reducing the burden is a key priority. Significant progress has been achieved by means of increased availability of nutritional interventions and new infection control strategies among others to prevent LBW (Bhutta, Das et al. 2013a, da Silva Lopes, Ota et al. 2017). Despite these efforts, approximately 20 million infants worldwide are still born with LBW each year (UNICEF and WHO 2019). Moreover, the progress in the reduction of LBW has been stalling over the last two decades (UNICEF and WHO 2019). Reducing LBW has therefore increased in urgency with the world committed to achieve a 30% reduction in LBW by year 2025 (WHO 2014).

A multitude of factors ranging from maternal to foetal to environmental factors have been identified as causes of LBW (Kramer 1987, Okah, Cai et al. 2005, Unger, Ome-Kaius et al. 2015, Ashorn, Hallamaa et al. 2018). Such factors include but are not limited to foetal sex, ethnicity, maternal undernutrition, foetal exposure to toxins from smoking and alcohol, infections such as malaria and sexually transmitted infections (STIs), inflammation and conditions such as pre-eclampsia. These factors may cause LBW through mechanisms that alter the supply of nutrients to the foetus leading to nutrient deprivation and consequently growth retardation or by creating an unhealthy and unfavourable intrauterine environment causing the baby to be born too early.

In Papua New Guinea, about 11% - 15% of all livebirths each year are LBW (Klufio, Amoa et al. 1997, Unger, Ome-Kaius et al. 2015) and the important predictors of LBW are malaria and anaemia during pregnancy, maternal nutrition and socioeconomic status (Brabin and Piper 1997, Allen, Raiko et al. 1998, Mueller 2002, Stanisic, Moore et al. 2015, Unger, Ome-Kaius et al. 2015, Fowkes, Moore et al. 2018). Malaria in pregnancy is particularly common in the

lowlands of PNG where malaria transmission is highly endemic. The factors common to all women across PNG are diet, nutrition, anaemia and socioeconomic status (Klufio, Amoa et al. 1997, Mueller 2002). Many women have limited education and live in rural areas. While food is abundant in PNG, the typical diet in many PNG families is often of low quality lacking essential nutrients such as protein that are required to grow a healthy baby in utero (A. P. C. Oomen 1971, Klufio, Amoa et al. 1997, Mueller, Vounatsou et al. 2001, Mueller 2002).

Since 2012, the world leaders pledged during the 65th World Health Assembly to reduce LBW by 30% between 2012 and 2025 (WHO 2014). Achieving this ambitious target will require strategies and interventions that can prevent causes of LBW. ~~E~~xisting interventions, such as nutritional interventions and intermittent preventive treatment for malaria in pregnancy (Bhutta, Das et al. 2013a, Unger, Ome-Kaius et al. 2015, da Silva Lopes, Ota et al. 2017), were developed to target specific causes that contribute to LBW. These interventions generally assume a single dominant cause. It is however important to realise the inter-relatedness of these factors; whereas some factors may directly cause LBW, others may operate through other factors or mechanisms to cause LBW. A recent study demonstrated that maternal infections, inflammation and nutrition and other constitutional factors had a joint influence on newborn size (Ashorn, Hallamaa et al. 2018). Another clear indication of this concept of risk factor inter-relatedness and co-causation of LBW was observed in our recent study of prevention of LBW and maternal anemia using IPTp in PNG women (Unger, Ome-Kaius et al. 2015), thus providing the motivation underlying the analysis presented in this chapter. Despite the very low prevalence of malaria observed in our study population, administering three doses of IPTp with SPAZ for malaria reduced LBW by about 30%, indicating that the effect of IPTp on LBW was not only through malaria but was through other mechanisms as well.

The majority of research investigating causes of LBW use standard statistical methods including linear and logistic regressions to identify factors associated with birthweight and LBW. The challenge with these models is that they cannot estimate both direct and indirect effects of the risk factor, i.e., the effects that are directly exerted on birthweight and LBW and those exerted via alternate indirect pathways acting through other risk factors. Interventions based on such results assume a single dominant cause and do not address the effect of other contributing factors and therefore whilst these interventions may be effective; they are likely to have minimal impact on the overall burden of LBW. This may explain the little to no benefits observed in studies conducted with some of these interventions (Bhutta, Das et al. 2013a, da

Silva Lopes, Ota et al. 2017) and could be one potential explanation for the persistence of the high burden of LBW despite the implementation of existing interventions.

Strategies and interventions that can concurrently address multiple exposures may therefore provide a way forward in reducing the burden of LBW. Developing such interventions however will require insight and knowledge into direct and indirect effects of risk factors and quantifying these effects. Only a few studies have explored this (Kramer 1987, Ashorn, Hallamaa et al. 2018, Fowkes, Moore et al. 2018) and none in the PNG setting. Using a structural equation modelling approach, we investigated the direct and indirect relationships between selected key risk factors, factors that are amenable to intervention during pregnancy, and pregnancy outcomes including birthweight and LBW, using data from our large IPTp clinical trial in PNG pregnant women (Unger, Ome-Kaius et al. 2015).

3.2 Methodology

3.2.1 Data source

Data for this analysis comes from a large single-blinded block-randomised controlled trial of IPTp (Clinicaltrials.gov, NCT01136850). The study was conducted in PNG between November 2009 and August 2012 and the aim of the study was to evaluate the efficacy of IPTp using a SPAZ combination regimen in preventing LBW, malaria, anaemia and STIs in PNG pregnant women (Unger, Ome-Kaius et al. 2015). Ethical approval for human participation in the study was granted by PNG Medical Research Advisory Committee (MRAC number 08.01). Detailed methodology of the study had been published elsewhere (Unger, Ome-Kaius et al. 2015, Unger, Hansa et al. 2019).

The study included pregnant women between 16 and 49 years of age residing in Sumkar and Madang districts of Madang Province who had voluntarily provided a written consent for participation in the study. The study area is located on the north coast of PNG where malaria transmission is usually high (Cattani, Tulloch et al. 1986, Muller, Bockarie et al. 2003) with malaria and anaemia during pregnancy being major causes of LBW (Brabin and Piper 1997, Allen, Raiko et al. 1998). The recent estimate of the prevalence of LBW in the area was 15% (Unger, Ome-Kaius et al. 2015).

3.2.2 Data for analysis

Women were enrolled into the study at gestational age of 26 weeks or less (determined by fundal height measurement) and followed up at two subsequent study visits (2nd and 3rd) 4-6 weeks apart until delivery.

At enrolment, the risk factor data collected included the mother's sociodemographic status (i.e., ethnicity, place of residence, education, employment status, partner's employment), obstetrics and medical history (e.g., gravidity, parity, recent illness) and maternal behavioural habits (e.g., smoking, betel nut chewing, alcohol drinking). The mother's clinical and physical examination data included uterine fundal height, weight, height, MUAC and blood pressure. From the venous blood sample provided by the women, levels of inflammation (CRP & AGP) and angiogenesis (sEng, sFlt-1 & PlGF) biomarkers as well as ferritin levels were measured. Ferritin level was measured using a novel in-house ELISA that was developed, validated and published by the Rogerson group at Doherty Institute (Bleicher, Unger et al. 2018). The inflammation and angiogenesis biomarkers were measured using commercially available ELISA (Human Quantikine ELISA kits; R&D Systems, Minneapolis, MN, USA) as described in chapter 2 and elsewhere (Unger, Hansa et al. 2019). Haemoglobin level was measured using a portable haemoglobin machine (HemoCue Ltd, Angelholm, Sweden). The collected blood slides were evaluated for malaria infection as described in chapter 2. Women then received the study treatment (i.e., IPTp with SPAZ or single treatment course of SP and chloroquine) as per their random treatment allocation (Unger, Ome-Kaius et al. 2015).

On the two subsequent study visits following enrolment, women provided blood for determination of malaria infection and received their study treatment. If the women presented to the study team with a febrile illness outside of the scheduled study visits, blood samples were collected for determination of malaria infection and haemoglobin level was measured and women were treated according to the national treatment guidelines. At delivery, birthweight was measured to the nearest 10 g using digital infant scales (Cupid 1, Charder Medical, Taiwan).

3.2.3 Selected risk factors for assessment

Previous studies have focused on identifying a multitude of factors as causes of LBW (Kramer 1987, Brodsky and Christou 2004) and as such, this analysis was not focussed on identifying

all risk factors but rather on risk factors that are amenable to intervention during pregnancy in a PNG setting to improve birthweight and thus prevent LBW. The factors were selected based on the past literature for this study area and our previous analyses of this data set (Brabin and Piper 1997, Allen, Raiko et al. 1998, Unger, Ome-Kaius et al. 2015, Fowkes, Moore et al. 2018, Unger, Hansa et al. 2019) and included maternal nutrition, infection, inflammation, plasma angiogenic factors, and intervention with IPTp during pregnancy. Selected variables to represent these key intervenable risk factors therefore included MUAC, haemoglobin, ferritin, malaria infection, CRP, AGP, sEng, sFlt-1 and PlGF ratio and IPTp with SPAZ. These variables will be referred to as 'test variables' in this chapter unless specified. Other factors including STIs, blood pressure and other maternal diseases are important but due to lack of sufficient data for these factors, they were not selected for analysis. Malaria infection in this chapter refers to the peripheral plasmodium infection detectable by light microscopy. IPTp will be used throughout the paper to refer to IPTp with SPAZ and interval haemoglobin and interval malaria will be used to refer to haemoglobin change from enrolment to delivery and peripheral plasmodium infection during the study respectively. The outcome variables included birthweight and LBW.

3.2.4 Statistical methods

Only birthweights from congenitally normal singleton livebirths that were measured within 7 days of delivery (n=2021) were included in the analyses. Descriptive analysis was performed to appreciate the distribution characteristics of the different variables to be evaluated in the models. Categorical variables were tabulated providing proportions which are expressed as frequencies or percentages. Histograms and boxplots were used to visualise the distribution of the continuous variables and the shape of these distributions (i.e., normal versus skewed distributions). They were summarised and expressed as means with standard deviations for normally distributed data while median with interquartile range were provided for skewed data.

To allow for interpretations of clinical and public health relevance, continuous variables under evaluation were categorised except for the interval heamoglobin. In maintaining consistency with definitions used in the previous analyses of this same data and on past literature (Thurnham, McCabe et al. 2010, Unger, Ome-Kaius et al. 2015, Zeisler, Llurba et al. 2016, Namaste, Aaron et al. 2017, Unger, Hansa et al. 2019), we defined maternal undernutrition as indicated by low mid-upper arm circumference as MUAC <23cm and anaemia as haemoglobin

<11g/dL. Iron deficiency is defined by WHO as ferritin <15µg/l (WHO 2011 (WHO/NMH/NHD/MNM/11.2) however, we increased the cut-off to <30µg/l to account for inflammation (Namaste, Rohner et al. 2017) that is likely to exist in a setting like PNG where high burden of infections including malaria and their associated inflammation are highly common. A raised CRP and AGP, an indication of inflammation was defined as a CRP ≥ 5 mg/L and AGP ≥ 360 mg/L while markers of angiogenesis were considered elevated if levels of sEng ≥ 28 pg/ml or a sFlt-1: PlGF ratio ≥ 38 were detected. Each of these markers were included in the analyses as separate variables.

We performed univariable and multivariable linear and logistic regression analyses to evaluate associations between the risk factors and birthweight and LBW respectively as well as among each other. Factors with a tendency for an association with birthweight or LBW (defined as p<0.1) in the univariable analyses were included in the respective multivariable analyses. The associations are expressed as difference in the mean birthweight and odds ratio (OR) for birthweight and LBW respectively and were considered to be statistically significant if the p-value was below the nominal level of significance of 0.05.

For SEM, we first developed a conceptual framework of pathway maps which specified pathways through which the factors of interest (test variables) may influence birthweight/LBW (Figure 3.1). This included direct paths whereby the risk factor may directly affect birthweight/LBW or indirectly, that is the influence of a factor on birthweight/LBW through one or more risk factors along the path.

All the test variables except interval haemoglobin were included as binary variables with zero denoting the reference group and one denoting the group of interest. We accounted for timing of birthweight measurement to account for the weight changes that occur during the first few days after birth (Rijken, Rijken et al. 2011). Haemoglobin level is believed to be gestation-age-dependent due to pregnancy associated haemodilution (from plasma volume expansion) but this is believed to stabilize at about 6 months and onwards to the end of pregnancy (Sato, Fujimori et al. 2014). Given the majority of our study population were enrolled and had haemoglobin measured at about 6 months of gestation, we believe the influence of haemodilution on the association between haemoglobin and birthweight/LBW will be minimal and consequently did not account for it in our models. To support this, we compared standard multivariable linear and logistic regression model, one adjusting for gestation age to that not

adjusting for gestation age using likelihood ratio test (lrtest) and no difference was seen between the models.

Mplus was used to estimate the model parameters using the weighted least square mean variance adjusted (WLSMV) estimator (see Chapter 2, section 2.2.1 for further details). The path coefficients (Figure 2.1) are analogous to regression coefficients and, depending on the outcome variable, can be interpreted as a difference in mean birthweight (in kg) and change in the probability of LBW. For the LBW analysis, the bootstrap method (number of draws set to 1000) was used to estimate standard errors and derive confidence intervals for the probit regression models used by Mplus to estimate the direct and indirect effects of the risk factors. Given no indirect paths are specified for the markers of angiogenesis and the interval heamoglobin, the coefficients will be interpreted as total effects.

The fit of the model was evaluated with the comparative fit index (CFI) (Bentler 1990), a Tucker– Lewis index (TLI) (Tucker and Lewis 1973) and root mean square error of approximation (RMSEA) (Steiger 1990). A model was a good fit if the CFI and TLI were >0.95 with RMSEA < 0.05, while an acceptable fit was indicated by CFI and TLI > 0.90 and RMSEA <0.08. If the model fit statistics were not acceptable, we examined the modification indices to decide if additional pathways should be identified to improve model fit. The models were re-specified and re-estimated. Note this was only done if these pathways were biologically logical and were in line with past literature.

The descriptive as well as linear and logistic regression analyses were conducted using Stata version 15 (StataCorp, USA) while the SEM models were built and estimated in Mplus software, version 8.2 (Muthen & Muthen, Los Angeles, CA). R studio was used to export SEM output from mplus into excel for use.

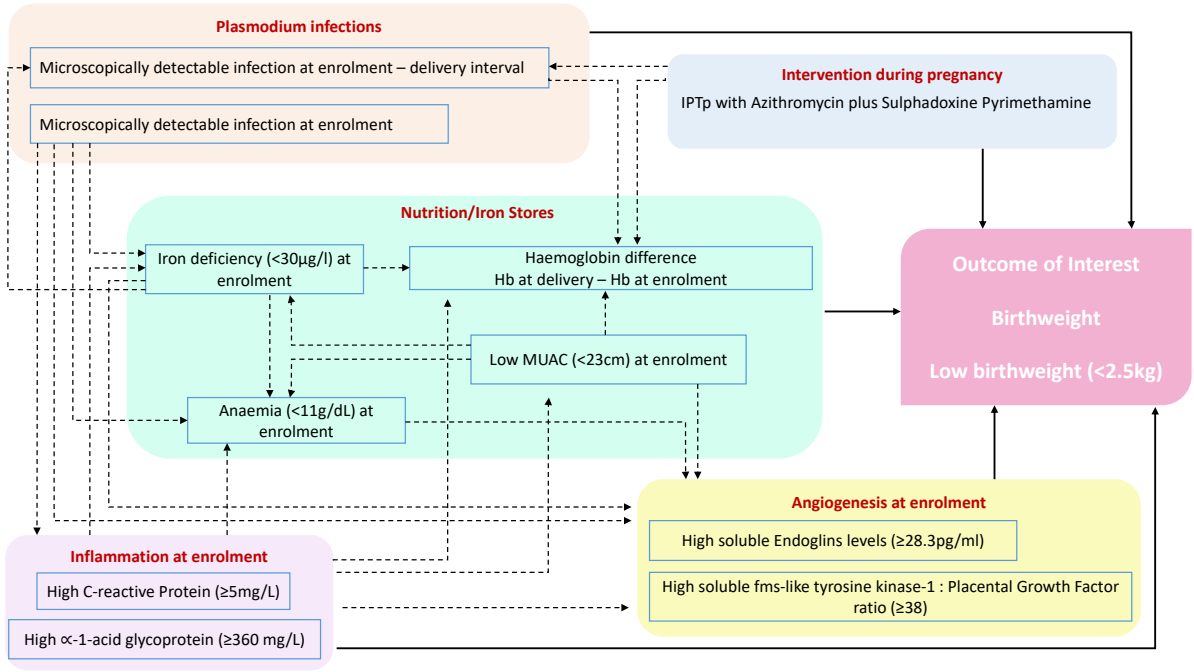

Figure 3-1: Conceptual model of structural equation modelling pathways of study-defined key factors contributing to birth weight and LBW.

Abbreviations: MUAC=mid-upper arm circumference; IPTp=Intermittent Preventive Treatment in pregnancy. The concept model reflects how the different factors measured at the first antenatal care visit (enrolment) and during (post-enrolment to pre-delivery interval) the study may have directly (solid lines) or indirectly (dotted lines) affected neonatal weight at birth. Large grey boxes represent each concept of infections, inflammation, angiogenesis, maternal nutrition and interventions, containing within each box the factors that are measures of these concepts. Arrows pointing to or away from the large grey boxes indicate pathways that involve each of the factors that are within the grey box. Arrows originating from or ending at each factor within the grey boxes indicate pathways that are specific to factors involved and not to all factors within the same grey box. In the SEM analytical model, all factors and their corresponding specific pathways were included into the model separately and modelled as single variables. Birthweight was modelled separately to low birthweight.

3.3 Results

3.3.1 Cohort characteristics

Detailed characteristics of the original study are published elsewhere (Unger, Ome-Kaius et al. 2015, Unger, Wangnapi et al. 2016, Unger, Hansa et al. 2019), some of which are summarised for the purpose of this present analysis in Table 3.1. Of the 2793 pregnant women enrolled into the parent study, 2021 were included in this analysis. The 772 exclusions were due to protocol violations (n=110), withdrawals (n=510) mostly due to loss of follow-up, and the lack of birthweight data for analysis (n=152) (Figure 3.2). Women included in the analysis were mostly rural residents (60.7%), 90.6% were literate, 49.6% were primigravid and half (50.1%)

of them were in the intervention arm (AZ plus SP) of the parent study. At enrolment, about 28% of the women were undernourished, 82% were anaemic, and two-thirds had iron deficiency (66.1%). A small number of women had microscopically detected plasmodium infection and up to 26% of the women had increased levels of biomarkers (CRP and AGP) that are indicative of inflammation (Table 3.1).

3.3.2 Pregnancy outcomes

The mean (SD) gestational age at delivery was 39.1 (1.8) weeks. Over half (55.8%) of the newborns were girls, about 15% had LBW and 8.6% were born preterm. The mean (SD) weight at birth was 2943 (479) grams (Table 3.1).

3.3.3 Summary statistics of study defined risk factors

The 1013 women in the intervention arm of the study received on average 3 doses of SPAZ during the study. Four percent of the women had malaria parasites detectable by light microscopy during the study, i.e. after enrolment and before delivery. Mean (SD) haemoglobin at enrolment was 9.7 (1.5) g/dL while mean MUAC was 24.0 (2.6). For the biomarkers, the median (interquartile range) for CRP, AGP, soluble endoglins, sFlt-1: PlGF ratio and ferritin were 1.3 (0.5 - 3.6), 233.4 (147.6 - 365.0), 15.8 (7.9 - 28.3), 15.5 (7.4 - 33.8) and 19.4 (10.7 - 39.7) respectively. Additional descriptive statistics for these variables are presented in Table 3.1.

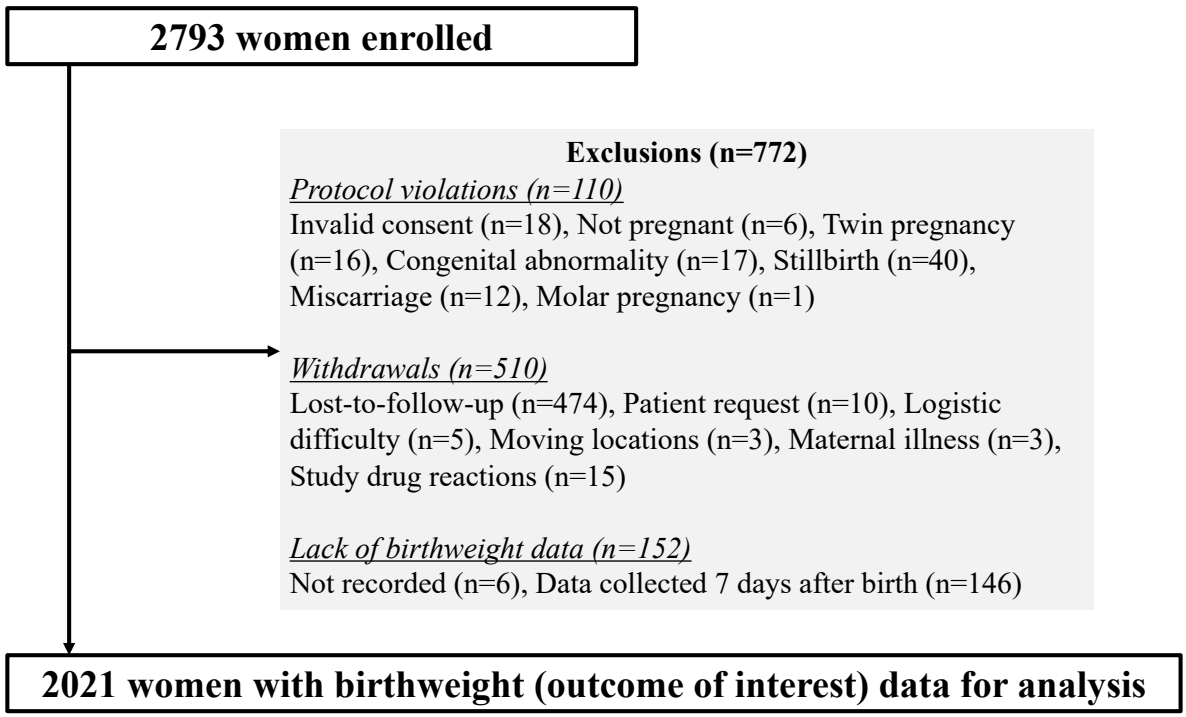

Figure 3-2: Flowchart showing the women included in the final cohort for analysis in this study and also describes the reasons for participant exclusion from the analysis.

Table 3.1: Cohort characteristics

Variables	N	Summary
At enrolment		
Maternal age (years)	2021	24.5 (5.5)
Literate	2016	90.6
Rural residence	2021	60.7
Highlands origin	2021	9.3
Smoking	2019	19.3
Betel nut chewing	2009	83.1
IPTp with azithromycin & sulphadoxine pyrimethamine	2021	50.1
Gestational age by ultrasound (weeks)	1320	21.8 (4.7)
First pregnancy (primipara)	2018	49.6
Maternal undernutrition (MUAC <23 cm)	1978	27.9
Anaemia (haemoglobin <11 g/dL)	1935	82.0
Low iron levels (Ferritin <30 µg/l)	1981	66.1
[a]Peripheral plasmodium infection by microscopy	2015	6.7
Inflammation and angiogenesis status at enrolment	2012	
High C-reactive protein levels (≥5 mg/L)		18.1
High ∝-1-acid-glycoprotein levels (≥ 360 mg/L)		25.8
High soluble endoglin levels (≥ 28.3 pg/ml)		25.0
High soluble fms-like tyrosine kinase-1 levels (sFlt-1, ≥ 4.3 ng/ml)		25.3
Low placental growth factor levels (PlGF, ≤ 93.5 pg/ml)		25.0
High sFlt1: PlGF ratio (≥ 38)		22.0
At delivery - birth outcomes		
Gestational age by ultrasound (weeks)	1320	39.1 (1.8)
Female neonate	2011	55.8
Birthweight (g)	2021	2942.6 (478.5)
Low birthweight (<2500g)		15.1
Preterm delivery (<37 weeks)	1320	8.6
Small-for-gestational-age		24.6

Summary are % for categorical variables and mean (SD) for continuous variables; MUAC: mid-upper arm circumference; IPTp: intermittent preventive treatment in pregnancy; [a]Infections of any plasmodium species and of any density

3.3.4 Univariate and multivariable associations with birthweight and low birthweight

In univariate analyses of birthweight, IPTp (coef 41.9; CI_{95}: 0.19 - 83.6) and iron deficiency (coef 60.0; CI_{95}: 15.5 - 104) were positively associated with birthweight while low MUAC (coef -147; CI_{95}: -193 - 101), high soluble endoglin levels (coef -52.4; CI_{95}: -100 - -4.01) and

elevated sFlt-1:PlGF ratio (coef -67.5; CI_{95}: -118 - -17.0) were all negatively associated with birthweight. Other factors of interest including malaria infection (both at enrolment and during the study), anaemia at enrolment, interval heamoglobin and inflammation did not show an association with birthweight (Table 3.2). Significant associations were also observed for other factors including maternal age (coef 14.0; CI_{95}: 10.3 - 17.8), partner had a wage/salary earning employment (coef 63.1; CI_{95}: 18.0 - 108), rural residence (coef -42.4; CI_{95}: -85.1 - 0.35), highlands origin (coef 218; CI_{95}: 147 - 289), primigravidity (coef -217; CI_{95}: -257 - -176), total antenatal care visits attended (coef 37.9; CI_{95}: 27.5 - 48.4), female sex of baby (coef -67.8; CI_{95}: -109 - -25.7) and short maternal height (coef -170; CI_{95}: -224 - -116). IPTp and iron deficiency remained significant after adjusting for these factors in the multivariable analysis, whereby women receiving IPTp during the study and being iron deficient at enrolment delivered babies 46g (CI_{95}: 1.69 - 89.9) and 59g (CI_{95}: 10.1 - 108) heavier than babies born to women who did not receive IPTp and who were not iron deficient, respectively (Table 3.2). Similarly, low MUAC remained significant such that having a low MUAC was associated with 121g (CI_{95}: -171 - -70.5) decrease in mean birthweight when compared to women with normal MUAC (Table 3.2). Associations with soluble endoglin levels and sFlt-1: PlGF ratio were no longer significant in the multivariable analysis (Table 3.2).

As observed with birthweight, IPTp and low MUAC were also strongly associated with LBW both in univariable and multivariable logistic regression analyses (Table 3.2). In univariate analysis, odds of delivering a LBW baby was reduced by 30% (OR 0.70; CI_{95}: 0.55 - 0.90) for women receiving IPTp when compared to women who didn't, while women with low MUAC had 1.71 (OR 1.71, CI_{95}: 1.32 - 2.21) times higher odds of delivering a LBW baby than those with normal MUAC (Table 3.2). Maternal age (OR 0.94; CI_{95}: 0.92 - 0.96), partner had a wage/salary earning employment (OR 0.67; CI_{95}: 0.52 - 0.86), highlands origin (OR 0.43; CI_{95}: 0.24 - 0.75), primigravidity (OR 2.86; CI_{95}: 2.19 - 3.73), total antenatal care visits attended (OR 0.84; CI_{95}: 0.78 - 0.90), female sex (OR 1.53; CI_{95}: 1.19 - 1.97) and maternal height (OR 1.75; CI_{95}: 1.31 - 2.32) were significantly associated with LBW in univariate analyses and were thus adjusted for in the multivariable logistic regression. Multivariable regression revealed that women receiving IPTp had a 35% (OR 0.65, CI_{95}: 0.48 - 0.88) reduced risk of delivering a LBW baby than the women who did not receive IPTp. Compared to women with normal MUAC, the odds of delivering a LBW baby was significantly increased for women with low MUAC (OR 1.72, CI_{95}: 1.25 - 2.37).

Contrary to what may be expected, we found anaemia at enrolment to be associated with decreased likelihood of LBW, such that the odds of delivering LBW among anaemic women was significantly reduced by 35% (OR 0.65; CI_{95}; 0.43 - 0.97, Table 3.2). Similarly, we also observed an increase in the odds of delivering a LBW baby for every 1g/dl increase in the difference in the haemoglobin measured between enrolment and delivery (adjusted OR: 1.12, CI_{95}: 1.03 - 1.22, Table 3.2).

Table 3.2: Crude and adjusted associations of risk factors with birthweight and low birthweight

	Birthweight (grams)		Low birthweight	
	Crude	**Multivariable**	**Crude**	**Multivariable**
	coef	[a]coef (CI_{95})	OR	[b]OR (CI_{95})
IPTp with azithromycin & sulphadoxine pyrimethamine	41.9 *	45.8 (1.69, 89.9) *	0.7 **	0.65 (0.48, 0.88) **
Plasmodium infection at enrolment (by microscopy)	-55.0	57.2 (-36, 150)	1.04	0.57 (0.29, 1.12)
[c]Plasmodium infection during study (by microscopy)	-51.6	10.9 (-104, 126)	0.95	0.76 (0.32, 1.79)
Low mid upper arm circumference (<23 cm)	-147 ***	-121 (-171, -70.5) ***	1.72 ***	1.72 (1.25, 2.37) **
Enrolment anaemia (Haemoglobin <11 g/dL)	-16.3	15.8 (-45.3, 76.9)	0.83	0.65 (0.43, 0.97) *
[d]Haemoglobin change during study (g/dL)	-10.4 #	-11.8 (-24.3, 0.75) #	1.08 *	1.12 (1.03, 1.22) **
Low ferritin levels (<30 µg/l)	60.0 **	59.1 (10.1, 108) *	0.87	0.87 (0.63, 1.22)
High C-reactive protein levels (≥5 mg/L)	-43.2	-37.8 (-97.8, 22.2)	1.28	1.44 (0.97, 2.13) #
High ∝-1-acid-glycoprotein levels (≥ 360 mg/L)	-33.0	-9.61 (-61.5, 42.3)	1.16	1.21 (0.86, 1.72)
High soluble endoglin levels (≥ 28.3 pg/ml)	-52.3 *	-22.2 (-73.8, 29.4)	1.20	1.05 (0.75, 1.49)
High sFlt-1: PlGF ratio (≥ 38)	-67.5 **	-39.3 (-93.1, 14.5)	1.31 #	1.17 (0.82, 1.67)

Abbreviations: coef =coefficients; OR = odds ratio; CI_{95} = 95% confidence interval; sFlt-1 = soluble fms-like tyrosine kinase-1; PlGF = Placental growth factor; IPTp = intermittent preventive treatment in pregnancy; [a]coefficients are mean differences in birthweight in grams and estimated using linear regression and adjusted for maternal age, partner's employment status, rural residence, highlands origin, primigravidity, total antenatal care visits attended, female sex and maternal height; [b]Odds ratio estimated using logistic regression and adjusted for maternal age, partner's employment status, highlands origin, primigravidity, total antenatal care visits attended, female sex and maternal height; [c]Infection post-enrolment and pre-delivery; [d]Difference in haemoglobin between enrolment and delivery;

$0.05 \leq p < 0.1$; * $0.01 \leq p < 0.05$; **$0.001 \leq p < 0.01$; ***$p<0.001$

3.3.5 Direct and indirect effects by structural equation modelling

The confounders included in the SEM models included time of birthweight measurement, foetal sex, maternal age, place of residence, ethnicity, partner's employment status, height, number of antenatal care visits attended and primiparity. Estimates of the fitted SEM model for birthweight based on the conceptual model (Figure 3.1) of the study defined variables are presented in Table 3.3. IPTp and iron deficiency were associated with a 52g (CI_{95}: 010 - 0.089) and 44g (CI_{95}: 009 - 077) increase in mean birthweight respectively while a 54g (CI_{95}: -0.081 - -0.025) decrease was observed for low MUAC. The SEM analysis shows that the significant effects of IPTp, low MUAC and iron deficiency were independently directly exerted. Birthweight was directly predicted positively by IPTp and negatively by low MUAC whereby babies of women receiving IPTp during the study were 50 grams (coef 0.05; CI_{95}: 0.004 - 0.092) heavier than their counterparts, while women with low MUAC delivered babies 57g (coef -0.057; CI_{95}: -0.086 - -0.029) lighter than babies born to women with normal MUAC. We also observed a tendency for soluble endoglins at enrolment to directly negatively predict birthweight (coef -0.027; CI_{95}: -0.057 - 0.002). Iron deficiency was noted to have an overall positive influence on birthweight and there was a tendency for this effect to be exerted directly (coef 0.037; CI_{95}: -0.003 - 0.079). We did not observe statistically significant direct and indirect associations of other factors that were of interest (Table 3.3).

In the SEM analysis of LBW, probit regression models are used in Mplus to estimate the direct and indirect effects. However, in probit regression models the probability of having a LBW baby (outcome variable) and the covariates have a nonlinear relationship, and the coefficients in Table 3.4 are not as straightforward to interpret as those from a linear or logistic regression model. For example, a positive coefficient means that an increase in the risk factor leads to an increase in the probability of LBW baby and a negative coefficient indicates a decreased probability.

IPTp and MUAC appear to only have direct effects on LBW (Table 3.4). For the women who received IPTp during their pregnancy, their propensity of delivering a LBW was 0.22 standard deviations lower than women who did not receive IPTp (coef: 0.22; CI_{95}: -0.0356 - -0.076). The propensity of having a LBW baby was increased in women with low MUAC (coef: 0.12; CI_{95}: 0.024 - 0.214) than the women who had normal MUAC (Table 3.4). We also observed anaemia (coef -0.174; CI_{95}: -0.332 - -0.019) and CRP levels (coef 0.140; CI_{95}: 0.002 - 0.267)

at enrolment to have an overall influence on LBW (Table 3.4). Whether the effects of CRP are exerted directly or indirectly was not established in this model (Table 3.4). Anaemia at enrolment was associated with a reduced probability of LBW (coef -0.169; CI_{95}: -0.325 - -0.014), suggesting that anaemia was protective against LBW and the effects appeared to be directly exerted (Table 3.4). On that same note, we see an increase in the probability of LBW with every 1g increase in the difference in the haemoglobin (coef 0.151; CI_{95}: 0.042 - 0.266) between enrolment and delivery (Table 3.4).

Associations between the selected risk factors are given in Table 3.5. Briefly, malaria infections at enrolment were strongly associated with an increase in the probability of the mother being anaemic. Risk of being iron deficient was noted to be reduced if women had malaria infection or had high levels of CRP, a reflection of the inflammation associated increase in ferritin levels.

The model fit statistics confirm that the model fitted the data well: birthweight model, CFI=0.96, TLI=0.93, RMSEA=0.013 (low=0.007, high=0.019); LBW model, CFI=0.95, TLI=0.91, RMSEA=0.015 (low=0.009, high=0.020).

Table 3.3: Unstandardised direct, total indirect and total associations between risk factors and birthweight.

Predictors	Direct Effects (kg)	Total Indirect Effects (kg)	Total Effects (kg)
IPTp with azithromycin & sulphadoxine pyrimethamine	0.050 (0.004, 0.092) *	0.002 (-0.014, 0.020)	0.052 (0.010, 0.089) **
[a]Plasmodium infection at enrolment (by microscopy)	0.031 (-0.035, 0.105)	-0.030 (-0.068, 0.003)	0.002 (-0.042, 0.049)
[a]Plasmodium infection during study (by microscopy)	-0.008 (-0.062, 0.042)	0.001 (-0.003, 0.005)	-0.007 (-0.061, 0.043)
Low mid upper arm circumference (<23 cm)	-0.057 (-0.086, -0.029) ***	-0.003 (-0.005, 0.012)	-0.054 (-0.081, -0.025) ***
Enrolment anaemia (Haemoglobin <11 g/dL)	0.016 (-0.036, 0.062)	0.003 (-0.002, 0.008)	0.018 (-0.034, 0.061)
Haemoglobin change during study (g/dL)	-0.012 (-0.033, 0.008)		
Low ferritin levels (<30 µg/l)	0.037 (-0.003, 0.079) #	0.007 (-0.009, 0.024)	0.044 (0.009, 0.077) *
High C-reactive protein levels (≥5 mg/L)	-0.020 (-0.062, 0.020)	-0.011 (-0.026, 0.001)	-0.031 (-0.070, 0.009)
High ∝-1-acid-glycoprotein levels (≥ 360 mg/L)	-0.006 (-0.04, 0.024)	0.002 (-0.009, 0.013)	-0.004 (-0.036, 0.025)
High soluble endoglin levels (≥ 28.3 pg/ml)	-0.027 (-0.057, 0.002) #		
High sFlt-1: PlGF ratio (≥ 38)	-0.023 (-0.052, 0.006)		

Abbreviations: IPTp=Intermittent Preventive Treatment in pregnancy; sFlt-1=soluble fms-like tyrosine kinase-1; PlGF=Placental Growth Factor. Numbers are unstandardised coefficients (95% confidence interval) estimated using structural equation modelling, adjusting for maternal age, height, ethnicity, place of residence, gravidity, number of antenatal care visits, partner's employment status, timing of birthweight measurement, and neonatal sex. [a]Peripheral microscopically detected infections (any species and any density). For the variables that we did not specify an indirect path (i.e., haemoglobin change during study, soluble endoglin levels, sFlt-1: PlGF ratio), only the direct effects were estimated.

[#] 0.05 ≤ p <0.1; * 0.01 ≤ p <0.05; **0.001 ≤ p <0.01; ***p<0.001

Table 3.4: Standardised direct, total indirect and total associations between risk factors and low birthweight.

	Direct	Total Indirect	Total
IPTp with azithromycin & sulphadoxine pyrimethamine	-0.220 (-0.356, -0.076) **	0.013 (-0.036, 0.076)	-0.207 (-0.335, -0.081) **
[a]Plasmodium infection at enrolment (by microscopy)	-0.086 (-0.31, 0.142)	0.046 (-0.079, 0.165)	-0.040 (-0.200, 0.095)
[a]Plasmodium infection during study (by microscopy)	-0.037 (-0.24, 0.124)	-0.003 (-0.026, 0.018)	-0.040 (-0.256, 0.121)
Low mid upper arm circumference (<23cm)	0.122 (0.024, 0.214) *	-0.005 (-0.038, 0.022)	0.117 (0.019, 0.205) *

Enrolment anaemia (Haemoglobin <11g/dL)	-0.169 (-0.325, -0.014) *	-0.005 (-0.022, 0.010)	-0.174 (-0.332, -0.019) *
Haemoglobin change during study (g/dL)	0.151 (0.042, 0.266) *		
Low ferritin levels (<30µg/l)	-0.037 (-0.192, 0.109)	-0.048 (-0.114, 0.017)	-0.085 (-0.222, 0.042)
High C-reactive protein levels (≥5 mg/L)	0.110 (-0.038, 0.244)	0.030 (-0.011, 0.077)	0.140 (0.002, 0.267) *
High ∝-1-acid-glycoprotein levels (≥ 360 mg/L)	0.016 (-0.102, 0.118)	-0.011 (-0.047, 0.023)	0.004 (-0.104, 0.106)
High soluble endoglin levels (≥ 28.3 pg/ml)	0.054 (-0.043, 0.152)		
High sFlt-1: PlGF ratio (≥ 38)	0.045 (-0.06, 0.138)		

Abbreviations: IPTp=Intermittent Preventive Treatment in pregnancy; sFlt-1=soluble fms-like tyrosine kinase-1; PlGF=Placental Growth Factor. Numbers are standardised coefficients (95% confidence interval) estimated using structural equation modelling, adjusting for maternal age, height, ethnicity, gravidity, number of antenatal care visits, partner's employment status, timing of birthweight measurement, and neonatal sex. [a]Peripheral microscopically detected infections (any species and density); For the variables that we did not specify an indirect path (i.e., haemoglobin change during study, soluble endoglin levels, sFlt-1: PlGF ratio), only the direct effects were estimated.

[#] 0.05 ≤ p <0.1; * 0.01 ≤ p <0.05; **0.001 ≤ p <0.01; ***p<0.001

Table 3.5: Unstandardised direct, total indirect and total associations between the different risk factors.

Predictors	Outcome	Summary	Estimate	CI_{95}	p-value
Plasmodium infection at enrolment (by microscopy)	Enrolment anaemia (Hb <11g/dL)	Total	0.177	(0.054, 0.356)	0.018
Plasmodium infection at enrolment (by microscopy)	Enrolment anaemia (Hb <11g/dL)	Total indirect	-0.097	(-0.225, -0.028)	0.049
Plasmodium infection at enrolment (by microscopy)	Enrolment anaemia (Hb <11g/dL)	Direct	0.274	(0.116, 0.520)	0.01
Low MUAC (<23 cm)	Enrolment anaemia (Hb <11g/dL)	Total	0.076	(-0.021, 0.169)	0.119
Low MUAC (<23 cm)	Enrolment anaemia (Hb <11g/dL)	Total indirect	0.023	(0.004, 0.052)	0.061
Low MUAC (<23 cm)	Enrolment anaemia (Hb <11g/dL)	Direct	0.053	(-0.042, 0.146)	0.294
High CRP levels (≥5 mg/L)	Enrolment anaemia (Hb <11g/dL)	Total	-0.028	(-0.185, 0.103)	0.702
High CRP levels (≥5 mg/L)	Enrolment anaemia (Hb <11g/dL)	Total indirect	-0.042	(-0.094, -0.012)	0.038
High CRP levels (≥5 mg/L)	Enrolment anaemia (Hb <11g/dL)	Direct	0.014	(-0.152, 0.158)	0.857
High AGP levels (≥ 360 mg/L)	Enrolment anaemia (Hb <11g/dL)	Total	-0.047	(-0.154, 0.064)	0.401

High AGP levels (≥ 360 mg/L)	Enrolment anaemia (Hb <11g/dL)	Total indirect	-0.023	(-0.057, 0.001)	0.12
High AGP levels (≥ 360 mg/L)	Enrolment anaemia (Hb <11g/dL)	Direct	-0.024	(-0.132, 0.094)	0.676
Low ferritin levels (<30 µg/l)	Enrolment anaemia (Hb <11g/dL)	Total	0.236	(0.113, 0.397)	0.001
Low ferritin levels (<30 µg/l)	Enrolment anaemia (Hb <11g/dL)	Total indirect	none specified		
Low ferritin levels (<30 µg/l)	Enrolment anaemia (Hb <11g/dL)	Direct	Not applicable		
Plasmodium infection at enrolment (by microscopy)	Low ferritin levels (<30 µg/l)	Total	-0.419	(-0.584, -0.313)	<0.001
Plasmodium infection at enrolment (by microscopy)	Low ferritin levels (<30 µg/l)	Total indirect	-0.086	(-0.131, -0.041)	<0.001
Plasmodium infection at enrolment (by microscopy)	Low ferritin levels (<30 µg/l)	Direct	-0.333	(-0.517, -0.209)	<0.001
High CRP levels (≥5 mg/L)	Low ferritin levels (<30 µg/l)	Total	-0.185	(-0.307, -0.077)	0.002
High CRP levels (≥5 mg/L)	Low ferritin levels (<30 µg/l)	Total indirect	0.004	(-0.006, 0.017)	0.549
High CRP levels (≥5 mg/L)	Low ferritin levels (<30 µg/l)	Direct	-0.188	(-0.310, -0.080)	0.001
High AGP levels (≥ 360 mg/L)	Low ferritin levels (<30 µg/l)	Total	-0.073	(-0.167, 0.019)	0.119
High AGP levels (≥ 360 mg/L)	Low ferritin levels (<30 µg/l)	Total indirect	-0.01	(-0.025, 0.000)	0.122
High AGP levels (≥ 360 mg/L)	Low ferritin levels (<30 µg/l)	Direct	-0.063	(-0.157, 0.031)	0.183
IPTp with azithromycin & sulphadoxine pyrimethamine	Haemoglobin level during study (g/dL)	Total	0.023	(-0.068, 0.121)	0.646
IPTp with azithromycin & sulphadoxine pyrimethamine	Haemoglobin level during study (g/dL)	Total indirect	0.005	(-0.031, 0.048)	0.798
IPTp with azithromycin & sulphadoxine pyrimethamine	Haemoglobin level during study (g/dL)	Direct	0.018	(-0.080, 0.123)	0.734
Low ferritin levels (<30 µg/l)	Haemoglobin level during study (g/dL)	Total	-0.064	(-0.145, 0.009)	0.1
Low ferritin levels (<30 µg/l)	Haemoglobin level during study (g/dL)	Total indirect	0.002	(-0.011, 0.042)	0.851
Low ferritin levels (<30 µg/l)	Haemoglobin level during study (g/dL)	Direct	-0.066	(-0.161, 0.007)	0.115
Plasmodium infection at enrolment (by microscopy)	Haemoglobin level during study (g/dL)	Total	0.057	(0.019, 0.103)	0.007
Plasmodium infection at enrolment (by microscopy)	Haemoglobin level during study (g/dL)	Total indirect	0.057	(0.019, 0.103)	0.007
High CRP levels (≥5 mg/L)	Haemoglobin level during study (g/dL)	Total	0.122	(0.034, 0.211)	0.007
High CRP levels (≥5 mg/L)	Haemoglobin level during study (g/dL)	Total indirect	0.014	(-0.001, 0.035)	0.124
High CRP levels (≥5 mg/L)	Haemoglobin level during study (g/dL)	Direct	0.108	(0.012, 0.203)	0.026
High AGP levels (≥ 360 mg/L)	Haemoglobin level during study (g/dL)	Total	-0.086	(-0.162, -0.010)	0.033
High AGP levels (≥ 360 mg/L)	Haemoglobin level during study (g/dL)	Total indirect	-0.001	(-0.014, 0.012)	0.886

High AGP levels (≥ 360 mg/L)	Haemoglobin level during study (g/dL)	Direct	-0.085	(-0.167, -0.005)	0.04
Low MUAC (<23 cm)	Haemoglobin level during study (g/dL)	Total	0.048	(-0.021, 0.118)	0.17
Low MUAC (<23 cm)	Haemoglobin level during study (g/dL)	Total indirect	-0.006	(-0.018, 0.001)	0.218
Low MUAC (<23 cm)	Haemoglobin level during study (g/dL)	Direct	0.054	(-0.017, 0.124)	0.125
Plasmodium infection at enrolment (by microscopy)	High sEng levels (≥ 28.3 pg/ml)	Total	0.113	(-0.008, 0.225)	0.063
Plasmodium infection at enrolment (by microscopy)	High sEng levels (≥ 28.3 pg/ml)	Total indirect	-0.039	(-0.127, 0.037)	0.336
Plasmodium infection at enrolment (by microscopy)	High sEng levels (≥ 28.3 pg/ml)	Direct	0.152	(-0.015, 0.320)	0.079
Low MUAC (<23 cm)	High sEng levels (≥ 28.3 pg/ml)	Total	-0.018	(-0.103, 0.070)	0.69
Low MUAC (<23 cm)	High sEng levels (≥ 28.3 pg/ml)	Total indirect	0.006	(-0.007, 0.023)	0.43
Low MUAC (<23 cm)	High sEng levels (≥ 28.3 pg/ml)	Direct	-0.024	(-0.110, 0.065)	0.604
Low ferritin levels (<30 µg/l)	High sEng levels (≥ 28.3 pg/ml)	Total	0.063	(-0.048, 0.183)	0.285
Low ferritin levels (<30 µg/l)	High sEng levels (≥ 28.3 pg/ml)	Total indirect	-0.001	(-0.029, 0.027)	0.951
Low ferritin levels (<30 µg/l)	High sEng levels (≥ 28.3 pg/ml)	Direct	0.064	(-0.058, 0.199)	0.313
High CRP levels (≥5 mg/L)	High sEng levels (≥ 28.3 pg/ml)	Total	-0.06	(-0.178, 0.061)	0.329
High CRP levels (≥5 mg/L)	High sEng levels (≥ 28.3 pg/ml)	Total indirect	-0.013	(-0.039, 0.013)	0.344
High CRP levels (≥5 mg/L)	High sEng levels (≥ 28.3 pg/ml)	Direct	-0.047	(-0.166, 0.079)	0.447
High AGP levels (≥ 360 mg/L)	High sEng levels (≥ 28.3 pg/ml)	Total	0.048	(-0.045, 0.146)	0.322
High AGP levels (≥ 360 mg/L)	High sEng levels (≥ 28.3 pg/ml)	Total indirect	-0.002	(-0.019, 0.013)	0.79
High AGP levels (≥ 360 mg/L)	High sEng levels (≥ 28.3 pg/ml)	Direct	0.05	(-0.047, 0.152)	0.314
Plasmodium infection at enrolment (by microscopy)	High sFlt-1: PlGF ratio (≥ 38)	Total	0.01	(-0.106, 0.134)	0.865
Plasmodium infection at enrolment (by microscopy)	High sFlt-1: PlGF ratio (≥ 38)	Total indirect	0.018	(-0.081, 0.101)	0.679
Plasmodium infection at enrolment (by microscopy)	High sFlt-1: PlGF ratio (≥ 38)	Direct	-0.008	(-0.200, 0.182)	0.931
Low MUAC (<23 cm)	High sFlt-1: PlGF ratio (≥ 38)	Total	0.098	(0.009, 0.188)	0.03
Low MUAC (<23 cm)	High sFlt-1: PlGF ratio (≥ 38)	Total indirect	-0.02	(-0.042, -0.003)	0.056
Low MUAC (<23 cm)	High sFlt-1: PlGF ratio (≥ 38)	Direct	0.118	(0.026, 0.214)	0.012
Low ferritin levels (<30 µg/l)	High sFlt-1: PlGF ratio (≥ 38)	Total	-0.141	(-0.247, -0.029)	0.011
Low ferritin levels (<30 µg/l)	High sFlt-1: PlGF ratio (≥ 38)	Total indirect	-0.027	(-0.067, -0.003)	0.097

Low ferritin levels (<30 µg/l)	High sFlt-1: PlGF ratio (≥ 38)	Direct	-0.114	(-0.233, 0.005)	0.056
High CRP levels (≥5 mg/L)	High sFlt-1: PlGF ratio (≥ 38)	Total	-0.005	(-0.124, 0.120)	0.929
High CRP levels (≥5 mg/L)	High sFlt-1: PlGF ratio (≥ 38)	Total indirect	0.029	(-0.001, 0.066)	0.095
High CRP levels (≥5 mg/L)	High sFlt-1: PlGF ratio (≥ 38)	Direct	-0.034	(-0.159, 0.088)	0.578
High AGP levels (≥ 360 mg/L)	High sFlt-1: PlGF ratio (≥ 38)	Total	0.03	(-0.064, 0.123)	0.536
High AGP levels (≥ 360 mg/L)	High sFlt-1: PlGF ratio (≥ 38)	Total indirect	0.002	(-0.025, 0.025)	0.903
High AGP levels (≥ 360 mg/L)	High sFlt-1: PlGF ratio (≥ 38)	Direct	0.028	(-0.065, 0.130)	0.571

Abbreviations: CI_{95} = 95% confidence interval; Hb = haemoglobin; MUAC = mid upper arm circumference; CRP = C-reactive protein; AGP = ∝-1-acid-glycoprotein; IPTp=Intermittent Preventive Treatment in pregnancy; sEng = soluble endoglins; sFlt-1=soluble fms-like tyrosine kinase-1; PlGF=Placental Growth Factor.

3.4 Discussion

Low birthweight is perhaps the most important determinant of childhood morbidities and mortality and a significant contributor to growth faltering in early childhood and adverse health, developmental, social and economic outcomes throughout life (Black, Allen et al. 2008, Victora, Adair et al. 2008, Christian, Lee et al. 2013, Katz, Lee et al. 2013, Lawn, Blencowe et al. 2014). The causes of LBW are multifactorial and inter-related hence presenting a major challenge for prevention of LBW, for clinical management of infants born with LBW, and for the use of public health interventions to address the associated factors. Teasing apart these inter-relationships between factors and determining and quantifying the magnitude of each factor's direct and indirect effects is greatly needed to inform policies and strategies targeted at improving birth weight.

We undertook this analysis to determine the direct and indirect effects of selected modifiable risk factors that are important in the PNG setting including maternal nutrition conditions (namely iron deficiency, anaemia, and maternal undernutrition (indicated by low MUAC)), malaria infections, inflammation and IPTp on birthweight and LBW. In this cohort of 2021 PNG pregnant women, iron deficiency, low MUAC and IPTp were significantly associated with birthweight and were observed to be direct predictors, although this relationship was less pronounced for iron deficiency. Correspondingly, IPTp and maternal undernutrition but not iron deficiency also directly predicted LBW. Anaemia and high CRP levels at enrolment were associated with LBW but not birthweight and while anaemia was observed to be a direct predictor of LBW, we did not establish whether CRP had direct or indirect effects on LBW in our model. No statistically significant associations were established for malaria infections from our models. We did not establish any indirect effects for any of these selected factors in our models.

In the original analysis of this dataset (Unger, Ome-Kaius et al. 2015), administering three courses (1 g twice daily for 2 days) of SPAZ to prevent malaria was observed to reduce LBW by about 30% in spite of the low prevalence of malaria observed in the study. This indicated that IPTp may be operating through mechanisms other than malaria to improve birthweight hence reducing the risk of LBW. The notion of IPTp operating through other mechanisms was shown in a follow up analysis of this same data investigating the impact of IPTp on inflammation and angiogenesis in influencing birth outcomes (Unger, Hansa et al. 2019). We

showed in that analysis that women receiving IPTp intervention had lower levels of inflammatory biomarkers at delivery which may indicate that IPTp prevents LBW by reducing inflammation. Apart from its antimalarial properties, azithromycin is a broad-spectrum antibiotic that can treat a wide range of bacterial infections such as STIs and genital infections during pregnancy, infections which are known to be associated with adverse birth outcomes (Mullick, Watson-Jones et al. 2005, Chico, Hack et al. 2013, Romero, Dey et al. 2014). It may also have anti-inflammatory properties (Ratjen, Saiman et al. 2012). In this analysis, we observed IPTp to have a direct impact on birthweight and risk of LBW independent of its effects on malaria infections detected during the study and the haemoglobin change. The lack of data regarding the sexually transmitted and genital infections did not allow us to explore whether IPTp could be influencing birthweight and LBW through effects on these infections. The mechanism underlying the effect of IPTp on birthweight and risk of LBW observed in original analysis therefore remains to be determined. Given this analysis was focussed on factors that are measurable before delivery so they can be intervened at prior to measuring birth outcomes at delivery, we did not explore the effects of IPTp on placental malaria, anaemia and inflammation at delivery. Gestational weight gain was proposed from one of our analysis (Unger, Wangnapi et al. 2016) as a possible mechanism through which IPTp may influence birthweight however, due to limited data points, we did not investigate this mechanism.

Nutrition has long been recognised to play a major role in maternal and child health, and optimal nutrition in the periconception period and during pregnancy is considered key to growing a healthy baby in utero and hence giving the child a healthy start to life (Barker, Gluckman et al. 1993, Black, Allen et al. 2008, Abu-Saad and Fraser 2010, Black, Victora et al. 2013, Young, Nguyen et al. 2015). In developing countries such as PNG, optimal nutrition which includes consistent intake of nutritious diet with adequate amounts of nutrients needed to meet maternal and foetal needs is a major challenge, primarily due to the underlying poverty. Nutritional deficiencies, often involving multiple rather than single nutrients, are therefore highly prevalent in these settings and are considered major contributors to the high burden of LBW seen (Kramer 1987, Fall, Yajnik et al. 2003, Black, Allen et al. 2008, Abu-Saad and Fraser 2010, Ashorn, Hallamaa et al. 2018, Abera, Ejara et al. 2019). Inadequate nutrition may result in IUGR and or preterm labour, and result in LBW through its impact on the growth and development of placenta, mother-to-foetal nutrient transfer and maternal as well as foetal metabolic and endocrine status (Harding 2001, Fall, Yajnik et al. 2003, Wu, Bazar et al. 2004, Belkacemi, Nelson et al. 2010, Wu, Imhoff-Kunsch et al. 2012).

In this study, low MUAC, which is indicative of maternal undernutrition, strongly predicts decreased birthweight and LBW via mechanisms independent of its effects through anaemia, iron deficiency, angiogenesis and the change in haemoglobin during the study. This has also been demonstrated elsewhere, in studies where maternal nutritional status was a direct predictor of newborn size independent of its effects on placental weight and other factors (Ashorn, Hallamaa et al. 2018). In the same study, the authors indicated that nutrition, inflammation, infections and other constitutional factors acted both directly and indirectly through each other to jointly influence size at birth; providing evidence that interventions addressing multiple exposures may be an appropriate strategy in improving birthweight. We did not establish significant indirect associations between maternal undernutrition and risk of LBW, particularly effects operating through anaemia and/or iron deficiency, both of which are very common nutritional problems faced by pregnant women and which have been the focus of many nutrition interventions over the years (Merialdi, Carroli et al. 2003, WHO 2008, Allen, Peerson et al. 2009, Stevens, Finucane et al. 2013, Bhutta, Das et al. 2013a, da Silva Lopes, Ota et al. 2017).

Anaemia during pregnancy is generally considered a risk factor for LBW and other adverse birth outcomes (Levy, Fraser et al. 2005, Rahman, Abe et al. 2016, Figueiredo, Gomes-Filho et al. 2018, Jung, Rahman et al. 2019). However, in our analysis, we observed anaemia at enrolment (which occurred mostly during the second trimester) to be a direct predictor of reduced odds of LBW. We cannot accurately explain this observation, but it is possible that majority of the cases defined in our study as anaemia are of haemoglobin levels that may be considered optimal to achieve better outcomes (Steer, Alam et al. 1995, Steer 2000, Dewey and Oaks 2017). In a large study conducted in United Kingdom among women across different racial ethnicities, the authors observed a maximum mean birthweight among women with haemoglobin levels ranging from 85-95 g/l; and lowest incidence of LBW at haemoglobin levels 95-105 g/L (Steer, Alam et al. 1995). Given we defined our anaemia as haemoglobin <11 g/dL as per the WHO definitions (WHO Vitamin and Mineral Nutrition Information System (VMNIS)), all the women in our study with these haemoglobin levels are defined as anaemic. This included 69% of women defined as having anaemia in the study. Only a very small proportion of the women (3%) were severely anaemia precluding analysis to investigate the association between severe anaemia and birthweight and LBW. It has been postulated that the relationship between the maternal haemoglobin levels and risk of adverse pregnancy

outcomes is U-shaped; i.e. risk of adverse birth outcomes increases as haemoglobin level approaches the extreme ends of haemoglobin levels (Steer, Alam et al. 1995, Dewey and Oaks 2017). Women with severe anaemia are thus more likely to have an adverse birth outcome than women with mild or moderate anaemia. In our study, severe anaemia was observed in 3% of the women while 54. 6% had moderate anaemia. The relationship between haemoglobin and adverse outcomes however is said to differ by trimester of pregnancy owing to the effect of haemodilution (from plasma volume expansion) (Garn, Ridella et al. 1981, Murphy, O'Riordan et al. 1986, Lu 1991, Steer, Alam et al. 1995, Steer 2000, Dewey and Oaks 2017). In the studies that reported an adverse association of anaemia on LBW, the adverse effects of haemoglobin were more marked if haemoglobin was measured in the first trimester while there are mixed reports of such associations in the second and third trimesters of pregnancy (Rasmussen and Oian 1993, Hamalainen, Hakkarainen et al. 2003, Dewey and Oaks 2017, Rahmati, Delpishe et al. 2017, Badfar, Shohani et al. 2019).

It is also possible that the protective effect of anaemia on LBW may be may be mediated through iron deficiency which is the most common cause of anaemia (Haider, Olofin et al. 2013, Stevens, Finucane et al. 2013). Recently, a study conducted in PNG showed iron deficiency to be associated with reduced odds of LBW which the authors proposed to be through malaria-independent protective mechanisms (Fowkes, Moore et al. 2018). This association was subsequently investigated by Verhoef and group who propose that the protective effect of iron deficiency on LBW may be due to the improvement in the iron status of women from the iron supplementation they received, particularly the women who were initially iron deficient (Verhoef, Mwangi et al. 2019). This is consistent with findings from another study that followed women with iron deficiency and found iron supplementation to increase birthweight (Mwangi, Roth et al. 2015). In our study, two-thirds of the women were iron deficient at enrolment and iron deficiency was associated with an increase in mean birthweight while no associations were observed when we examined the relationship between iron deficiency and LBW. Haemoglobin is often used as a proxy for iron deficiency and in PNG, iron supplementation is often indicated for anaemic women, although the cut-offs used often vary and thus not all women may receive iron supplementation. Based on the high prevalence (69%) of anaemia we observed in this study, it may be possible that the positive effects of anaemia as well iron deficiency on birthweight we observe is due to iron supplementation which many of these women may have received for their anaemia during the antenatal care visits.

Malaria in pregnancy has long being recognised as an important risk factor for LBW. When we explored the relationship between malaria infections at enrolment and birthweight and risk of LBW through interactions with anaemia and iron deficiency, we did not observe any significant direct or indirect associations. Considering very little malaria infection was observed in trial participants (6.7% at enrolment), it is likely that we did not have sufficient statistical power to detect an association between episodes of parasitaemia and birthweight or LBW. Although malaria infection was associated with increased risk of anaemia, consistent with literature (Brabin and Piper 1997, Allen, Raiko et al. 1998, Ouedraogo, Koura et al. 2012), this did not appear to have an impact on birthweight nor risk of LBW. An inverse association was observed between presence of parasitaemia and iron deficiency such that the odds of being iron deficient was reduced if women had malaria infection.

We also investigated the interaction between malaria, inflammation and angiogenesis on birth outcomes. Malaria induced inflammation had been shown to be associated with adverse birth outcomes (Fried, Muga et al. 1998, Rogerson, Hviid et al. 2007, Umbers, Boeuf et al. 2011a, Unger, Hansa et al. 2019). Inflammation during pregnancy, whether sterile or due to other infections, has been linked to IUGR; to pre-eclampsia (which is an important cause of preterm delivery (Ernst, de Jonge et al. 2011) (Karinen, Pouta et al. 2005, Rebelo, Schlussel et al. 2013)); and has also been linked to impaired placental angiogenesis (Fiedler and Augustin 2006, Conroy, Silver et al. 2013). We did not observe any significant associations between malaria and LBW through its effect on inflammation or angiogenesis nor did we observe associations between inflammation and LBW through angiogenesis. Nevertheless, malaria has consistently been demonstrated in other studies to be common and significant risk factor for LBW (Desai, ter Kuile et al. 2007, Umbers, Aitken et al. 2011b, Rijken, McGready et al. 2012) and therefore prevention of malaria in pregnancy must remain a priority in PNG and globally.

The study has several limitations. Firstly, it was not designed for the purpose of this analysis and there are key risk factors such as STIs, chorioamnionitis and other nutrient deficiencies such as folate deficiency that could not be evaluated in the model. Restricting our analysis to only the selected risk factors that are measurable and intervenable during pregnancy precluded us from investigating key relationships such as the effect of IPTp on placental malaria and inflammation and their influence on birth outcomes. There is also the chance of bias in our results, particularly of the associations between anaemia and iron deficiency and the risk of

LBW considering we did not adjust for gestational age in our models. However, we don't anticipate significant differences in results considering we did not observe any difference in our standard multivariable regression models when we adjusted for gestational age.

3.5 Conclusion

This analysis did not reveal any joint or indirect effects of the selected factors on birthweight and risk of LBW that could guide future strategies that may adopt the concept of interventions that can concurrently address multiple exposures. Nevertheless, this analysis provides insight into the direct and indirect effects of some of the common risk factors of LBW that we normally would not gain from standard regression models. We show that low MUAC, indicative of maternal undernutrition is a key predictor of reduced birthweight and increased odds of LBW highlighting the value of such simple to measure indicator that could be routinely measured and used to identify women at risk of adverse birth outcomes allowing for timely interventions. We have also observed that IPTp with SPAZ improves birth outcomes by mechanisms independent of the currently measured risk factors. A further interesting observation is the apparent positive influence of anaemia and iron deficiency on birthweight, two of the conditions generally considered risk factors for adverse birth outcomes. These findings raise additional questions as to what haemoglobin level may be considered optimal and at what level of haemoglobin can we consider a mother as anaemic hence the need for interventions such as iron supplementation.

4 Chapter 4: Investigating the association between episodes of acute malaria, pneumonia and diarrhoeal illness and early child growth in an intervention trial of malaria prevention in Papua New Guinea infants.

4.1 Introduction

Childhood undernutrition as a result of infectious diseases and inadequate dietary intake is an important cause of morbidity and mortality during early childhood (Black, Allen et al. 2008, Black, Cousens et al. 2010, Olofin, McDonald et al. 2013, G. B. D. Mortality and Causes of Death Collaborators 2016) and is associated with long-term adverse health, developmental, social and economic outcomes later in life and has intergenerational influences (Grantham-McGregor, Cheung et al. 2007, Black, Allen et al. 2008, Shonkoff, Garner et al. 2012, Hoddinott, Behrman et al. 2013). It is indicated by deficits in WHO child growth indices (WHO Multicentre Growth Reference Study Group) and comprises low birthweight, underweight, stunting, wasting and deficiencies of essential micronutrients.

As in many developing countries elsewhere (Shrimpton, Victora et al. 2001, Victora, de Onis et al. 2010), children in Papua New Guinea experience significant growth faltering in the first 1000 days of their lives (Mueller and Smith 1999, Mueller, Vounatsou et al. 2001). From the 2011 estimates, 49.5% of the children aged under 5 years were stunted, 14% and 28% were wasted and underweight, respectively (WHO). In 2018, severe malnutrition (i.e. severe wasting and/or MUAC ≤ 115 mm and/or bilateral pitting oedema) was among the five most common problems seen in the hospitals and contributed either directly or indirectly to about 18.7% of all deaths (PNG Paediatric Society and PNG Department of Health 2018). Furthermore, modelling estimates that childhood undernutrition would have cost the country over $USD 500 million in the financial year 2015-2016 through loss of economic productivity of the human workforce and increased health care expenditure in treating associated diseases (Save the Children). Reducing the burden of childhood undernutrition in PNG is therefore a very urgent priority and as such, it is imperative to understand the common risk factors and how best to intervene.

Childhood undernutrition as a result of inadequate dietary intake is well documented (Bailey and Whiteman 1963, A. P. C. Oomen 1971, Ferro-Luzzi, Norgan et al. 1975). Inadequate dietary intake may result from consistently insufficient consumption of food to meet energy requirements, low nutrient quality of consumed food, or inadequate absorption of nutrients. In PNG, there is evidence although that the quantity of food consumed may be adequate but the quality is not, with food consumed often lacking essential nutrients such as protein and zinc that are needed, particularly for linear growth (A. P. C. Oomen 1971, Gibson, Heywood et al. 1991, Mueller and Smith 1999, Mueller, Vounatsou et al. 2001). Infectious diseases are also

highly prevalent in PNG, with a high burden of morbidity experienced by infants and children (Genton, D'Acremont et al. 2008, Manning, Laman et al. 2011, Laman, Manning et al. 2012, Paediatric Society of Papua New Guinea 2017). Their contribution to stunting, wasting and underweight is less appreciated due to the paucity of data in the country.

Infectious diseases account for over two-thirds of death among children under 5 years of age globally (Black, Cousens et al. 2010, Walker, Rudan et al. 2013, G. B. D. Mortality and Causes of Death Collaborators 2016). The leading causes include diarrhoea, pneumonia and malaria (Black, Cousens et al. 2010, Walker, Rudan et al. 2013, G. B. D. Mortality and Causes of Death Collaborators 2016). In PNG, these three are among the top 5 common reasons for hospital admissions (PNG Paediatric Society and PNG Department of Health 2018). Although the overall burden of malaria has significantly declined due to the scale-up of the national malaria control program (Hetzel, Pulford et al. 2017), malaria remains an important cause of childhood morbidity and mortality (Genton, D'Acremont et al. 2008, Manning, Laman et al. 2011, Paediatric Society of Papua New Guinea 2017). The rates of pneumonia are particularly high in the highlands region of PNG (Greenhill, Pomat et al. 2010, Pomat, Greenhill et al. 2010, Riley 2010). Of importance is the introduction of the conjugate *Haemophilus influenza* type b vaccine in 2008 and the 13-valent *Streptococcus pneumoniae* vaccine (13vPCV) in 2014 to prevent the two most common causes of pneumonia and meningitis in the country, *Streptococcus pneumoniae* and *Haemophilus influenzae* (Witt, Montgomery et al. 1990, Lehmann 1992). In spite of these achievements, pneumonia remains the most common cause of admission among young children (PNG Paediatric Society and PNG Department of Health 2018). Diarrhoeal illness is the most common and widespread amongst the three. It is a manifestation of intestinal bacterial, viral or parasitic infections, often as a result of limited access to clean drinking water, poor hygiene and sanitation (PNG Department of National Planning and Monitoring , Bukenya and Nwokolo 1991).

The relationship between nutrition and infection has long been recognized to be bi-directional, often synergistic and in some instances antagonistic (Scrimshaw, Taylor et al. 1959, Scrimshaw, Taylor et al. 1968). It is well recognised that undernutrition increases vulnerability to infections likely due to reduced immunity and lack of essential micronutrients to protect against infections (Keusch 2003, Bhutta 2006, Chandra 2007). Moreover, severity of illness and risk of death due to infectious diseases are heightened when undernutrition is an underlying condition (Smith 1991, Caulfield, de Onis et al. 2004, Olofin, McDonald et al. 2013). The

notion that infectious diseases may be a cause of undernutrition has often been debated (Briend 1990, Briend, Hasan et al. 1990) and an area of active research (Scrimshaw, Taylor et al. 1968, Mata, Urrutia et al. 1972, Black 1991, Katona and Katona-Apte 2008). During an infection, the associated immune response and complex systemic physiological and metabolic adjustments occur leading to decreased nutrient intake due to anorexia, increased metabolic nitrogen and nutrient losses, malabsorption of nutrients (intestinal infections), internal diversion of nutrients for metabolic and immune responses and increased metabolism (in the presence of fever) (Scrimshaw 2003, Schaible and Kaufmann 2007, Katona and Katona-Apte 2008).

Associations of common childhood diseases such as diarrhoea malaria, acute respiratory infections and measles with childhood growth have been demonstrated. Likely due to its high prevalence and its tight link with feeding practices in young children in developing countries, diarrhoea is the most common infectious disease that has been used to investigate this relationship. Consequently, a vast amount of existing evidence examines whether and how diarrhoea may negatively impact growth (Black 1991, Checkley, Epstein et al. 2003, Checkley, Buckley et al. 2008). There have also been growing evidence of the association of malaria with undernutrition (Marsden 1964, Cole and Parkin 1977, Rowland, Cole et al. 1977, Sharp and Harvey 1980, Williams, Maitland et al. 1997, Nyakeriga, Troye-Blomberg et al. 2004, Lee, Yori et al. 2012, Kang, Kreuels et al. 2013, Alexandre, Benzecry et al. 2015, Gari, Loha et al. 2018) with one study establishing a causal relationship between the two (Kang, Kreuels et al. 2013). Some of the reported associations of malaria and undernutrition were drawn from studies with small sample sizes (Marsden 1964, Cole and Parkin 1977, Rowland, Cole et al. 1977) often lacking data to fully adjust for confounding or in areas of low malaria transmission (Cole and Parkin 1977, Sharp and Harvey 1980) which in Cole et al, malaria's contribution to undernutrition was reported to be only 1% (Cole and Parkin 1977). Evidence on adverse effects on growth from pneumonia and respiratory infections are inconsistent (Black 1991), with some studies observing an adverse effect (Rowland, Rowland et al. 1988, Moffat 2003, Batiro, Demissie et al. 2017) while some do not (Black, Brown et al. 1984, Smith 1991, Assis, Barreto et al. 2005). Data on the associations of measles with undernutrition is limited (Cole and Parkin 1977, Ahmed, Ahmed et al. 2012, Rahman, Rahman et al. 2019). In one study that reported an association between measles and undernutrition, measles was very rare in the study population (Cole and Parkin 1977) and observation association may have been biased by other factors.

Generating evidence that will improve our understanding of the association between acute episodes of infectious diseases such as diarrhoea, pneumonia and malaria with childhood undernutrition (i.e. stunting, wasting and underweight) is essential to inform the development of interventions, programs and guidelines that are necessary to address the burden and adverse outcomes of undernutrition among young children. Using a large dataset of morbidity and anthropometric data from a clinical trial of infants in the coastal and lowlands of PNG (Senn, Rarau et al. 2012), we investigated the association between acute malaria, pneumonia and diarrhoeal illness and stunting, wasting and underweight.

4.2 Methodology

4.2.1 Data collection

This study analyses data from a large infant intervention trial (Clinicaltrials.gov, NCT00285662) conducted in PNG. The details of the study are summarised in chapter 2 and detailed methodology is published elsewhere (Senn, Rarau et al. 2012).

4.2.2 Enrolment and active follow-up

At enrolment, the child's demographic information was recorded, were clinically assessed for presence of illnesses, their immunization status was reviewed, and appropriate vaccinations were given. The child's weight was measured using hanging scales. Blood was collected for malaria determination by light microscopy and PCR and haemoglobin levels were measured using portable HemoCue 201 machine (Angelholm, Sweden).

Following enrolment, children were actively followed up every three months for another eight visit time points. At each visit, the child's weight was measured and was clinically examined for presence of any illness. Their vaccination schedules were also reviewed, and appropriate vaccinations were given. Blood samples were collected to evaluate for presence of malaria parasites by LM or PCR. At the 5th and 9th visit time points, the child's haemoglobin level was measured using the portable HemoCue 201 machine (Angelholm, Sweden) and their heights measured using locally made portable height measuring board. Details of the study schedule and procedures are illustrated in Figure 4.1.

4.2.3 Morbidity surveillance

To establish a continuous morbidity surveillance of the child throughout the study, children were queried and examined for presence of illness during all routine 3-monthly study visits including enrolment (Figure 4.1). Passive morbidity surveillance was also maintained at the health centres and the aid posts.

Upon presentation with complaints of illness, children were examined by study staff using a standard questionnaire of signs and symptoms including danger signs as per the integrated management of childhood illness (IMCI) guidelines. Children with a febrile illness, defined as a history of fever in the preceding 2 days and/or an axillary temperature ≥37.5^{0}C were tested for malaria by RDT (ICT Combo, South Africa). Artemether-lumefantrine (Coartem®, Novartis, Switzerland) was prescribed to those parasitologically confirmed for malaria, irrespective of the species. Haemoglobin was measured using a portable Hemocue 201 machine (Angelholm, Sweden) and children with moderate to severe anaemia (haemoglobin <8g/dL) were supplied iron supplementation at the dose of 5 mg/kg/day of ferrous sulphate for 6 weeks. All other illnesses including pneumonia and diarrhoeal illness were treated according to IMCI-based PNG Standard Treatment for Childhood Illness guidelines (PNG).

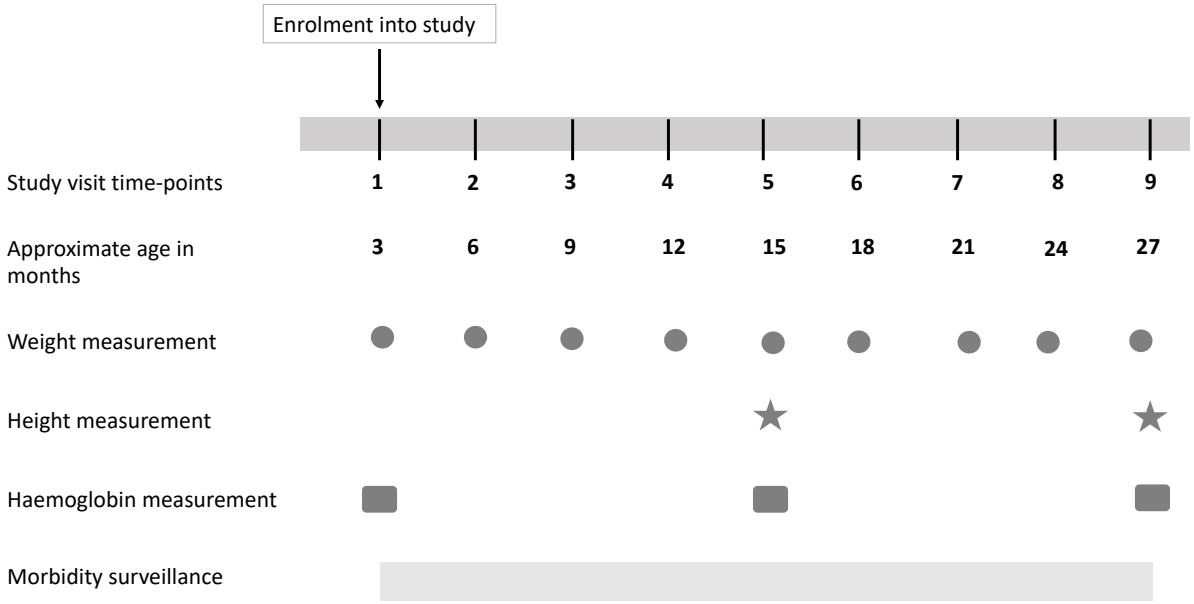

Figure 4-1: Study timeline showing the study contact time points when weight and height as well the morbidity data were collected.

4.2.4 Data for current analysis

Morbidity data was collated from the records of acute illness episodes captured during morbidity surveillance over the study period. The recorded signs and symptoms were used to define acute malaria, pneumonia and diarrhoeal illness using WHO definitions (WHO 2018). An acute malaria episode was defined as history of febrile illness in the past 2 days and/or axillary temperature ≥37.5^{0}C in the presence of a RDT or microscopically detectable infection of any species and density. Presence of diarrhoea was defined as an acute episode of diarrhoea. Episodes with diarrhoea lasting more than 14 days were not included in this definition. The definition for pneumonia was the presence of cough or difficulty breathing and fast breathing or chest indrawing.

Anthropometric data comprises weight and height, estimated in kilograms (kg) and centimetres (cm), respectively. Weight-for-age, length- and height-for-age and weight-for-height and weight-for-length Z-scores were calculated using WHO Child Growth Standards igrowup package implemented via Stata 15.0 software (StataCorp, USA) (WHO). The weight-for-age Z-score <-6 and >5 (n=1), height-for-age Z-score <-6 and >6 (n=33) and weight-for-height Z-score <-5 & >5 (n=7) were considered as extreme biological implausible values and were not included in the analysis. Underweight was defined as weight-for-age Z-score less than -2 standard deviations below WHO growth standards median and severe underweight if weight-for-age Z-score was less than -3 standard deviations. Stunting was defined as height-for-age Z-score or length-for-age Z-score more than two standard deviations below the WHO growth standard median of the same age and sex and severe if more than three standard deviations below the standard. Wasting and severe wasting were defined as weight-for-height Z-score / weight-for-length Z-score less than -2 and -3 standard deviations below WHO growth standards median, respectively (WHO Multicentre Growth Reference Study Group).

4.2.5 Statistical Methods

Descriptive statistics were derived from 2x2 crosstabulation of binary variables and expressed as frequencies or proportions/percentages. For continuous variables, the mean and standard deviation were calculated if the variable was normally distributed, otherwise the median and range were calculated. The population averaged prevalence of underweight, stunting and wasting for the entire study period was estimated using generalized estimating equations (GEE) with a logit link and an exchangeable working correlation matrix. The average prevalence for

underweight was calculated using all the weight measurements collected at the 3-monthly visit time points for the 1511 children while 2 height measurements from 1411 children for stunting and again 2 height and corresponding weight measurements for 1410 for wasting.

4.2.6 Analysis of the association between acute malaria, pneumonia and diarrhoeal illness and weight

A two-staged analysis plan was used to examine the effects of acute malaria, pneumonia and diarrhoeal illness on weight. The first stage of analysis investigated the effects of these illnesses occurring within each of the routine 3-monthly intervals on the child's weight and their risk of being underweight. Given the longitudinal nature of the study with repeated measures of weight and illness episodes, we used GEE with a logit link for the binary outcome variable (underweight) and identity link for the continuous outcome variable (weight and weight-for-age Z-score), all with an exchangeable working correlation matrix; to account for the dependency between observations from the same child. Robust standard errors were also used to correct for working correlation matrix misspecification.

Exploratory and descriptive analysis revealed that within each 3-monthly interval, a child experienced at most only one episode of each of the three acute illnesses; only a small number of children experienced two episodes within an interval (malaria: n=96 (6.3%); pneumonia: n=62 (4.1%); diarrhoea: n=35 (2.3%)). As such, for each morbidity, the number of morbidities within each 3-month interval was collapsed into a binary variable: 0 if the morbidity did not occur within a particular 3-month interval and 1 if one or more morbidity episodes occurred in an interval. The potential effect of the timing of the three acute illnesses on weight and risk of underweight was explored by interacting the acute illness variables with study visits and Wald test was used to assess the statistical significance of the interaction term.

In the second stage of this analysis, the objective was to determine if these acute illnesses had delayed effects on weight. In other words, that their effect on weight may not be limited to the period of acute illness but may in fact manifest much later. This was an exploratory analysis considering this study was not designed to estimate delayed morbidity effects. The relationship between weight and a morbidity was investigated using distributed lag models (DLM), which assume the outcome (weight) can be explained in terms of contributions from multiple exposure (morbidity) events in the past (Gasparrini, Armstrong et al. 2010, Gasparrini 2011).

The term lag is used to represent the period of time elapsed between measurement of the exposure and outcome. For each child in the study, a series of 12 lagged morbidity variables were constructed for each morbidity. The lagged morbidity variables record the number of episodes that occurred within 30-day intervals preceding a weight measurement, i.e. 0-30 (lag 1), 30-60 (lag 2), 60-90 (lag 3), …, 330-360 (lag 12) days before a weight measurement. Due to the low frequency of morbidity episodes at each lag, these variables were collapsed/transformed to binary lag variables, where 1 indicates the presence of one or more acute illness episodes at a lag and 0 indicates that no acute illness was observed at a lag. The DLM allows the 12 regression coefficients for each lagged morbidity variable to be modelled parsimoniously as a function of lag, which is represented as discrete values from 1 to 12. Note in this case these discrete values can be interpreted as months. A natural cubic spline with 3 knots placed at the quartiles of the discrete lag variable (3.75 (25^{th}), 6.50 (50^{th}) and 9.25 (75^{th}) months) was used to model this relationship.

As the maximum lag was 330-360 days prior to a weight measurement, only children with at least one weight measurement at or after 12 months in the study could be included in the analysis. We restricted this analysis to weight measurements that had complete illness history for the twelve lagged morbidity variables for all three illnesses. This was to ensure that the same participants were included in the analysis for each illness. A total of 508 children with 1927 weight measurements were included in this analysis. We additionally explored the impact of lagged morbidity variables, where the interval had been increased to 3-month (~90 day) intervals preceding a weight measurement, i.e. 0-90 (lag 1), 90-180 (lag 2), 180-270 (lag 3), 270-360 (lag 4) days before a weight measurement. These variables were included in an unconstrained DLM, where a regression coefficient is estimated for each lagged variable. The relationship between weight at or after 12 months in the study and time (days) was assumed to be linear for each child, and modelled by a linear mixed effects model, which allows the baseline weight and growth rate (change in weight over time) to vary between children. This was implemented via the dlnm and nlme packages in R (Gasparrini 2011). A detailed description of fitting a DLM in a mixed effects model framework was described in chapter 2.

4.2.7 Analysis of the impact the association between acute malaria, pneumonia and diarrhoeal illness and odds of stunting.

We employed a repeated measures analysis approach to assess the impact of malaria, pneumonia and diarrhoeal illness on the odds of stunting. We used GEEs with a logit link and an exchangeable working correlation matrix to account for the correlations between repeated measurements in each child. Robust standard errors were also used to correct for working correlation matrix misspecification.

Acute illness variables were included in the model as continuous variables representing the total number of each illness detected in the interval preceding the height measurement. Potential confounding by bednet use, haemoglobin level, study province, IPTi intervention and gender were accounted for. An interaction term between the binary illness variables and visit (either 5^{th} or 9^{th}) was included in the model to investigate whether the effect of an illness on odds of stunting depends on when the illness episode occurred. Wald test was used to test the statistical significance of the interaction term.

4.2.8 Analysis of the association between acute malaria, pneumonia and diarrhoeal illness and odds of wasting.

Weight-for-height measurements recorded at the 5^{th} visit (n=1188) were used for this analysis as this data provided a larger sample size than the data at 9^{th} visit time point. Wasting is generally considered to be an acute form of undernutrition and as such we investigated the effect of these acute illnesses occurring within a month of the measurements. The acute illness variables were binary with 1 denoting presence of the illness within the past 30 days and 0 for the illness not present.

The crude relationships between wasting and the acute illness episodes were explored using a chi-squared test and logistic regression. The latter was also used for multivariate analysis when haemoglobin level, study province, gender and IPTi intervention group were included.

All analyses were conducted using Stata 15.0 (StataCorp, USA) and R v3.4.0 (2017) (R Core Team, R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria). A p-value below the nominal level of significance of 0.05 was considered as a guide to determine if the observed differences were significant.

4.3 Results

Of the 1605 children that enrolled into the study, 54 were excluded post-hoc due to having had only enrolment visit done thus not sufficient data to include in the analysis. A sample size of 1551 children were included in the final cohort (Figure 4.2). Of these, 52% were males and 70% resided in Madang Province. Mean age, haemoglobin and weight at enrolment were 3.2 (0.5) months, 9.5 (0.1) g/dL and 5.8 (0.9) kg, respectively. The characteristics of the cohort are given in Table 4.1.

The population averaged prevalence of underweight, stunting and wasting in the study was 16.1% (CI_{95}: 15% - 18%), 52.9% (CI_{95}: 51% - 55%) and 6.1% (CI_{95}: 5% - 7%) respectively. A low prevalence of severe underweight (3%, CI_{95}: 3 – 4) and severe wasting (1%, CI_{95}: 1 – 2) was observed while the prevalence of severe stunting was 25% (CI_{95}: 23 – 27).

Table 4.1: Cohort characteristics

No. of children	**1551**
Male (%)	52
Age at enrolment in months (mean, SD)	3.2 (0.5)
Madang Province (%)	70
Haemoglobin level at enrolment (g/dL)	9.5 (1.0)
Total illness episodes recorded over the study period (n)	8902
Malaria	26
Pneumonia	33
Diarrhoeal illness	21
[a]Under nutrition (z-scores <-2) (%)	
Weight-for-age <-2 (underweight)	38
Height-for-age <-2 (stunting)	61
Weight-for-height <-2 (wasting)	17

4.3.1 Anthropometric data summary

A total of 11 263 weight measurements were collected from the 1551 children in this dataset, with over two-thirds (n=1067) of the children having 7-9 out of the 9 expected weight measurements over the study period. The number of weight measurements varied from 2-9 for

each child. At enrolment, the mean weight-for-age Z-score for all the children was -0.6 (1.1) with no difference in the means for female and male children (crude coef: 0.05, CI_{95} (-0.16 – 0.06), p=0.41; Figure 4.3A). Over the study period, there was a slight decrease in the mean weight-for-age Z-score with increasing age; the decline approaching the -2 cut-off for underweight but did not reach it by the end of the study period (Figure 4.3A). Female children had slightly higher mean weight-for-age Z-score than the males throughout the study period (Figure 4.3A). The proportion of the underweight children increased with increasing age, and at each time point when weight was measured a higher proportion was observed among male children (Figure 4.3B). Overall, 38.1% (n=591) of the child were underweight at least once during the study period.

Height was measured at least once in 1411 children with a total of 2024 height measurements recorded over the study period. Of this, 1198 were taken at the 5th visit while 826 were made at the 9th visit (Figure 4.2). Children who did not have any height data (n=140) were excluded from subsequent analysis of stunting. The mean height-for-age Z-score was -2.2 (1.5) and -2.0 (1.3) at the 5th and 9th visits, respectively. At the 5th visit when the children were 15 months of age, 49.1% (n=692) of the children were classified as too short for their age. Of these 692 children, 48.3% (n=334) were severely stunted. For the 826 children who had height measurements at the 9th visit time point (children aged 27 months), 47.1% (n=389) of them were stunted and 165 (42.4%) of these children were considered severely stunted. Male children in general appeared to be on average shorter than the female children (crude coef: -0.39, CI_{95} (-0.51 - -0.26), p<0.001). Overall, 61.1% (862/1411) of the children were found to be stunted at least once during the study period.

In addition, a subset of children had height measurements for both visit time points (n=613). Among these 613 children, 34.3% (n=210) of them were stunted at both the 5th visit time point when they were 15 months of age and again at the 9th visit time point when they were 27 months of age, a possible indication of failure to regain the lost linear growth. Of these 210 children, 24.3% were classified as severely stunted at both visit time points. There were also children who were stunted at the earlier time point (i.e. 5th visit) but were no longer stunted at the 9th visit (n=141, 23.0%), indicating that these children caught up to their expected linear growth rate at age 27 months.

A total of 2005 weight and corresponding height data (i.e. weight-for-height Z-score data) was available for 1410 children; 1, 188 measured at the 5th visit and 817 at the 9th visit time point. The mean weight-for-height Z-score was -0.05 (1.3) and -0.18 (1.2) at the 5th and 9th visit time points, respectively. At the 5th visit, 75 children (6.3%) were classified as too thin for their height and 17 (22.7%) of these 75 children were considered severely wasted. Similar proportions were observed at the 9th visit (wasted: 5.5% (n=45), severe wasting: 24.4% (n=11)). Correspondingly, wasting was quite rare in this cohort; 8.2% (n=115) of the children were classified as wasted at least once during the study.

Figure 4-2: Data flowchart.

Of the 1605 children that were initially enrolled into the study, 54 were excluded due to having had only one weight and no other measurements. The flow chart shows the number of measurements that were taken at each visit for both weight and height. Height was measured at two timepoints.

4.3.2 Morbidity data descriptive statistics

A total of 8902 illness episodes were recorded over the course of the study. A child experienced 7 (range: 1-28) illness episodes on average during the study. There were 67 children who did not report any illness episodes at all during the study. Of the 8902 episodes, 60.1% (n=5348) were due to either malaria and/or pneumonia and/or diarrhoeal illnesses. Malaria was diagnosed in 26% (n= 2326) of the illness episodes, pneumonia in 33% (n=2901) and 21% (n=1899) were due to diarrhoeal illnesses. Overall, 53%, 72% and 62% of the children

experienced an acute episode of malaria, pneumonia and diarrhoeal illness, respectively, at least once during the study. Amongst these children, each child experienced on average 3 episodes of malaria (range: 1 - 12), 3 episodes of pneumonia (range: 1 – 15) and 2 episodes of diarrhoeal illness (range: 1 – 11) over the study period.

Of the 5348 illness episodes that were due to any of the 3 illnesses of interest, a single diagnosis of malaria, pneumonia or diarrhoea was more common than co-morbidities (Figure 4.4). Specifically, a single diagnosis of malaria, pneumonia and diarrhoea was made in 30%, 24% and 17%, respectively (Figure 4.4). Two or more concomitant diagnoses occurred in 29% (n=1591) with only 196 episodes during which all 3 illnesses were diagnosed (Figure 4.4). The latter was observed in 158 children with 128 of them having had this only once (range: 1 – 4) during the study. Diagnosis of co-morbidity with malaria/pneumonia was made in 411 children, malaria/diarrhoeal illness in 220 children and pneumonia/diarrhoeal illness in 381 children. The majority of these children (70%, 81%, 73%, respectively) experienced these co-morbidities only once throughout the study period.

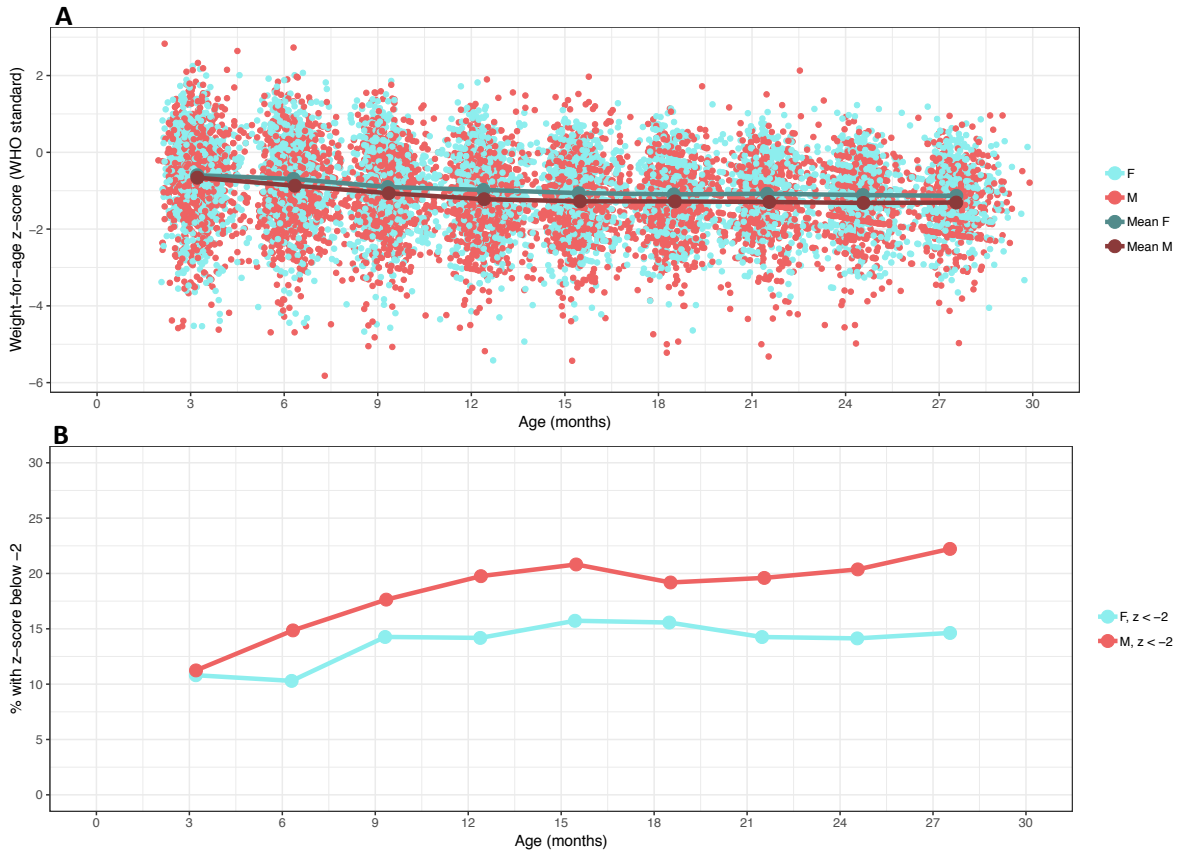

Figure 4-3: Plots showing A) the distribution of weight-for-age Z-score with the mean Z-scores across the ages and by sex in reference to the WHO growth standards median at each time point when weight was measured and B) the trend in the proportion of children (by sex) with weight-for-age Z-score <-2 (underweight) at the various ages at the time points when weight was measured.

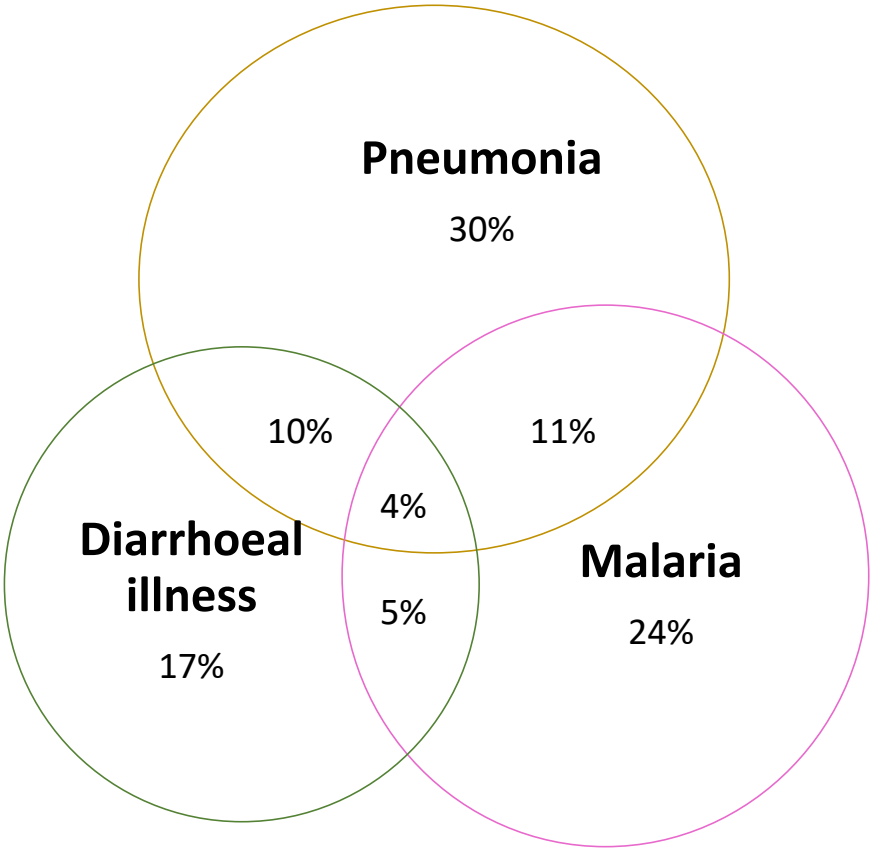

Figure 4-4: A venn diagram illustrating the proportion of acute malaria, pneumonia and diarrhoeal illness episodes and the proportion of co-morbidities. % are percentage out of the 5348 illness episodes that were due to either one of the three illnesses as a single morbidity or as co-morbidities.

4.3.3 Association of acute malaria, pneumonia or diarrhoeal illness with weight and odds of being underweight

Children who had one or more episodes of pneumonia or diarrhoeal illness within 3 months of their weight measurement were on average 110 ($p<0.001$) and 50 ($p<0.017$) grams lighter than those that did not have pneumonia or diarrhoeal illness respectively (Table 4.2). Consequently, the odds of being underweight was increased by 32% among children who had pneumonia (OR=1.32, $p<0.001$) and 15% for those who had diarrhoeal illness (OR=1.15, p=0.065) (Table 4.2). An association between acute malaria and weight-for-age Z-score was not observed (Coef: 0.003, p=0.878), nor was there any association between acute malaria and odds of underweight (OR=1.0, p=0.984) within 3 months of its occurrence (Table 4.2). Potential confounding effects of haemoglobin, age, gender, IPTi intervention and the study province were adjusted for in the models (Table 4.2).

While some of the effects of these acute illnesses on child health and growth are immediate, some may be delayed and manifest much later than the time of their occurrence. With the DLM that allowed modelling of these lag or delayed effects, we show that approximately 6-9 months following an acute episode of malaria, children were observed to be underweight for their age compared to other lag intervals (Figure 4.5B). This is demonstrated more clearly in the 3-month interval lag model where we observe a reduction in the mean weight-for-age Z-score in the 6-9-month interval following a malaria episode (Figure 4.6B). Lagged effects of acute pneumonia and diarrhoeal illness on weight were only observed from 1 to 3 months after an episode with none thereafter (Figure 4.5A & 4.5C & 4.6A & 4.6C).

The occurrence of an acute illness of pneumonia, malaria or diarrhoeal illness at certain time points in the first 1000 days of a child's life could potentially influence associations observed between these acute illness episodes, weight and weight-for-age. In this analysis there was no evidence to suggest that timing of these illnesses influences the association between these three morbidities and weight (pneumonia: chi2: 10.2, p=0.17; malaria: chi2: 13.0, p=0.07; diarrhoea: chi2: 5.5, p=0.60) or weight-for-age (pneumonia: chi2: 13.2, p=0.07; malaria: chi2: 9.4, p=0.23; diarrhoea: chi2: 5.7, p=0.580).

Table 4.2: Association of acute malaria, pneumonia and diarrhoeal illness with weight and odds of being underweight.

	weight (kg)			underweight (< - 2 Z-score)		
	Coef.	CI_{95}	p-value	Odds Ratio	CI_{95}	p-value
[a]Acute malaria	0.003	[-0.05, 0.04]	0.878	1.00	[0.85, 1.17]	0.984
[a]Acute pneumonia	-0.11	[-0.14, -0.07]	<0.001	1.32	[1.15, 1.52]	<0.001
[a]Acute diarrhoeal illness	-0.05	[-0.09, -0.01]	0.017	1.15	[0.99, 1.34]	0.065

Abbreviation: CI_{95} = 95% confidence interval; Coef = coefficient; GEE model-based estimates of association between acute malaria, pneumonia and diarrheal illness episodes and the weight as a continuous outcome and the odds of underweight while adjusting for haemoglobin, bednet use, sex, IPTi intervention and study province. [a]Morbidity variables are binary for presence of 1 or more episodes versus absence of the illness within 3 months of weight measurement.

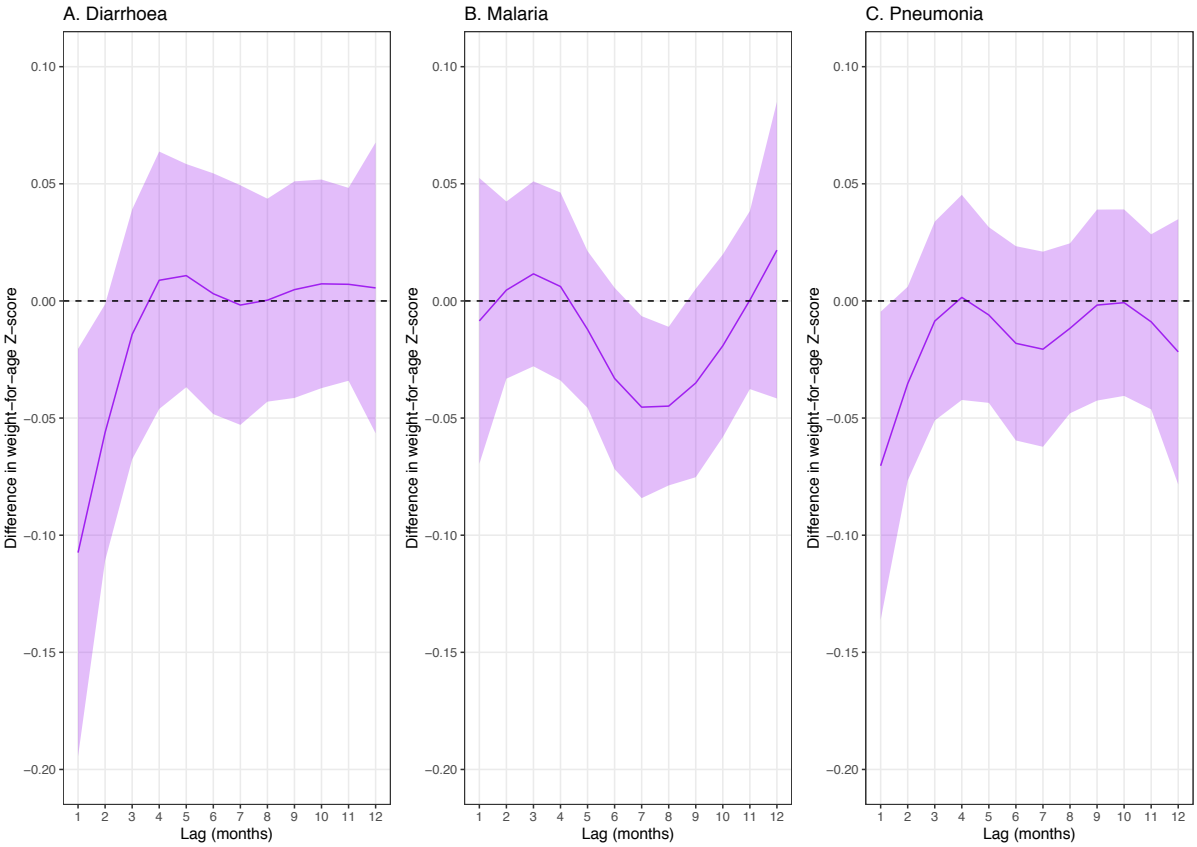

Figure 4-5: Estimates of distributed lag models for monthly intervals

Estimates (purple line) and 95% confidence intervals (purple region) for the association between weight difference and the lagged variables for diarrhea (column A), malaria (column B) and pneumonia (column C) variables from the distributed lag model (DLM). A natural cubic spline with 4 knots, was used to model how the weight difference caused by the presence of a morbidity at a particular lag changes as the lag period increases from 0-30 days (~1 month) to 330-360 days (~12 months) prior to a weight measurement. All estimates adjusted for sex and study site.

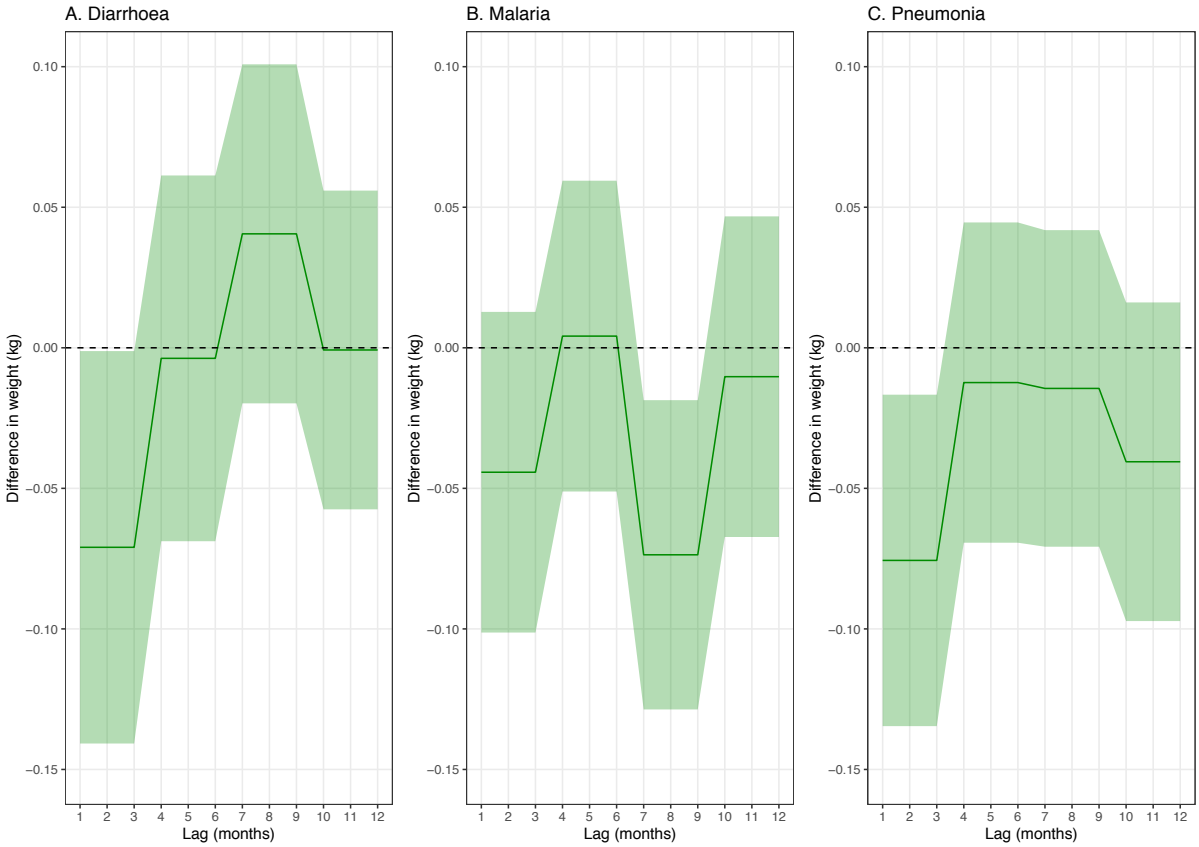

Figure 4-6: Estimates of distributed lag models for 3-monthly intervals.

Estimates (green line) and 95% confidence intervals (green region) for the association between weight difference and the lagged variables for diarrhea (column A), malaria (column B) and pneumonia (column C)derived for 3-month time intervals preceding a weight measurement, i.e. 0-3, 3-6, 6-9 and 9-12 month intervals. All estimates adjusted for sex and study site.

4.3.4 Association of acute illness with malaria, pneumonia or diarrhoea with odds of stunting.

Of the 1198 children with height measurements at the 5th visit, 511 (43%), 713 (60%) and 645 (54%) children experienced a total of 1005 (range: 1-8) malaria, 1417 (range: 1-9) pneumonia and 1083 (range: 1-6) diarrhoeal illness episodes respectively, prior to the height measurement. At the 5th visit and thereafter, 51% (n=421), 56% (n=459) and 35% (n=292) of the 826 children with height measurements at the 9th visit experienced a total of 876 (range: 1-8) malaria, 760 (range: 1-7) pneumonia and 397 (range: 1-4) diarrhoeal illness episodes, respectively.

Neither pneumonia or diarrhoeal illness showed any association with stunting while there was a tendency for an association of malaria with stunting after adjusting for mean haemoglobin level, average bednet use, study province, IPTi intervention and gender. For every one additional malaria episode experienced by the child, there was a tendency for their odds of

stunting to increase by 8% (OR 1.08, p=0.075; Table 4.3). A seven percent increase in the odds of stunting was observed for diarrhoeal illness but this was not statistically significant (OR 1.07, p=0.193; Table 4.3), while no difference was observed for pneumonia episodes (Table 4.3).

The effect of the timing of these 3 illnesses, that is, if occurring in the first 15 months of life versus occrring after 15 months of age on odds of stunting was investigated with no remarkable observations (interaction terms non significant; pneumonia: CI_{95} :0.93, 1.33, p=0.24; malaria: : CI_{95} :0.81, 1.10, p=0.26; diarrhoea: CI_{95} :0.74, 1.19, p=0.60). However, it was noted that the odds of a child being stunted at the 9th visit when they were approximately 27 months of age was significantly reduced by 28% (OR 0.72, p<0.001; Table 4.3), a possible indication that the odds of stunting may be much higher in the earlier months of life than after 15 months of life.

Table 4.3: Association of acute malaria, pneumonia and diarrhoeal illness with odds of stunting.

	Odds Ratio	CI_{95}	p-value
[a]Acute malaria	1.08	[0.99, 1.18]	0.075
[a]Acute pneumonia	0.97	[0.89, 1.04]	0.384
[a]Acute diarrhoeal illness	1.07	[0.97, 1.19]	0.193
9th visit time point	0.72	[0.60, 0.86]	<0.001

Abbreviation: CI_{95} = 95% confidence interval**;** GEE model-based estimates of association between acute malaria, pneumonia and diarrhoeal illness episodes and the odds of stunting while adjusting for haemoglobin, bednet use, sex, IPTi intervention and study province. [a]Morbidity variables are continuous variables denoting totals of episodes captured in each interval preceding height measurements, i.e. pre-5th visit interval and 5th - 9th visit interval.

4.3.5 Association of acute illness with malaria, pneumonia or diarrhoeal illness with odds of wasting.

Wasting was quite rare in this cohort with 8.2% (n=115) of the children observed to be wasted at least once during the study period. There were 1188 children with weight-for-length Z-score data at the 5th visit and 13.1% (n=156) of these had malaria, 15.9% (n=189) had pneumonia and 8.8% (n=105) had diarrhoeal illness in the previous month. None of the three acute illnesses were associated with wasting in unadjusted and multivariate analysis (Table 4.4). Given the small number of children meeting the definition of wasting and small number of acute illness episodes in the previous month, it is likely that this analysis is under-powered to detect a signficant association between acute illness episodes and wasting. The odds of wasting

for children with pneumonia (OR 1.10, CI_{95} [0.59, 2.04], p=0.768) or diarrhoeal illness (OR 1.42, CI_{95} [0.69, 2.94], p=0.343) in the preceding month were higher compared to those children that did not have pneumonia and diarrhoeal illness respectively and this may warrant further investigation in a larger dataset. There was no difference in the odds of wasting for those children who had malaria versus those who did not (OR 1.00, CI_{95} [0.50, 1.99], p=0.994; Table 4.4). The sample size at the 9th visit was much lower precluding analysis of the associations at that time point for reasons mentioned above.

Table 4.4: Association of acute malaria, pneumonia and diarrhoeal illness with odds of wasting

	Odds Ratio	CI_{95}	p-value
[a]Acute malaria	0.44	[0.0.15, 1.36]	0.156
[a]Acute pneumonia	0.84	[0.35, 2.02]	0.700
[a]Acute diarrhoeal illness	1.12	[0.36, 3.44]	0.845

Abbreviation: CI_{95} = 95% confidence interval**;** Logistic regression estimates of association between acute malaria, pneumonia and diarrhoeal illness episodes and the odds of wasting while adjusting for haemoglobin, bednet use, sex, IPTi intervention and study province. [a]Morbidity variables are binary for presence versus absence of the illness within a month of the weight and height measurements.

4.4 Discussion

The high prevalence of stunting observed in this study confirms previous findings (Gibson, Heywood et al. 1991, Keeble and Keeble 2006, Wand, Lote et al. 2012) and highlights the importance of addressing stunting to improve child health and development in PNG. At birth, children in PNG are believed to be similar to WHO reference growth standards but experience significant faltering thereafter (Mueller, Vounatsou et al. 2001). In particular, children experience significant growth faltering in their linear growth with the mean height-for-age approaching the cut-off for stunting (-2SD) in the second year of life with no evidence of catch up thereafter (Mueller, Vounatsou et al. 2001). In our study, the mean height-for-age at 15 months of age was 2.2 SD below the WHO growth standard. At 27 months of age, there was a slight improvement in the mean height-for-age Z-score to - 2.0 SD as compared to the that at 15 months of age. The odds of being stunted at 27 months of age was also noted to be lower when compared to the odds at 15 months of age. In a subset of children who had height measurements at both time points, 23.0% of the children who were stunted at 15 months of age were no longer stunted at 27 months of age. Taken together, these observations may indicate that the children in PNG could potentially catch up to their normal linear growth trajectory at

27 months of age provided they receive appropriate interventions and quality health care. These observations are made in the context of a clinical intervention trial with improved health care provision (which also includes mother's improved knowledge on child health and maternal health care) and hence could reflect the positive impact of this on the potential for the child to catch up on growth. This highlights the importance of adequate health care services, maternal care as well as their awareness of the importance in childcare and comprehensive vaccinations which among many other interventions which are known to be key in improving child growth (Bhutta, Das et al. 2013, Buisman, Van de Poel et al. 2019, Faye, Fonn et al. 2019). These however are major challenges in PNG with the failing health systems, low human capacity for adequate health care provisions and lack of political support among the many other challenges (PNG Department of Health 2010).

A much greater concern from our observations is that a high proportion of the children who were classified as stunted were severely stunted; 48% at 15 months of age and 42% at 27 months of age. Moreover, 24.3% of the children were severely stunted at 15 months of age and remained severely stunted at 27 months of age. An analysis by Olofin *et al* showed that the associated risk of mortality with severe stunting was 5.5-fold higher than the risk in children who were not stunted (Olofin, McDonald et al. 2013). What's even more concerning is that the risk of dying is much greater in severely stunted children than moderately wasted children (Olofin, McDonald et al. 2013) and yet, the children with the latter are more likely to be identified to receive treatment while severe stunting would often go unrecognised. This is because in many settings such as PNG where short stature is prevalent, parents and even health professionals often fail to recognise stunting as a problem (Emergency Nutrition Network (ENN) 2014, de Onis and Branca 2016). Our observation of the persistence of severe stunting at 27 months of age may reflect this, such that stunting was likely not recognised as a problem and was therefore not adequately managed at the earlier time point. Adequately addressing stunting in PNG as well as globally will require everyone involved in the care of children to recognise stunting as a problem and importantly, to identify it early for timely interventions. It has been indicated in some studies that reversal of stunting after it had occurred, although possible may be challenging to achieve (Golden 1994, Martorell, Khan et al. 1994, Checkley, Buckley et al. 2008). Preventing occurrence of stunting is therefore very important and understanding the causes is vital.

Child undernutrition and infectious diseases are a common co-occurrence in resource-constrained settings such as PNG and often of very high burden. The associated risks of stunting, wasting and underweight with infectious diseases have been demonstrated in other settings. We investigated the contribution of common infectious diseases, acute diarrhoea, pneumonia and malaria on child growth in the lowland settings of PNG, where there is scant information regarding their association. Care must be taken when interpreting the reported associations given this study was not specifically designed for this purpose and as such, potential confounding from unmeasured variables was unable to be adjusted for. The association between diarrhoea and stunting, wasting and underweight have been widely documented in many parts of the world (Becker, Black et al. 1991, Checkley, Epstein et al. 2003, Checkley, Buckley et al. 2008, Richard, Black et al. 2013, Fekadu, Mesfin et al. 2015). From our analysis, diarrhoea was observed to have adverse association with weight but not linear growth. We observed a reduction in weight at 1-3 months following an episode of diarrhoea. There was also a tendency for children who had 1 or more episodes of diarrhoea within 3 months of their weight measurement to be at increased risk of being underweight. Associations with underweight are often difficult to interpret given underweight can represent both stunting and wasting (Waterlow 1973, WHO 1995). However, short term change in weight often reflects change in weight-for-height Z-scores (WHO 1995). and as such, the effects of diarrhoea on weight demonstrated in this study are likely short term, as consistently documented elsewhere (Black, Brown et al. 1984, Briend, Hasan et al. 1989, Richard, Black et al. 2013). Although acute weight loss is often reflected in a change in weight-for-height Z-score and subsequent wasting, we did not see an association between diarrhoea and wasting in this study. Given the significantly low prevalence of wasting observed in the study, it is likely that our analysis is under-powered to detect any meaningful association between diarrhoea and wasting.

Effects of diarrhoea on weight are generally considered temporary (Black, Brown et al. 1984, Briend, Hasan et al. 1989, Richard, Black et al. 2013) and catch-up growth may occur provided there is adequate nutrient intake, prevention of further infections and prolonged time between infections (Briend, Hasan et al. 1989, Moy, de et al. 1994, Scrimshaw 2003). Observations of longer-term impact of diarrhoea on growth have been inconsistent and controversial (Black 1991, Richard, Black et al. 2013). In this study, the effects of diarrhoea on weight lasted up to 3 months following an episode indicating a delayed effect of diarrhoea on weight. The lack or associations following the 3 months suggests that children did catch up on their weight growth,

only that it is delayed by 3 months. It is possible that the worsening of nutrition during diarrhoea may prolong the duration of the ongoing illness thus delaying recovery (Smith 1991, Scrimshaw 2003, Katona and Katona-Apte 2008). In LMICs such as PNG, children tend to contract infections frequently which prevents the children from catching up their lost growth before they further lose more weight.

Although numerous studies have demonstrated an association between diarrhoea and stunting (Checkley, Epstein et al. 2003, Checkley, Buckley et al. 2008), we fail to show an association in our study. Researchers of the earlier nutritional studies in PNG noted that the prevalence of wasting was low because the food intake was not a problem in PNG. They indicated that the children experience growth faltering largely from the lack of essential nutrients for growth such as protein and zinc in their typical diets. (A. P. C. Oomen 1971, Ferro-Luzzi, Norgan et al. 1975, Gibson, Heywood et al. 1991, Mueller, Vounatsou et al. 2001). These nutrients are particularly essential for achieving normal linear growth and was therefore explaining the high burden of stunting observed in the country. Our lack of association between diarrhoea and stunting may indicate that diarrhoea does not have a bigger influence on the child's linear growth.

Malaria as an important risk factor for childhood undernutrition has been demonstrated in various studies. It has been associated with weight faltering (Marsden 1964, Cole and Parkin 1977, Rowland, Cole et al. 1977) and stunting (Kang, Kreuels et al. 2013) (Williams, Maitland et al. 1997, Nyakeriga, Troye-Blomberg et al. 2004, Lee, Yori et al. 2012, Alexandre, Benzecry et al. 2015) and wasting (Gari, Loha et al. 2018). In our analysis, we observed malaria to be have a delayed impact on weight-for-age. In addition, there was also a tendency that for every additional episode of malaria, risk of stunting increased 1.08-fold. No short-term associations of malaria with growth were observed in our analysis. Undernutrition may be a consequence of inflammation induced by malaria infection. During malaria, an acute inflammatory response is initiated resulting in secretion of proinflammatory cytokines such as TNF (Clark, Virelizier et al. 1981, Bate, Taverne et al. 1989, Schofield and Hackett 1993) . TNF has been shown to be pyrogenic thus increasing metabolism and induces anorexia and cachexia (Beutler, Milsark et al. 1985, Schofield and Hackett 1993) and may also be responsible for nausea, vomiting and diarrhoea (Jakubowski, Casper et al. 1989), which all together can significantly affect the nutritional status of the child.

The relationship between malaria however is complex (McGregor 1982, Das, Grais et al. 2018) given the bi directionality of their association, that undernutrition can predispose children to malaria (Deen, Walraven et al. 2002, Friedman, Kwena et al. 2005). In addition to this complexity, some studies even indicated that undernutrition may also be protective against malaria (Genton, Al-Yaman et al. 1998, Alexandre, Benzecry et al. 2015). Interpretation of findings from associations between malaria and undernutrition are therefore complicated.

Association between pneumonia and undernutrition remains to be clarified (Black 1991) in many settings. While there are some studies that have shown an association between the two (Rowland, Rowland et al. 1988, Sulaiman, Bushara et al. 2018, Rahman, Rahman et al. 2019), others did not demonstrate any association between acute respiratory infections and linear growth faltering (Martorell, Habicht et al. 1975, Black 1991, Owoaje, Onifade et al. 2014). The study by Martorell et al showed in a population where respiratory illness were present for 30-35% of the time, respiratory infections were not associated with undernutrition (Martorell, Habicht et al. 1975, Black 1991). In our study in a setting where the incidence of pneumonia was 0.85 episodes/child/year (Senn, Rarau et al. 2014), we demonstrated that one or more episodes of pneumonia occurring in the 3 months prior to the weight assessment was associated with 32% increased odds of being underweight consistent with observations made elsewhere (Bloss, Wainaina et al. 2004). The negative effects of pneumonia on weight-for-age were observed to be delayed up to 3 months following an acute episode. Worsening of nutrition during pneumonia may further increase the susceptibility of the child to other infections and diseases or prolonging the duration of the ongoing illness thus delaying recovery or increasing the severity of disease (Smith 1991, Scrimshaw 2003, Katona and Katona-Apte 2008) which may explain observed delay in our study.

Although studies have demonstrated associations of respiratory infections with stunting (Batiro, Demissie et al. 2017) and wasting (Sulaiman, Bushara et al. 2018), we did not observe such associations in this study. Stunting is a long-term process although it may initiate immediately following an illness episode and whether the effects of pneumonia on linear growth hence stunting manifest immediately may be questionable. For studies that report on the adverse effects of pneumonia on stunting, the effects are often observed in the short-term, some as short as 2 weeks (Batiro, Demissie et al. 2017). Catch up of growth is postulated to be within 4-6 weeks (Black 1991, Moy, de et al. 1994) and thus in these studies, the time may be too short for the child to catch up on his/her growth and the observed effects may be an

overestimation of the association between pneumonia and stunting. The lack of association with wasting may be explained by the low prevalence of wasting observed in this study.

It is important to note that this study was not specifically designed for this analysis and as such, important determinants of growth such as the socioeconomic status, dietary intake and feeding practices were not able to be included in the analysis. Secondly, this was a clinical trial where children were intensively monitored with early detection and prompt treatment of diseases which are likely to influence the observed associations; this pattern of health care is not reflective of standard care in a PNG setting and improved health care provided in the study may lead to an underestimation of associations between acute illness episodes and growth. Third, our analysis of the lagged effects of the 3 illnesses was limited to children who had complete illness exposure history resulting in a sample size that may be underpowered.

4.5 Conclusion

The data presented here re-affirms that there is a high burden of stunting among young children in PNG. Of greater concern is that the majority of children who are stunted are severely stunted. Unless recognised and addressed, these children are at an increased risk of dying. If they survive, they face the risk of remaining stunted past 2 years of age when reversal may be challenging to achieve. The effects of acute pneumonia and diarrhoea on weight can last up to 3 months while effects of malaria can manifest several months later highlighting the importance of regular monitoring of the child's wellbeing, health and nutritional status during the months following an episode of illness. This may be a crucial step to allow early detection of nutritional stressors and to introduce early intervention thus preventing deterioration of the nutritional status. Although not significant, there is a tendency for malaria to be associated with stunting. Malaria may therefore a potential contributor to the high burden of stunting in the country and its prevention must be priority.

5 Differential impact of malaria control interventions on *P. falciparum* and *P. vivax* infections in young Papua New Guinean children.

5.1 Introduction

Malaria poses a much greater threat in the lowlands of PNG as it not only inflicts high burden of disease on young children during infancy, it starts affecting the lives of these children at the time of conception. Infection with malaria during pregnancy and subsequent maternal anaemia, placental malaria, LBW and preterm delivery have been widely documented for this setting (Brabin, Ginny et al. 1990, Brabin and Piper 1997, Allen, Raiko et al. 1998, Stanisic, Moore et al. 2015, Lufele, Umbers et al. 2017). Historically, malaria has been the leading cause of childhood illness and death in PNG, although, children acquired immunity quickly (Michon, Cole-Tobian et al. 2007, Genton, D'Acremont et al. 2008, Lin, Kiniboro et al. 2010). This was attributed to high exposure to infections, particularly in the lowlands PNG where malaria transmission was hyperendemic in most parts (Muller, Bockarie et al. 2003, Michon, Cole-Tobian et al. 2007, Lin, Kiniboro et al. 2010). Since 2004, the national malaria control program has received increased support allowing scale-up of control interventions. In the period between 2004 and 2014 there was a significant reduction in the overall burden of malaria nationwide (Hetzel, Morris et al. 2015, Hetzel, Pulford et al. 2017, Koepfli, Ome-Kaius et al. 2017).

As the overall transmission of malaria declines, the epidemiology of malaria becomes complex with significant epidemiological changes including the risk profile of malaria infection and illness and immunological changes (Ceesay, Casals-Pascual et al. 2008, Brasseur, Badiane et al. 2011, Fowkes, Boeuf et al. 2016). For example, in areas that are transitioning from high-to-low-to-no malaria transmission, children are less frequently exposed to parasites which will ultimately impact their rate of acquiring clinical immunity and their risk of malaria as a whole These may lead to a shift in cases of malaria to older ages as seen in some settings (Ceesay, Casals-Pascual et al. 2008, Brasseur, Badiane et al. 2011, Griffin, Ferguson et al. 2014). In settings like PNG where *P. falciparum* and *P. vivax*, the two predominant human malaria species are co-endemic, evaluating the impact of the improved malaria control on these two species is important for guiding further interventions that can sustain and maintain these reductions. This is especially important for PNG considering it traditionally had very diverse transmission scenarios ranging hyper-endemic settings in coastal to malaria free area in the highlands (Muller, Bockarie et al. 2003, Kazura, Siba et al. 2012).

Understanding the changing epidemiology will require a detailed understanding of the relationships between transmission intensity, the prevalence of infection and the incidence of clinical malaria episodes. Transmission intensity is traditionally quantified through entomological measures of exposures and the gold standard metric for measuring transmission is the entomological inoculation rate (EIR). It is however labour intensive and is becoming increasingly difficult to establish particularly in low transmission settings (Tusting, Bousema et al. 2014). The two most commonly used traditional metrics, parasite prevalence and incidence of clinical illness are widely employed to measure the impact of interventions, although the confidence in the estimates significantly reduces as malaria becomes rare and that large sample size is required to achieve precision in these estimates (Gregory and Blackburn 1991, Hay, Smith et al. 2008). Force of infection (FOI) is the number of new infections acquired over time and has been a widely accepted alternative for measuring transmission. The molecular force of blood-stage infections ($_{mol}$FOB) has recently been demonstrated to be a sensitive and suitable proxy for measuring transmission at the population level and for measuring exposure at individual level (Mueller, Schoepflin et al. 2012, Koepfli, Colborn et al. 2013).

In the latter, presence of new blood-stage infections can be easily determined through sensitive molecular approaches (Smith, Beck et al. 1999, Smith and Vounatsou 2003, Sama, Owusu-Agyei et al. 2006). By genotyping parasites, multiple co-infecting parasite clones within one host can be distinguished and monitored over time hence allowing precise estimation of the number of new infections in the bloodstream over time ($_{mol}$FOB) (Mueller, Schoepflin et al. 2012, Koepfli, Colborn et al. 2013). It is therefore a measure of exposure to new blood-stage infections rather than a direct measure of infective mosquito bites. For *P. falciparum*, $_{mol}$FOB is a directly proportional measure of the FOI, that is, the number of new infections entering the blood-stream acquired through infective bites over time and therefore, may closely reflect *P. falciparum* transmission as most of the new infections acquired through a bite are likely to establish blood-stage infections. In contrast, *P. vivax* blood-stage infections do not only derive from a recent infective bite but also from the relapsing hypnozoites. Hence $_{mol}$FOB for *P. vivax* is a composite measure of exposure from new infections from a recent infective bite and from relapses. As such, $_{mol}$FOB for *P. vivax* is not a direct measure of mosquito-to-human *P. vivax* transmission.

Use of $_{mol}$FOB as an alternative to EIR alongside the commonly used metrics such as parasite prevalence and clinical incidence would provide a detailed insight into epidemiology of malaria. However, estimation of $_{mol}$FOB and clinical incidence rely on longitudinally collected data as collected in cohort studies. The benefit of using a longitudinal cohort study is that it allows detailed investigation into the dynamics of infection and illness in a host over time. More importantly, the ability to assess the impact of intensification on the incidence of infections and illness, both of which provide vital information on the rate of acquiring new infections and clinical illness over time. This is not achievable using cross-sectional studies which by their nature reflect a relatively short period providing only a snapshot of transmission or the burden of infections and illness through a single time point measurement. Extrapolating this single time point measurement to an overall contribution to the burden of infections and illness or transmission can be challenging. Many of the studies that have investigated the impact of the improved malaria control on epidemiology of malaria in PNG and elsewhere are cross-sectional in nature including nationwide household surveys, large community cross-sectional studies and health information systems (Pulford, Kurumop et al. 2013, Hetzel, Morris et al. 2015, Hetzel, Pulford et al. 2017, Koepfli, Ome-Kaius et al. 2017). There is currently limited data available on the impact of declining transmission that is investigated from a longitudinal context. Moreover, despite being the group that bears the highest burden of malaria in the country, there is currently no study in PNG that has specifically investigated in detail the impact of the observed declining transmission between 2004 and 2014 in children under 5 years of age.

5.2 Methodology

The detailed description of the methods are provided in the published article (included in this chapter) however, here we describe in brief the procedures involved in estimating the $_{mol}$FOB to account for the differences in the sampling schedules of the studies that we analysed. $_{mol}$FOI is defined as the number of distinct clones acquired by an individual throughout his follow-up period. Depending on the sampling interval, a clone may be detected or missed because it would have been acquired after a scheduled visit and cleared before the subsequent one. Since clones can be short-lived (White, Karl et al. 2018) it is expected that the $_{mol}$FOI would increase with smaller sampling intervals. We compared three studies, one of which used a different sampling scheme (larger sampling interval of ~2 months with double-bleeds 24 hours apart). This sampling difference was corrected by censoring the two studies that had shorter sampling

intervals, participant-wise, so that the mean interval between consecutive visits would match that observed in the third study. Additionally, we randomly discarded one of the two repetitive bleeds from the third study. Overall, this yielded a dataset comprised of three independent studies, now with comparable sampling scheme. We used Negative-Binomial regression stratified by study and offseted by the time at risk for each participant to estimate the incidence of new clones.

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Differential impact of malaria control interventions on *P. falciparum* and *P. vivax* infections in young Papua New Guinean children

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Abstract

Introduction: As malaria transmission declines, understanding the differential impact of intensified control on *Plasmodium falciparum* relative to *Plasmodium vivax* and identifying key drivers of ongoing transmission is essential to guide future interventions.

Methods: Three longitudinal child cohorts were conducted in Papua New Guinea before (2006/2007), during (2008) and after scale-up of control interventions (2013). In each cohort, children aged 1–5 years were actively monitored for infection and illness. Incidence of malaria episodes, molecular force of blood-stage infections ($_{mol}$FOB) and population-averaged prevalence of infections were compared across the cohorts to investigate the impact of intensified control in young children and the key risk factors for malaria infection and illness in 2013.

Results: Between 2006 and 2008, *P. falciparum* infection prevalence, $_{mol}$FOB, and clinical malaria episodes reduced by 47%, 59% and 69%, respectively, and a further 49%, 29% and 75% from 2008 to 2013 (prevalence 41.6% to 22.1% to 11.2%; $_{mol}$FOB: 3.4 to 1.4 to 1.0 clones/child/year; clinical episodes incidence rate (IR) 2.6 to 0.8 to IR 0.2 episodes/child/year). *P. vivax* clinical episodes declined at rates comparable to *P. falciparum* between 2006, 2008 and 2013 (IR 2.5 to 1.1 to 0.2), while *P. vivax* $_{mol}$FOB (2006, 9.8; 2008, 12.1) and prevalence (2006, 59.6%; 2008, 65.0%) remained high in 2008. However, in 2013, *P. vivax* $_{mol}$FOB (1.2) and prevalence (19.7%) had also substantially declined. In 2013, 89% of *P. falciparum* and 93% of *P. vivax* infections were asymptomatic, 62% and 47%, respectively, were sub-microscopic. Area of residence was the major determinant of malaria infection and illness.

Conclusion: Intensified vector control and routine case management had a differential impact on rates of *P. falciparum* and *P. vivax* infections but not clinical malaria episodes in young children. This suggests comparable reductions in new mosquito-derived infections but a delayed impact on *P. vivax* relapsing infections due to a previously acquired reservoir of hypnozoites. This demonstrates the need to strengthen implementation of *P. vivax* radical cure to maximise impact of control in co-endemic areas. The high heterogeneity of malaria in 2013 highlights the importance of surveillance and targeted interventions to accelerate towards elimination.

Keywords: *P. falciparum*, *P. vivax*, Papua New Guinea, Epidemiology, Malaria control, Incidence, Prevalence

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Background

Intensification of malaria control measures has been associated with marked reductions in transmission and infection and illness burden in many endemic areas [1]. In the Americas [1, 2] and some parts of Asia-Pacific [3, 4], these reductions have been associated with a marked shift to the predominance of *Plasmodium vivax* as the primary source of *Plasmodium* spp. infections. In parallel, the proportion of low-density, asymptomatic infections has been observed to increase [5–8] and transmission becomes more heterogeneous [9–11].

The reasons underlying these shifts are likely to be multifactorial. A major factor for the relative increase in *P. vivax* is the poor uptake and/or adherence of anti-hypnozoite therapy [12, 13]. As a result, *P. vivax* hypnozoites are able to cause repeated bouts of blood-stage parasitaemia and are responsible for up to 80% of all *P. vivax* blood-stage infections [14]. Even in low and very low transmission settings, most *P. vivax* infections are asymptomatic [15, 16] and often of very low density [16] but almost all carry detectable gametocytaemia [6, 17, 18]. These infections are thus not detected and treated by the health systems and can sustain transmission. *P. vivax* is also considered more easily transmissible given the rapid maturation and thus early presence of its gametocytes [19] and faster development cycle in its mosquito host [20]. Lastly, it has also been observed that *P. vivax*-infected mosquitoes may be younger and more likely to bite early and outdoors [21, 22]. All of these factors may render *P. vivax* transmission less susceptible to vector control and routine case management interventions.

The highly heterogeneous nature of malaria transmission across countries, between neighbouring villages and within the same village has long been recognised [23–25] and is driven by an interplay of host, vector and environmental factors [23, 26, 27]. As transmission declines, there is a tendency for malaria infections to become increasingly clustered in high-risk populations and high-risk areas [11, 28] and it becomes more important to be able to identify these clusters since they may be responsible for sustaining transmission [11]. There is growing evidence that despite achieving overall reductions in malaria transmission through improved malaria control, infections and illness burden in many hyperendemic areas remain unaltered [29–31] and that more targeted interventions may be necessary for elimination [11].

In the early 2000s, the overall burden of malaria in Papua New Guinea (PNG) was amongst the highest in the Asia-Pacific region, albeit with intensity of transmission geographically highly variable across the country [27, 32, 33]. *Plasmodium falciparum* and *P. vivax* are the two predominant species that account for most of the burden of malaria infections and illness in PNG [32, 34].

Beginning in 2004, with the support of Global Fund to Fight AIDs, Tuberculosis and Malaria, PNG scaled up its malaria control interventions through scheduled 3-yearly nationwide distribution of long-lasting insecticide treated nets (LLINs), introduction of a test-and-treat approach and a switch to artemether-lumefantrine (AL) as first-line treatment [35, 36]. Subsequent surveys revealed a substantial decline in the overall burden of malaria [6, 33], with the nationwide infection prevalence by light microscopy (LM) declining from 11.1% in 2009 to 0.9% in 2014 [33, 37]. Entomological studies also revealed a large decline in human biting rates from 83 bites/person/night to 31 bites/person/night [37, 38]. As elsewhere, these reductions in PNG have gone hand-in-hand with an increase in the proportion of asymptomatic and sub-microscopic infections [6] and a pronounced heterogeneity of residual transmission [39]. Although the prevalence of PCR-detectable *P. vivax* infections in community surveys has not declined to the same extent as *P. falciparum* infection [6], the shift towards *P. vivax* predominance has not yet been as pronounced as in neighbouring SE Asia- and SW Pacific countries [7].

To better understand the relationship between changing transmission and the risk profile of malaria infections and disease, it is vital to gain insight into the impact that control measures have on the two main species, *P. falciparum* and *P. vivax*. Using three consecutive longitudinal child cohorts (1–5-year-old children) conducted in the same study area, prior [40], during [41] and following 5 years of intensification (2013 cohort), we investigated the impact of improved malaria control on the breadth of metrics including clinical incidence, incidence of newly acquired infections (i.e. the molecular force of blood-stage infection, $_{mol}$FOB) [42, 43] and infection prevalence to better understand changing *P. falciparum* and *P. vivax* epidemiology in the context of rapid reductions in transmission. In order to guide continued reductions in transmission, we also investigated the key drivers of infection and illness in young children during the period of low transmission in 2013.

Methods

Study design and sites

Three longitudinal cohort studies of 1–5-year-old children were conducted in the same study area in the Ilahita area of Maprik District, East Sepik Province in 2006, 2008 and 2013. A detailed description of the study area is given elsewhere [40]. Briefly, the study area is located in northern PNG where malaria transmission is considered hyperendemic [34, 44] and all human malaria species are endemic [40, 41, 45, 46]. Health services are provided solely by the church-run Ilahita Health Centre with inconsistent services from a government aid post. The cohorts were conducted at three different time-points before and during the scale-up of malaria control interventions in the study area (Fig. 1).

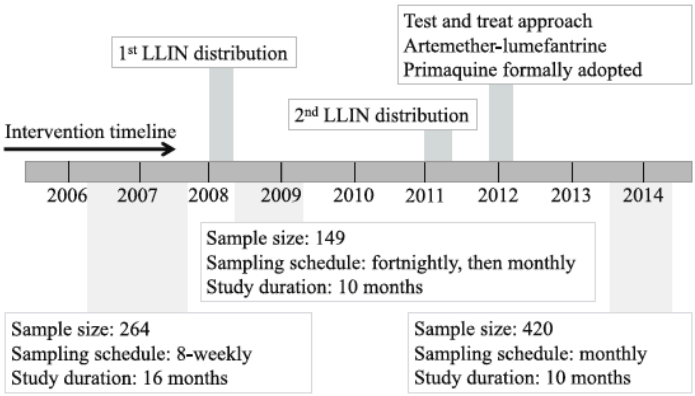

Fig. 1 Study and intervention timeline. Legend: The timeline shows the time-points when the three cohorts were conducted in reference to malaria control interventions that occurred in the study area

Cohorts

2006 cohort (pre-intensification)

Children aged 1–3 years were enrolled into the study and actively followed up for malaria infection and illness every 8 weeks for a total of 16 months from March 2006 to August 2007 [40, 42, 43]. Passive case detection at Ilahita Health Centre was maintained throughout the study for detection of clinical episodes. All rapid diagnostic test (RDT) or LM confirmed febrile illness episodes were treated with AL (Coartem®, Novartis) (if treated by study staff) or amodiaquine plus sulphadoxine-pyrimethamine as per the PNG standard treatment for common illnesses in children [47] (if receiving treatment from a non-study source). Children with *P. vivax* episodes were not treated with primaquine as it had not yet been introduced into PNG standard treatment guidelines [47]. Full details of the study methodology are published [40, 42, 43].

2008 cohort (during early intensification)

Children 1–5 years of age were enrolled into this randomised controlled trial in April 2008, a month after the first population-wide distribution of LLIN into the study area [41]. Analysis was restricted to the control arm to allow comparability to the other two observational studies. Children were actively checked for malaria infection and illness fortnightly for the first 3 months and monthly thereafter for another 7 months. All RDT or LM confirmed febrile illness episodes were treated with AL (Coartem®, Novartis) (if treated by study staff) or Amodiaquine plus sulphadoxine-pyrimethamine as per the PNG standard treatment guidelines [47] (if receiving treatment from a non-study source). Children with *P. vivax* episodes were not treated with primaquine as it had not yet been introduced into PNG standard treatment guidelines [47]. Full details of the study methodology are published [41].

2013 cohort (5 years after sustained control)

This cohort was conducted after 5 years of sustained malaria control in the study area (Fig. 1) A total of 465 children aged 1–5 years at enrolment from 12 villages (Ilahita 1–7, Kamanokor, Sunuhu 1 and 2, Balanga and Balif) in Ilahita area were enrolled from July to September, 2013, and followed for 12 months. Of these, 45 children were excluded post hoc (11 withdrawals, 26 lost to follow-up, 8 with erratic attendance), resulting in a final sample size of 420 children (90% retention rate). All 420 children ranging in age from 0.9–6.4 years during the study period were included in the analysis investigating the key drivers of infection and illness in 2013. A subset ($n = 371$) aged ≤ 55 months were age-matched to earlier two cohorts to investigate the changing burden of malaria across the intervention time-points.

At enrolment, demographic and clinical data on recent illness and medications, bednet use and current state of health were recorded. Axillary temperatures were measured using an electronic digital thermometer. A 5-ml (ml) venous blood sample and two blood slides were collected. Haemoglobin level was measured using a portable HemoCue machine (HemoCue, Angholm, Sweden). The location of each child's residence was recorded using a Garmin eTrex®.

Following enrolment, children were actively followed up fortnightly for morbidity surveillance and monthly for blood sampling (250 μl finger prick sample, two blood slides and haemoglobin measurement). If a child had a febrile illness at a morbidity surveillance visit, a finger prick sample of 250 μL blood and 2 blood slides were collected. RDT for malaria was performed and, if positive, children were treated with AL (Coartem®, Novartis) and occasionally AL plus primaquine for RDT positive *P. vivax*, as per PNG standard treatment guidelines [48]. Over the course of the study, 9 children were documented as receiving primaquine, suggesting that

primaquine was inconsistently administered by health workers. Anaemic children with haemoglobin < 7.5 g/dL were given an anthelminthic drug (albendazole) and iron supplementations while other ailments were treated according to PNG standard treatment [48].

Plasmodium spp. infections were detected by real-time quantitative PCR assay (qPCR), as previously described [40–43, 49] and LM. Briefly, parasite DNA was extracted from cell pellets (equivalent to 200 μL whole blood) using a Favorgen 96-well Genomic DNA Extraction Kit following the manufacturer's instructions and eluted in 200 μL elution buffer. The presence of *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale* infections were determined using two multiplex 2-species qPCR assays [49]. Infections with *P. falciparum* and *P. vivax* were further genotyped for *Pfmsp2*, *Pvmsp1F3* and *PvMS16* to identify individual parasite clones. All blood slides positive by first read and/or by *Plasmodium* screening qPCR [50], as well as 10% of the negatives, were independently examined by a second microscopist. Any discrepancies between the first and the second reads were then re-read by a third expert-level microscopist (WHO Level 1 certified). The final density was calculated by taking the geometric mean of the two concordant reads.

Statistical analysis

Analysis for this paper occurred in two parts and focussed on the two predominant species, *P. falciparum* and *P. vivax*. In the first part "Analysis of changing burden of malaria infections and illness: 2006 – 2013", we aimed to compare the prevalence, $_{mol}$FOB and clinical incidence across the three cohorts to determine patterns of decline for *P. falciparum* relative to *P. vivax* across the intervention time-points. In the second part, "Analysis of key determinants of malaria infection and illness during the time of low transmission 2013", the objective was to explore the full dataset of the 2013 cohort to identity factors that were key predictors of infection and illness during the period of low transmission in 2013. In both analyses, a clinical malaria episode was defined as history of febrile illness during the preceding 48 h and/or measured temperature ≥ 37.5 °C in the presence of a microscopically detectable infection of any density. The $_{mol}$FOB (number of genetically unique blood-stage infections) was calculated from the number of new infections acquired during the intervals between sampling time-points by counting all new *msp2* alleles for *P. falciparum* and *msp1F3* and *MS16* alleles for *P. vivax* per unit time that were not present in the preceding intervals.

Analysis of changing burden of malaria infections and illness: 2006–2013

Data from each cohort were analysed separately due to the differences in the sampling schedules and the length of follow-up between the studies. However, to allow direct comparison, we used the full dataset of the 2006 cohort as the baseline while age-matched subsets of the 2008 and 2013 cohorts were used.

The population-averaged prevalence (referred to as prevalence) of *P. falciparum* and *P. vivax* infections in the three cohorts was estimated using generalised estimating equations (GEE) with a logit link and an exchangeable working correlation matrix, to account for the dependency between observations from the same child. Robust standard errors were also used to correct for working correlation matrix misspecification. Incidence rates (IR) for clinical episodes were calculated from the total number of clinical episodes experienced by each child over the study period and was modelled using negative binomial regression for the 2006 and 2013 cohorts and Poisson regression for the 2008 cohort. The relative percentage change in the prevalence and incidence was calculated using the formula: percentage change = ((current estimate – previous estimate)/previous estimate) × 100. Both the frequency of sampling and duration of blood-stage infections [51] are important factors influencing the $_{mol}$FOB variable. Due to the differences in the frequency of sampling in the 2006, 2008 and 2013 cohorts, it was necessary to censor any sampling time-points that were not available across all three cohorts in order to be able to directly compare the $_{mol}$FOB estimate across the cohorts. Incidence of new clones was defined as the sum of all new clones over the study period and derived using negative binomial regression, adjusting for individual time of exposure.

Analysis of key determinants of malaria infection and illness during the time of low transmission 2013

Risk factors of infection and malaria episode investigated in 2013 included the child's age (years), timing of active detection of infection visits, area of residence, bednet use in the previous night, history of febrile illness in the past 2 weeks, presence of febrile illness, which is defined as the 2-day history of fever ± axillary temperature ≥ 37.5 °C, and haemoglobin levels.

For all risk factor analyses, both univariable and multivariable regression models including all risk factors were examined. The association between prevalence of infections at monthly time-points and the risk factors was estimated using GEEs with a logit link and exchangeable working correlation matrix. Incidence of new blood-stage infections was estimated using GEE with negative binomial regression and an exchangeable working correlation matrix. Due to a very low number of clinical episodes observed in 2013, we used the total number of clinical episodes for each child across the follow-up period to assess the association between incidence of clinical infections and the risk factors. This was

estimated using negative binomial regression. The risk factors were summarised across the study period for each child as follows: age at enrolment, residence (assumed not to vary across follow-up), mean haemoglobin level and $_{mol}$FOB. Two multivariable models of the incidence of clinical infections, one including all aggregated risk factors and $_{mol}$FOB ($_{mol}$FOB-adjusted model) and the other excluding $_{mol}$FOB (base model) were examined.

Due to reduced levels of transmission in 2013, several villages had few *P. falciparum* or *P. vivax* infections detected, no clinical *P. falciparum* or *P. vivax* episodes and very few new blood-stage clones. Therefore, villages were grouped into 4 areas with geographically similar characteristics (1 = Ilahita 1, 2, 3, 4, 6 and 7; 2 = Balanga and Balif; 3 = Kamanokor and Ilahita 5; and 4 = Sunuhu 1 and 2). Due to the universally high bednet use, analyses of their association with incidence of new blood-stage infections and clinical episodes did not converge and bednet use was excluded from both analyses. The associations are expressed as odds ratio (OR) and incidence rate ratios (IRR) and were considered to be statistically significant if the Wald test *p* value was below the nominal level of significance of 0.05.

The analyses were conducted using Stata 12.0 (StataCorp, USA) and R v2.12 (2011) [2006 cohort $_{mol}$FOB analysis] and v3.4.0 (2017) [2008 cohort analyses] (R Core Team, R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria).

Results

Changing burden of malaria infections and illness: 2006–2013

The prevalence of infection, $_{mol}$FOB and incidence of clinical malaria were compared across three independent age-matched child cohorts conducted before (cohort 1, $n = 264$) and during (cohort 2, $n = 149$; cohort 3, $n = 371$) the intensification of malaria control activities. The overall prevalence of all *Plasmodium* spp. infections by PCR was 79.4% (CI_{95} 76.7–81.9%) in 2006, 77.0% (CI_{95} 73.4–80.3%) in 2008 and 25.6% (CI_{95} 22.5–29.0%) in 2013, with *P. vivax* the predominant species across all timepoints.

In 2006, 2 years prior to the scale-up of control activities in the study area, prevalence of *P. falciparum* and *P. vivax* was 41.6% (CI_{95} 38.4–44.9%) and 59.6% (CI_{95} 56.6–62.4%) by PCR and 24.8% (CI_{95} 21.9–27.6%) and 45.3% (CI_{95} 42.3–48.3%) by LM, respectively (Fig. 2a, b). Two years later and within several months of the first population-wide distribution of LLIN by the National Malaria Control Program, the prevalence of *P. falciparum* almost halved [PCR 22.1% (CI_{95} 7.7–27.3%); LM 12.8% (CI_{95} 10.0–16.2%)), Fig. 2a, b], with little observed impact on *P. vivax* prevalence [PCR 65.0% (CI_{95} 61.4–68.4%); LM 49.4% (CI_{95} 45.4–53.5%), Fig. 2a, b]. However, after 5 years of sustained control in the area, the prevalence of *P. vivax* had also substantially declined (PCR 19.6% (CI_{95} 16.9–22.6%); LM 11.4% (CI_{95} 9.5–13.6%), Fig. 2a, b), and *P. falciparum* prevalence had continued to decline further to 11.2% (CI_{95} 9.2–13.0%) by PCR and 4.5% (CI_{95} 3.5–5.8%) by LM in 2013 (Fig. 2a, b). Infections due to *P. malariae* [2006 (7.9%), 2008 (4.1%), 2013 (0.3%)] and *P. ovale* [2006 (3.5%), 2008 (3.0%), 2013 (0.2%)] were only occasionally detected by PCR and also declined from 2006 to 2013.

As observed with the prevalence of infections, the incidence of *P. falciparum* genetically distinct blood-stage infections substantially declined following the first LLIN distribution. In contrast, *P. vivax* $_{mol}$FOB did not change over the same interval. *P. falciparum* $_{mol}$FOB decreased from 3.4 clones/child/year-at-risk (CI_{95} 3.1–3.6) in 2006 to 1.4 clones/child/year-at-risk (CI_{95} 0.9–1.8) in 2008, which further declined to 1.0 clones/child/year-at-risk (CI_{95} 0.9–1.2) in 2013 (Fig. 2c). In contrast, *P. vivax* $_{mol}$FOB was observed to increase from 9.8 clones/child/year-at-risk (CI_{95} 9.1–10.5) in 2006 to 12.1 clones/child/year-at-risk (CI_{95} 10.6–13.5) in 2008, before declining to 1.2 clones/child/year-at-risk (CI_{95} 1.0–1.5%) in 2013 (Fig. 2c).

Interestingly, a different pattern was observed for the incidence of clinical *P. vivax* episodes (Fig. 2d). In spite of the persistence of a relatively high *P. vivax* prevalence and $_{mol}$FOB following the first LLIN distribution, the incidence of clinical *P. vivax* declined by 55% in 2008 (2006, 2.46 episodes/child/year-at-risk (CI_{95} 2.27–2.66); 2008, 1.11 episodes/child/year-at-risk (CI_{95} 0.90–1.36)), before further declining to 0.23 episodes/child/year-at-risk (CI_{95} 0.13–0.24) in 2013. This corresponded to an overall reduction of 91% between 2006 and 2013. The incidence of clinical *P. falciparum* exhibited a similar pattern to that of the prevalence and $_{mol}$FOB, with a continuous decline (2006, 2.56 episodes/child/year-at-risk (CI_{95} 2.36–2.77); 2008, 0.76 episodes/child/year-at-risk (CI_{95} 0.57–1.01); 2013, 0.21 episodes/child/year-at-risk (CI_{95} 0.14–0.28)), corresponding to an overall reduction of 92% between 2006 and 2013 (Fig. 2d).

Key determinants of malaria infection and illness during the time of low transmission 2013

Demographic characteristics of enrolled participants

Of the 465 children enrolled into the 2013 cohort, data from 420 were available for analyses (retention rate 90%). These children ranged in age from 0.9 to 6.4 years (mean 3.3), 53.8% were male and 93% reported sleeping under a bednet the previous night. On average, the children attended 8 out of the 10 [range 1–10] active detection of infection visits.

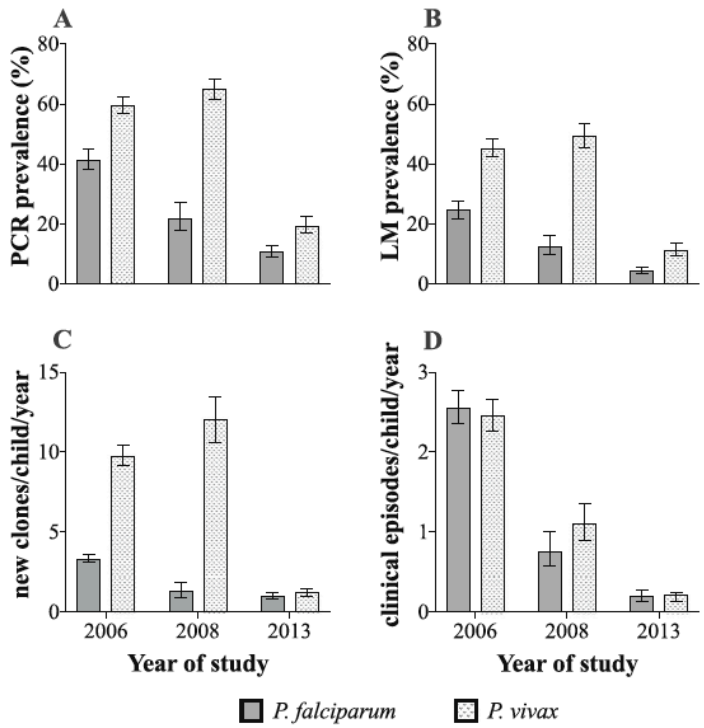

Fig. 2 Changing burden of malaria infections and illness across the different time-points of malaria control intensification in the study area. Legend: Impact of improved malaria control on prevalence of infections detectable by **a** polymerase chain reaction assay (PCR), **b** light microscopy (LM), **c** incidence of new blood-stage infections ($_{mol}$FOB) and **d** incidence of clinical malaria episodes. Error bars are 95% confidence intervals

Prevalence of infections during follow-up

Throughout the follow-up period, 47% children had at least one *P. falciparum* infection and 48% had at least one *P. vivax* infection (detected by PCR). Overall, the averaged prevalence of *P. vivax* was 19.9% by PCR and 10.8% by LM, while *P. falciparum* prevalence was 11.0% by PCR and 4.2% by LM. Sub-microscopic infections accounted for 64% of *P. falciparum* and 47% of *P. vivax* infections.

The prevalence of PCR-detectable infections varied markedly across the different areas (*Pf*: range 4.5–28.8%, *Pv*: range 6.0–45.2%; Table 1) with significantly higher risk of infection observed amongst children living in Sunuhu 1 and 2 compared to Ilahita 1, 2, 3, 4, 6 and 7 (*Pf* crude OR 8.49 (CI_{95} 6.14–11.8) $p < 0.001$, *Pv* 12.6 (CI_{95} 8.11–19.6) p value < 0.001); Additional file 1). Whereas the prevalence and the risk of *P. falciparum* infections also varied significantly over time (range 7.1–32.2%, $p < 0.0001$), *P. vivax* prevalence and risk was more stable over time (range 17.8–23.2%, $p = 0.1777$; see Table 1 and Additional file 1). The risk of both *P. falciparum* and *P. vivax* infections was higher in children experiencing a febrile illness in the last 2 weeks (*Pf*: crude OR 2.97 (CI_{95} 1.57–5.63) $p = 0.001$, *Pv* 1.68 (CI_{95} 1.06–2.66) $p = 0.028$), as well as those with an enlarged spleen (*Pf*: crude OR 2.25 (CI_{95} 1.23–4.11) $p = 0.009$, *Pv* 1.82 (CI_{95} 1.07–3.11) $p = 0.028$); see Additional file 1). The prevalence and risk of *P. falciparum* infections was also increased in children experiencing a concurrent febrile illness (crude OR 2.28 (CI_{95} 1.66–3.15) $p = 0.001$), increased linearly with age (crude OR 1.24 (CI_{95} 1.09–1.41) $p = 0.001$) but declined for every 1 g/dL increase in haemoglobin level (crude OR 0.72 (CI_{95} 0.64–0.80) $p < 0.001$; Additional file 1). Bednet use was associated with a reduced prevalence of infections for both species (*Pf* crude OR 0.58 (CI_{95} 0.27–1.29) $p = 0.182$, *Pv* 0.80 (CI_{95} 0.45–1.40) $p = 0.431$), but the very low number of non-users results in insufficient power. Having received recent antimalarial treatment was associated with a decrease in *P. vivax* (crude OR 0.36 (CI_{95} 0.15–0.85) $p = 0.021$; Additional file 1) prevalence and risk.

In multivariate analyses, area of residence, time of visit, age, haemoglobin level and the presence of a concurrent febrile illness remained independently associated with the

Table 1 Key predictors of infections due to *P. falciparum* and *P. vivax* as detected by qPCR in 2013

	P. falciparum				*P. vivax*			
	Observed positive (%; $n = 4363$)	OR	CI_{95}	p	Observed positive (%; $n = 4363$)	OR	CI_{95}	p
Areas of residence								
Ilahita 1–4, 6, 7	4.5		Reference group		6.1		Reference group	
Balanga and Balif	4.6	1.01	0.59–1.72	0.969	6.0	0.98	0.51–1.88	0.946
Kamanokor and Ilahita 5	12.9	2.29	1.38–3.80	0.001	38.0	9.22	5.55–15.3	< 0.001
Sunuhu 1 and 2	28.8	7.63	5.34–10.9	< 0.001	45.2	13.7	8.81–21.3	< 0.001
			$p < 0.0001$[a]				$p < 0.0001$[a]	
Age								
Linear		2.91	1.26–6.70	0.012		1.32	1.16–1.51	< 0.001
Quadratic		0.88	0.78–0.99	0.032				
ADI visit								
Enrolment	18.4		Reference group		23.2		Reference group	
Week 4	8.7	0.40	0.27–0.58	< 0.001	21.4	0.80	0.61–1.05	0.105
Week 8	8.7	0.39	0.19–0.46	< 0.001	21.3	0.85	0.65–1.11	0.238
Week 12	7.1	0.30	0.19–0.46	< 0.001	19.1	0.71	0.53–0.96	0.024
Week 16	8.1	0.34	0.22–0.53	< 0.001	17.8	0.65	0.49–0.88	0.004
Week 20	9.9	0.47	0.27–0.79	0.004	20.3	0.66	0.45–0.98	0.04
Week 24	7.6	0.31	0.19–0.52	< 0.001	21.8	0.83	0.59–1.19	0.312
Week 28	7.2	0.36	0.22–0.60	< 0.001	17.9	0.64	0.43–0.94	0.024
Week 32	8.8	0.40	0.25–0.65	< 0.001	18.1	0.57	0.40–0.83	0.004
Week 36	10.8	0.55	0.35–0.85	0.007	18.0	0.53	0.37–0.76	0.001
Week 40	32.2	3.20	2.15–4.74	< 0.001	22.9	0.82	0.56–1.19	0.298
			$p < 0.0001$[a]				$p = 0.0129$[a]	
Haemoglobin		0.65	0.57–0.74	< 0.001				
Recent antimalarial								
No	11.5				20.3			
Yes	21.3				15.0	0.34	0.17–0.71	0.004
Enlarged spleen								
No	10.8				19.2			
Yes	38.6				54.6	1.66	0.98–2.79	0.059
Febrile illness								
No	10.9				19.7			
Yes	25.0	1.84	1.30–2.62	0.001	29.2			
[b]2 weeks history of febrile illness								
No	11.5				20.0			
Yes	28.6	2.24	0.93–5.38	0.073	38.1	1.84	1.02–3.32	0.042

Multivariate GEE model-based estimates of risk of infection detected at each monthly active case detection visit time-point via backward selection of significant risk factors. *OR* multivariate adjusted odds ratio, CI_{95} 95% confidence interval, *p* *p* value, *ADI* active detection of infection. [a]Overall significance level for the variable estimated using Wald chi-square test. [b]Excluding febrile illness at the time of visit

presence of a *P. falciparum* infection (Table 1). Area of residence, time of visit, recent antimalarial use, age and having an episode of febrile illness in the previous 2 weeks were all associated with the risk of carrying a *P. vivax* infection (Table 1). Risk factors of LM-detectable infections were similar (see Additional file 2).

Molecular force of blood-stage infections in monthly intervals

Incidence of new blood-stage infections was determined for a total of 303.4 person-years of follow-up with each child at risk of acquiring new blood-stage infections for an average of 0.73 years during the cohort. The mean

$_{mol}$FOB for *P. falciparum* was 1.6 (CI_{95} 1.4–1.9) new infections per child per year-at-risk and 2.2 (CI_{95} 1.9–2.6) infections/child/year-at-risk for *P. vivax*.

The rate of acquiring new *P. falciparum* clones was higher in Sunuhu 1 and 2 compared to Ilahita 1, 2, 3, 4, 6 and 7 (*Pf* IRR 3.10 (CI_{95} 2.08–4.63) *p* value < 0.001) and also in those with recent antimalarial use (IRR 10.4 (CI_{95} 5.92–18.2) *p* value < 0.001, Table 2). Age was not associated with *P. falciparum* $_{mol}$FOB in multivariate analysis despite the significant linear association observed in the crude analysis. The *P. vivax* $_{mol}$FOB was increased in both Sunuhu 1 and 2 and Kamanokor and Ilahita 5 compared to Ilahita 1, 2, 3, 4, 6 and 7 (IRR 8.16 (CI_{95} 5.38–12.4) *p* value < 0.001 and 6.66 (CI_{95} 4.24–10.5) *p* value < 0.001, respectively), and also increased linearly with age (IRR 1.26 (CI_{95} 1.13–1.40) *p* value < 0.001, Table 2). Both *P. falciparum* and *P. vivax* incidence varied markedly over the follow-up time period (both $p < 0.0001$, Table 2).

Predictors of clinical malaria episodes

Over the 10 months of follow-up, a total of 366 febrile illness episodes were observed, of which 109 (30%) were associated with microscopically confirmed infections (IR, 0.36/child/year), with 51 *P. vivax* (any density: IR, 0.19) and 49 *P. falciparum* (any density: IR, 0.18) episodes. Another 7 were *P. falciparum* and *P. vivax* mixed infections (any density: IR 0.02), 2 were *P. malariae* (any density: IR, 0.07). Clinical episodes with high-density parasitaemia (≥ 2500 for *P. falciparum* and ≥ 500 for *non-falciparum* infections) accounted for 63.3% (35 *Pf*,

Table 2 Multivariate predictors of molecularly determined new *P. falciparum* and *P. vivax* blood-stage infections in 2013

	P. falciparum				*P. vivax*			
	IR	IRR	CI_{95}	p	IR	IRR	CI_{95}	p
Areas of residence								
Ilahita 1–4, 6,7	1.09		Reference group		0.65		Reference group	
Balanga and Balif	1.25	1.22	0.70–2.12	0.485	0.94	1.46	0.81–2.64	0.213
Kamanokor and Ilahita 5	1.82	1.61	0.92–2.80	0.096	4.83	6.66	4.24–10.5	< 0.001
Sunuhu 1 and 2	3.57	3.10	2.08–4.63	< 0.001	5.37	8.16	5.38–12.4	< 0.001
			$p < 0.0001$[a]				$p < 0.0001$[a]	
Age						1.26	1.13–1.40	< 0.001
ADI visit interval								
Enrolment–week 4	1.23		Reference group		3.20		Reference group	
Week 4–week 8	0.85	0.58	0.27–1.23	0.153	2.62	0.71	0.52–0.98	0.035
Week 8–week 12	0.22	0.15	0.06–0.38	< 0.001	1.60	0.44	0.30–0.63	< 0.001
Week 12–week 16	0.77	0.50	0.23–1.09	0.081	2.30	0.59	0.43–0.82	< 0.001
Week 16–week 20	1.23				3.20	0.87	0.60–1.25	0.446
Week 20–week 24	2.58	1.99	1.00–3.96	0.049	3.60			
Week 24–week 28	1.17	0.83	0.43–1.61	0.583	2.20	0.57	0.41–0.79	< 0.001
Week 28–week 32	1.13	0.84	0.43–1.64	0.602	1.98	0.48	0.34–0.69	< 0.001
Week 32–week 36	1.28	0.89	0.49–1.64	0.719	2.46	0.58	0.42–0.80	< 0.001
Week 36–week 40	7.19	5.55	3.33–9.25	< 0.001	2.26	0.56	0.39–0.79	< 0.001
			$p < 0.0001$[a]				$p < 0.0001$[a]	
Recent antimalarial use[b]	8.50	10.4	5.92–18.2	< 0.001	2.79			
Febrile illness	2.05				3.02			
2 weeks history of febrile illness[c]	2.11				2.31			
Haemoglobin								
≥ 10 g/dL	1.60				2.15			
9–9.9 g/dL	1.88				2.48			
≤ 9 g/dL	2.06				2.87			

Estimates from a multivariate negative binomial regression with GEE model predicting risk of acquiring new species-specific clones for *P. falciparum* and *P. vivax* in a 4-week interval when the child was considered at risk. A backward selection approach was used with the best fitting model consisting of the significant associations. *IR* incidence rate, *IRR* incidence rate ratio, CI_{95} 95% confidence interval, *p* *p* value, *g/dL* grams/decilitre, *ADI* active detection of infections. [a]Overall significance level for the variable estimated using Wald chi-square test. [b]Antimalarial treatment within 28 days before the start of the interval. [c]Excluding febrile illness at the time of visit

27 *Pv*, 7 *PfPv* mixed) of all the clinical episodes. There were no *P. ovale* clinical episodes observed.

The incidence of clinical *P. falciparum* episodes was significantly higher in Kamanokor, Ilahita 5 and Sunuhu 1/2 compared to Ilahita 1, 2, 3, 4, 6 and 7 (IRR 4.30 (CI_{95} 1.59–11.6) *p* value 0.004 and 8.15 (CI_{95} 3.40–19.6) *p* value < 0.001, respectively; Table 3). Each 1 g/dL increase in haemoglobin was associated with a 48% reduction in the incidence of clinical *P. falciparum* (CI_{95} 0.35–0.77, *p* value: 0.001, Table 3), and each 1-year increase in age was associated with a 38% increase in the rate of clinical *P. falciparum* (CI_{95} 1.10–1.73, *p* value: 0.006, Table 3). After adjustment for $_{mol}$FOB, all remained associated with the rate of clinical *P. falciparum* episodes, and a unit increase in $_{mol}$FOB (i.e. one new *P. falciparum* infection per child per year-at-risk) was associated with a 10% (CI_{95} 1.02–1.18, *p* value 0.008) increase in the rate of clinical *P. falciparum* infections (Table 3).

The rate of clinical *P. vivax* episodes was also significantly higher in Kamanokor, Ilahita 5 and Sunuhu 1/2 compared to Ilahita 1, 2, 3, 4, 6 and 7 (IRR 8.01 (CI_{95} 3.23–19.9) *p* value < 0.001 and 3.71 (CI_{95} 1.53–8.99) *p* value 0.004, respectively; Table 3). Each 1 g/dL increase in haemoglobin was associated with a 69% reduction in the rate of clinical *P. vivax* (CI_{95} 0.19–0.48, *p* value < 0.001). After adjustment for $_{mol}$FOB, only area of residence and haemoglobin remained associated with the rate of clinical *P. vivax* episodes (Table 3). A unit increase in $_{mol}$FOB (i.e. one new *P. vivax* infection per child per year-at-risk) was associated with a 17% (CI_{95} 1.09–1.25, *p* value < 0.001) increase in the rate of clinical *P. vivax* infections. Age was not associated with the rate of clinical *P. vivax* episodes, either before or after adjustment for $_{mol}$FOB.

Discussion

This is the first study in a *P. falciparum/P. vivax* co-endemic area and amongst very few studies globally [52] to examine the impact of improved malaria control on the epidemiology of malaria in young children using longitudinal cohorts rather than the widely used nationwide and community household surveys and routine health information systems [6, 33, 37]. Longitudinal cohort studies allow for a detailed investigation into the dynamics of infection, and illness, as well as the rate of acquiring new infections ($_{mol}$FOB) and clinical illness over time.

By analysing these metrics in three consecutive longitudinal cohorts in young PNG children, we demonstrate a differential impact of control interventions on *P. vivax* compared to *P. falciparum* that may be overlooked in routine surveillance. Following the first LLIN distribution, the prevalence of *P. falciparum* infection and both *P. falciparum* and *P. vivax* clinical episodes declined immediately and continuously across the time period of the three cohorts. Contrastingly, the prevalence and force of *P. vivax* blood-stage infections did not decline, remaining initially relatively high with a substantial decline only evident in the most recent cohort that was conducted 5 years after commencement of intensified control in the area. These observations confirm that key biological differences between the two species render them differentially susceptible to standard control tools such as LLINs and case management, highlighting the need for *P. vivax*-focused interventions in co-endemic regions.

Notably, the relationship between transmission and $_{mol}$FOB differs for *P. falciparum* and *P. vivax*. *P. falciparum* metrics are directly linked to blood-stage infections, which are always mosquito-derived, hence closely reflecting current levels of transmission. The reductions

Table 3 Key predictors of clinical malaria episodes due to *P. falciparum* and *P. vivax* in 2013

	P. falciparum					*P. vivax*				
		Base model		$_{mol}$FOB adjusted			Base model		$_{mol}$FOB adjusted	
	IR	IRR (CI_{95})	p	IRR (CI_{95})	p	IR	IRR (CI_{95})	p	IRR (CI_{95})	p
Areas of residence										
Ilahita 1–4, 6, 7	0.05		Reference group			0.06		Reference group		
Balanga & Balif	0.03	0.63 (0.13–3.14)	0.575	0.56 (0.11–2.81)	0.485	0.06	1.15 (0.32–4.06)	0.833	1.08 (0.30–3.91)	0.909
Kamanokor & Ilahita 5	0.25	4.30 (1.59–11.6)	0.004	3.96 (1.46–10.8)	0.007	0.52	8.01 (3.23–19.9)	< 0.001	3.86 (1.44–10.3)	0.007
Sunuhu 1&2	0.50	8.15 (3.40–19.6)	< 0.001	6.48 (2.65–15.8)	< 0.001	0.33	3.71 (1.53–8.99)	0.004	2.00 (0.77–5.17)	0.152
		$p < 0.0001^a$		$p < 0.0001^a$			$p < 0.0001^a$		$p < 0.0234^a$	
Age		1.38 (1.10–1.73)	0.006	1.30 (1.03–1.64)	0.026					
Haemoglobin		0.52 (0.35–0.77)	0.001	0.61 (0.40–0.92)	0.017		0.31 (0.19–0.48)	< 0.001	0.38 (0.24–0.59)	< 0.001
FOB[b]				1.10 (1.02–1.18)	0.008				1.17 (1.09–1.25)	< 0.001

Multivariate negative binomial regression model-based estimates predicting risk of clinical *P. falciparum* and *P. vivax*. Backward selection approach was used to derive significant associations. Base models included all variables except $_{mol}$FOB. Incidence is based on aggregated clinical data for entire 10-month study period thus precluding analysis of recent antimalarial treatment as a covariate. Bednet use was not analysed due to non-converge of data when included into models. [a]Overall significance level for the variable estimated using wald chi-square test; $_{mol}$FOB: molecular force of blood-stage infections; [b]$_{mol}$FOB was included as a rate; IR: Incidence rate; IRR: Incidence rate ratio. CI_{95} 95% confidence interval; *p p* value

in *P. falciparum* $_{mol}$FOB observed across these three cohorts confirm reductions in *P. falciparum* prevalence and EIR observed through monitoring and evaluation of the national programme [37, 38]. Due to the biological ability of *P. vivax* to remain dormant in liver cells as hypnozoites and to serve as a continuing source of relapsing infections, *P. vivax* metrics are not able to differentiate between mosquito-derived and relapsing infections and therefore do not reflect active transmission as closely as *P. falciparum* metrics. This is particularly relevant in PNG, where *P. vivax* is the predominant species detectable in young children and relapses account for more than 50–80% of *P. vivax* infections in preschool and primary school children [14, 41]. As a consequence, the *P. vivax* $_{mol}$FOB is a composite measure reflecting the joint burden of new, mosquito-derived and relapsing infections [42, 43]. This metric therefore reveals a high burden of persisting low-density relapsing infections in young children, contrasting results of nationwide surveys that showed a comparable decline in *P. falciparum* and *P. vivax* prevalence detectable by LM in both children under 5 years and the general population [37].

Given the persistence of a high burden of *P. vivax* infections following the initial LLIN distribution, the observation that the burden of clinical *P. vivax* dropped and continued to decline over the years of intensification marked a striking difference. Clinical immunity to *P. vivax* is acquired rapidly, even under relatively low transmission [15]. In malaria therapy patients, only few mild febrile symptoms were observed when they were re-infected with a homologous infection [53]. As relapsing infections are either genetically identical or meiotic siblings of the primary infection [54, 55], it is generally thought that clinical episodes are more likely to be caused by new mosquito-bite-acquired infections. Considering that reduction in transmission results in the acquisition of fewer new mosquito-derived infections, the observation that the immediate impact of LLIN was exclusively on incidence of *P. vivax* clinical episodes and not on risk of infection strongly suggests that the majority of clinical episodes due to *P. vivax* may indeed be associated with mosquito-derived rather than relapsing infections.

The observation of a delayed impact of LLIN scale-up on *P. vivax* compared to *P. falciparum* blood-stage infections in co-endemic areas is important evidence for control programmes. It suggests that the large reservoir of hypnozoites acquired when transmission is high (prior to scale-up of control) gives rise to a sufficient burden of relapsing infections that may be transmissible, although often not symptomatic, such that minimal impact may be observed on *P. vivax* prevalence in the years immediately following scale-up even though transmission is being reduced. This highlights the importance of strengthening the implementation of radical cure of *P. vivax* in order to accelerate reduction in the burden of *P. vivax* [56]. Reluctance to prescribe primaquine without G6PD testing and poor adherence to the 14-day regime are major issues limiting the effectiveness of *P. vivax* radical cure in many settings, including PNG.

The observed impact on clinical incidence and the comparable longer-term reduction in *P. vivax* and *P. falciparum* burden of infections does however provide reassurance that vector control with LLINs can reduce the burden of *P. vivax*, at least in countries where malaria transmission is largely peri-domestic [57], even if coverage needs to be maintained for a longer period of time before the full effectiveness is observed. Interestingly, in many countries in Asia and the Americas where dramatic shifts to *P. vivax* predominance have been observed, programmes rely upon clinical case management (often with poor coverage of anti-hypnozoite therapy) as their primary malaria control strategy [2, 58] and/or have highly exophilic vectors with transmission occurring mainly in forested areas where LLIN and other traditional vector control tools such as indoor-residual spraying have limited efficacy [59–61].

During the period of reduced transmission in 2013, the individual level of exposure to new blood-stage infections ($_{mol}$FOB) and the geographical location of the child's residence were the two key determinants of infection and illness. In the previous 2006 and 2008 cohorts, age-dependent decreases in the incidence of clinical *P. vivax* were observed [40, 41], suggestive of rapid acquisition of clinical immunity due to high *P. vivax* $_{mol}$FOB during those periods. Conversely, we did not observe any age association in 2013, which may be explained by the substantial decline in the force of *P. vivax* infection.

As documented in other settings, declining transmission leads to increasing transmission heterogeneity [60, 62] and an increasing proportion of asymptomatic low-density infections [6–8]. In 2013, over two thirds all PCR-detected infections were sub-microscopic and the risk of clinical malaria was highly dependent on where the child lived, with higher risk of clinical illness observed in areas with higher force of infection. This pronounced spatial heterogeneity in the risk of infections and malaria illness has also been observed in the two previous cohorts [40–43] indicating that despite the declining transmission between 2006 and 2013, the high burden areas remained stable. In particular, we observed marked geographical clustering of infections and illness in two areas, Sunuhu 1/2 and Kamanokor/Ilahita 5 in 2013, the same geographical locations that were identified as highest burden areas before [40, 42, 43] and during scale-up of interventions [41]. The persistence of high-burden areas such as these despite the ongoing implementation of control interventions is supported by observations

made elsewhere [29, 30] and strengthens the rationale for surveillance strategies that target interventions to these potential transmission hotspots in order to accelerate control. Such strategies will clearly need to identify the characteristics of hotspots that fuel sustained transmission and address the diagnostic challenge imposed by asymptomatic, low-density infections [5, 63–65].

A limitation of this study is the differences in the study designs, sampling schedules and the length of follow-up as well as the non-uniform structuring of the individual datasets. Consequently, each cohort was analysed separately and the calculated burden of malaria infection and disease were compared between the cohorts to determine the patterns of decline for *P. falciparum* and *P. vivax* across the intervention time-points. As such, we did not statistically test the differential patterns of decline exhibited by *P. falciparum* and *P. vivax* across the intervention time-points. However, confidence intervals of the prevalence, $_{mol}$FOB and clinical incidence across the three cohorts are provided illustrating when differences are statistically significant. It should also be noted that the cohorts were conducted in the same study area with a stable population and the cohorts were age-matched hence minimising variation between the cohorts.

Lastly, the impact of malaria control interventions on transmission are a function of diverse social and ecological settings leading to differences in mosquito abundance, mosquito behaviour and human-mosquito interaction. While improvements in the quality of housing have occurred over the past decade in many urban areas of PNG, housing for PNG's rural majority remains largely dependent on bush material. Higher quality of housing and socio-economic status was associated with reduced risk of malaria in previous studies in PNG [37]; however, such data is not available from the child cohorts analysed. The association between weather patterns and long-term malaria trends in PNG have also been investigated using site-specific satellite weather variables and did not explain variations in observed malaria incidence over time (Rodríguez-Rodríguez & Hetzel, unpublished data). Further work understanding the dynamic, complex and responsive ecological niches driving ongoing malaria transmission in certain areas of PNG will inform the development of targeted control and elimination efforts.

Conclusions

Scale-up of standard malaria control interventions in PNG substantially reduced the burden of malaria infection and disease in the most vulnerable 1–5-year-old age group. Data presented here suggests comparable reductions in new mosquito-derived infections for both *P. falciparum* and *P. vivax* but a delayed impact on *P. vivax* relapsing infections due to the previously acquired reservoir of hypnozoites. We confirm the effectiveness of sustained implementation of LLINs and case management in reducing transmission of both species in PNG but highlight the critical need to strengthen case management, radical cure, surveillance and targeted intervention strategies in order to accelerate control of malaria in co-endemic settings.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12916-019-1456-9.

Additional file 1: Table S1. Bivariate associations between risk factors and the prevalence, $_{mol}$FOB and clinical incidence. Estimates of bivariate associations calculated via generalised estimating equation (GEE) models for prevalence and molecular force of blood-stage ($_{mol}$FOB) infections and negative binomial regression model used for clinical malaria episodes. Recent antimalarial use was not tested in the model for clinical malaria episodes due to aggregated clinical data. [a]Age in years at enrolment was used for clinical malaria incidence while at the start of interval was used for $_{mol}$FOB. [b]Comparison group; For multilevel variables, comparison group estimates are presented as odds or incidence rate. PCR: Polymerase chain reaction assay; LM: light microscopy; OR: odds ratio; CI_{95}: 95% confidence interval; IRR: incidence rate ratio.

Additional file 2: Table S2. Key predictors of the prevalence of infections due to *P. falciparum* and *P. vivax* as diagnosed by light microscopy. Multivariate generalised estimating equation (GEE) model-based estimates of the risk of infection detected at each monthly active detection of infection visits via backward selection of significant risk factors. [a]Overall significance level for the variable estimated using wald chi-squared test. AOR: multivariate adjusted odds ratio. CI_{95}: 95% confidence interval; [b]Excluding febrile illness at the time of visit; Data for observed positive are %.

Abbreviations

AL: Artemether-lumefantrine; CI_{95}: 95% confidence interval; DNA: Deoxyribonucleic acid; GEE: Generalised estimating equations; GPS: Global Positioning System; IR: Incidence rate; IRR: Incidence rate ratio; LLIN: Long-lasting insecticide treated nets; $_{mol}$FOB: Molecular force of blood-stage infection; OR: Odds ratio; *p*: *p* value; PCR: Polymerase chain reaction; Pf: *P. falciparum*; PNG: Papua New Guinea; Pv: *P. vivax*; qPCR: Quantitative polymerase chain reaction; RDT: Rapid diagnostic test; spp.: Species

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Authors' contributions

LJR, IM and JK conceived and designed the study; MOK, LJR, MS and DM supervised enrolment and follow-up of participants; MOK, LJR, IM, SZ and TO analysed and interpreted the data: MOK, LJR, SZ and IM wrote the first draft of the manuscript. All authors critically revised the manuscript and read and approved the final draft.

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Availability of data and materials
Anonymised data is available upon reasonable request by contacting the PNG Medical Research Advisory Committee and the PNG Institute of Medical Research IRB. The contact is Dr. William Pomat, secretary PNGIMR IRB: William.Pomat@pngimr.org.pg.

Ethics approval and consent to participate
Ethical approvals for the three cohort studies were granted by Papua New Guinea Institute of Medical Research Institutional Review Board (2006, 09.24: 2008, 07.20; 2013, 11.16) and PNG Medical Research and Advisory Committee [2006, 05.19; 2008, 07.34; 2013, 11.21]. Voluntary written informed consent was obtained from the parents or guardians of the children following community awareness and individual study information sessions.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References

1. W.H.O. World Malaria Report 2018. Geneva: World Health Organization; [https://apps.who.int/iris/bitstream/handle/10665/275867/9789241565653-eng.pdf?ua=1. Accessed: 01 May 2019.
2. Coura JR, Suarez-Mutis M, Ladeia-Andrade S. A new challenge for malaria control in Brazil: asymptomatic Plasmodium infection--a review. Mem Inst Oswaldo Cruz. 2006;101(3):229–37.
3. Nguitragool W, Karl S, White M, Koepfli C, Felger I, Singhasivanon P, et al. Highly heterogeneous residual malaria risk in western Thailand. Int J Parasitol. 2019;49(6):455–62.
4. W.H.O. W.P.R.O. Meeting Report: Malaria Programme Managers Meeting to review progress on implementation of the regional action framework for malaria control and elimmination in the Western Pacific 2016–2020 [https://iris.wpro.who.int/bitstream/handle/10665.1/14326/RS-2018-GE-31-PHL-eng.pdf. Accessed: 02 May 2019.
5. Harris I, Sharrock WW, Bain LM, Gray KA, Bobogare A, Boaz L, et al. A large proportion of asymptomatic Plasmodium infections with low and sub-microscopic parasite densities in the low transmission setting of Temotu Province, Solomon Islands: challenges for malaria diagnostics in an elimination setting. Malar J. 2010;9:254.
6. Koepfli C, Ome-Kaius M, Jally S, Malau E, Maripal S, Ginny J, et al. Sustained malaria control over an 8-year period in Papua New Guinea: the challenge of low-density asymptomatic Plasmodium infections. J Infect Dis. 2017; 216(11):1434–43.
7. Waltmann A, Darcy AW, Harris I, Koepfli C, Lodo J, Vahi V, et al. High rates of asymptomatic, sub-microscopic Plasmodium vivax infection and disappearing Plasmodium falciparum malaria in an area of low transmission in Solomon Islands. PLoS Negl Trop Dis. 2015;9(5):e0003758.
8. Sattabongkot J, Suansomjit C, Nguitragool W, Sirichaisinthop J, Warit S, Tiensuwan M, et al. Prevalence of asymptomatic Plasmodium infections with sub-microscopic parasite densities in the northwestern border of Thailand: a potential threat to malaria elimination. Malar J. 2018;17(1):329.
9. Ernst KC, Adoka SO, Kowuor DO, Wilson ML, John CC. Malaria hotspot areas in a highland Kenya site are consistent in epidemic and non-epidemic years and are associated with ecological factors. Malar J. 2006;5:78.
10. Bousema T, Drakeley C, Gesase S, Hashim R, Magesa S, Mosha F, et al. Identification of hot spots of malaria transmission for targeted malaria control. J Infect Dis. 2010;201(11):1764–74.
11. Bousema T, Griffin JT, Sauerwein RW, Smith DL, Churcher TS, Takken W, et al. Hitting hotspots: spatial targeting of malaria for control and elimination. PLoS Med. 2012;9(1):e1001165.
12. Khantikul N, Butraporn P, Kim HS, Leemingsawat S, Tempongko MA, Suwonkerd W. Adherence to antimalarial drug therapy among vivax malaria patients in northern Thailand. J Health Popul Nutr. 2009;27(1):4–13.
13. Takeuchi R, Lawpoolsri S, Imwong M, Kobayashi J, Kaewkungwal J, Pukrittayakamee S, et al. Directly-observed therapy (DOT) for the radical 14-day primaquine treatment of Plasmodium vivax malaria on the Thai-Myanmar border. Malar J. 2010;9:308.
14. Laman M, Aipit S, Bona C, Siba PM, Robinson LJ, Manning L, et al. Ultrasonographic assessment of splenic volume at presentation and after anti-malarial therapy in children with malarial anaemia. Malar J. 2015;14:219.
15. Mueller I, Galinski MR, Tsuboi T, Arevalo-Herrera M, Collins WE, King CL. Natural acquisition of immunity to Plasmodium vivax: epidemiological observations and potential targets. Adv Parasitol. 2013;81:77–131.
16. Cheng Q, Cunningham J, Gatton ML. Systematic review of sub-microscopic P. vivax infections: prevalence and determining factors. PLoS Negl Trop Dis. 2015;9(1):e3413.
17. Hofmann NE, Gruenberg M, Nate E, Ura A, Rodriguez-Rodriguez D, Salib M, et al. Assessment of ultra-sensitive malaria diagnosis versus standard molecular diagnostics for malaria elimination: an in-depth molecular community cross-sectional study. Lancet Infect Dis. 2018; 18(10):1108–16.
18. Koepfli C, Schoepflin S, Bretscher M, Lin E, Kiniboro B, Zimmerman PA, et al. How much remains undetected? Probability of molecular detection of human plasmodia in the field. PLoS One. 2011;6(4):e19010.
19. McKenzie FE, Jeffery GM, Collins WE. Plasmodium vivax blood-stage dynamics. J Parasitol. 2002;88(3):521–35.
20. Jeffery GM. The infection of mosquitoes by Plasmodium vivax (Chesson strain) during the early primary parasitemias. Am J Trop Med Hyg. 1952;1(4): 612–7.
21. Bockarie MJ, Alexander N, Bockarie F, Ibam E, Barnish G, Alpers M. The late biting habit of parous Anopheles mosquitoes and pre-bedtime exposure of humans to infective female mosquitoes. Trans R Soc Trop Med Hyg. 1996; 90(1):23–5.
22. Bockarie MJ, Dagoro H. Are insecticide-treated bednets more protective against Plasmodium falciparum than Plasmodium vivax-infected mosquitoes? Malar J. 2006;5:15.
23. Greenwood BM. The microepidemiology of malaria and its importance to malaria control. Trans R Soc Trop Med Hyg. 1989;83(Suppl):25–9.
24. Strickland GT, Zafar-Latif A, Fox E, Khaliq AA, Chowdhry MA. Endemic malaria in four villages of the Pakistani province of Punjab. Trans R Soc Trop Med Hyg. 1987;81(1):36–41.
25. Burkot TR, Graves PM, Paru R, Wirtz RA, Heywood PF. Human malaria transmission studies in the Anopheles punctulatus complex in Papua New Guinea: sporozoite rates, inoculation rates, and sporozoite densities. Am J Trop Med Hyg. 1988;39(2):135–44.
26. Hii JL, Smith T, Mai A, Mellor S, Lewis D, Alexander N, et al. Spatial and temporal variation in abundance of Anopheles (Diptera:Culicidae) in a malaria endemic area in Papua New Guinea. J Med Entomol. 1997;34(2): 193–205.
27. Muller I, Bockarie M, Alpers M, Smith T. The epidemiology of malaria in Papua New Guinea. Trends Parasitol. 2003;19(6):253–9.
28. Mwesigwa J, Okebe J, Affara M, Di Tanna GL, Nwakanma D, Janha O, et al. On-going malaria transmission in the Gambia despite high coverage of control interventions: a nationwide cross-sectional survey. Malar J. 2015;14:314.
29. Roca-Feltrer A, Kwizombe CJ, Sanjoaquin MA, Sesay SS, Faragher B, Harrison J, et al. Lack of decline in childhood malaria, Malawi, 2001-2010. Emerg Infect Dis. 2012;18(2):272–8.
30. Bautista CT, Chan AS, Ryan JR, Calampa C, Roper MH, Hightower AW, et al. Epidemiology and spatial analysis of malaria in the Northern Peruvian Amazon. Am J Trop Med Hyg. 2006;75(6):1216–22.

31. Jagannathan P, Muhindo MK, Kakuru A, Arinaitwe E, Greenhouse B, Tappero J, et al. Increasing incidence of malaria in children despite insecticide-treated bed nets and prompt anti-malarial therapy in Tororo. Uganda Malar J. 2012;11:435.
32. Kazura JW, Siba PM, Betuela I, Mueller I, et al. Research Challenges and Gaps in Malaria Knowledge in Papua New Guinea. Acta Trop. 2012;121(3):274–280.
33. Hetzel MW, Morris H, Tarongka N, Barnadas C, Pulford J, Makita L, et al. Prevalence of malaria across Papua New Guinea after initial roll-out of insecticide-treated mosquito nets. Tropical Med Int Health. 2015;20(12):1745–55.
34. Genton B, al-Yaman F, Beck HP, Hii J, Mellor S, Narara A, et al. The epidemiology of malaria in the Wosera area, East Sepik Province, Papua New Guinea, in preparation for vaccine trials. I. Malariometric indices and immunity. Ann Trop Med Parasitol. 1995;89(4):359–76.
35. Hetzel MW, Choudhury AA, Pulford J, Ura Y, Whittaker M, Siba PM, et al. Progress in mosquito net coverage in Papua New Guinea. Malar J. 2014;13:242.
36. Hetzel MW, Gideon G, Lote N, Makita L, Siba PM, Mueller I. Ownership and usage of mosquito nets after four years of large-scale free distribution in Papua New Guinea. Malar J. 2012;11:192.
37. Hetzel MW, Pulford J, Ura Y, Jamea-Maiasa S, Tandrapah A, Tarongka N, et al. Insecticide-treated nets and malaria prevalence, Papua New Guinea, 2008-2014. Bull World Health Organ. 2017;95(10):695–705B.
38. Reimer LJ, Thomsen EK, Koimbu G, Keven JB, Mueller I, Siba PM, et al. Malaria transmission dynamics surrounding the first nationwide long-lasting insecticidal net distribution in Papua New Guinea. Malar J. 2016;15:25.
39. Hofmann NE, Karl S, Wampfler R, Kiniboro B, Teliki A, Iga J, et al. The complex relationship of exposure to new Plasmodium infections and incidence of clinical malaria in Papua New Guinea. Elife. 2017;6:e23708.
40. Lin E, Kiniboro B, Gray L, Dobbie S, Robinson L, Laumaea A, et al. Differential patterns of infection and disease with P. falciparum and P. vivax in young Papua New Guinean children. PLoS One. 2010;5(2):e9047.
41. Betuela I, Rosanas-Urgell A, Kiniboro B, Stanisic DI, Samol L, de Lazzari E, et al. Relapses contribute significantly to the risk of Plasmodium vivax infection and disease in Papua New Guinean children 1-5 years of age. J Infect Dis. 2012;206(11):1771–80.
42. Koepfli C, Colborn KL, Kiniboro B, Lin E, Speed TP, Siba PM, et al. A high force of plasmodium vivax blood-stage infection drives the rapid acquisition of immunity in Papua new guinean children. PLoS Negl Trop Dis. 2013;7(9):e2403.
43. Mueller I, Schoepflin S, Smith TA, Benton KL, Bretscher MT, Lin E, et al. Force of infection is key to understanding the epidemiology of Plasmodium falciparum malaria in Papua New Guinean children. Proc Natl Acad Sci U S A. 2012;109(25):10030–5.
44. Hay SI, Guerra CA, Gething PW, Patil AP, Tatem AJ, Noor AM, et al. A world malaria map: Plasmodium falciparum endemicity in 2007. PLoS Med. 2009;6(3):e1000048.
45. Mueller I, Widmer S, Michel D, Maraga S, McNamara DT, Kiniboro B, et al. High sensitivity detection of Plasmodium species reveals positive correlations between infections of different species, shifts in age distribution and reduced local variation in Papua New Guinea. Malar J. 2009;8:41.
46. Mehlotra RK, Lorry K, Kastens W, Miller SM, Alpers MP, Bockarie M, et al. Random distribution of mixed species malaria infections in Papua New Guinea. Am J Trop Med Hyg. 2000;62(2):225–31.
47. Paediatric society of PNG. Standard treatment for common illness of children in Papua New Guinea: a manual for nurses, community health workers, health extension officers, and doctors. 8th ed.2006.
48. Paediatric society of PNG. Standard treatment for common illness of children in Papua New Guinea: a manual for nurses, community health workers, health extension officers, and doctors 9th ed.2011.
49. Rosanas-Urgell A, Mueller D, Betuela I, Barnadas C, Iga J, Zimmerman PA, et al. Comparison of diagnostic methods for the detection and quantification of the four sympatric Plasmodium species in field samples from Papua New Guinea. Malar J. 2010;9:361.
50. Wampfler R, Mwingira F, Javati S, Robinson L, Betuela I, Siba P, et al. Strategies for detection of Plasmodium species gametocytes. PLoS One. 2013;8(9):e76316.
51. White MT, Karl S, Koepfli C, Longley RJ, Hofmann NE, Wampfler R, et al. Plasmodium vivax and Plasmodium falciparum infection dynamics: re-infections, recrudescences and relapses. Malar J. 2018;17(1):170.
52. Kamya MR, Arinaitwe E, Wanzira H, Katureebe A, Barusya C, Kigozi SP, et al. Malaria transmission, infection, and disease at three sites with varied transmission intensity in Uganda: implications for malaria control. Am J Trop Med Hyg. 2015;92(5):903–12.
53. Collins WE, Jeffery GM, Roberts JM. A retrospective examination of reinfection of humans with Plasmodium vivax. Am J Trop Med Hyg. 2004;70(6):642–4.
54. White NJ. Determinants of relapse periodicity in Plasmodium vivax malaria. Malar J. 2011;10:297.
55. Chen N, Auliff A, Rieckmann K, Gatton M, Cheng Q. Relapses of Plasmodium vivax infection result from clonal hypnozoites activated at predetermined intervals. J Infect Dis. 2007;195(7):934–41.
56. Wells TN, Burrows JN, Baird JK. Targeting the hypnozoite reservoir of Plasmodium vivax: the hidden obstacle to malaria elimination. Trends Parasitol. 2010;26(3):145–51.
57. Cooper RD, Waterson DG, Frances SP, Beebe NW, Sweeney AW. Speciation and distribution of the members of the Anopheles punctulatus (Diptera: Culicidae) group in Papua New Guinea. J Med Entomol. 2002;39(1):16–27.
58. Alves FP, Gil LH, Marrelli MT, Ribolla PE, Camargo EP, Da Silva LH. Asymptomatic carriers of Plasmodium spp. as infection source for malaria vector mosquitoes in the Brazilian Amazon. J Med Entomol. 2005;42(5):777–9.
59. Somboon P, Lines J, Aramrattana A, Chitprarop U, Prajakwong S, Khamboonrua C. Entomological evaluation of community-wide use of lambdacyhalothrine-impregnated bed nets against malaria in a border area of north-west Thailand. Trans R Soc Trop Med Hyg. 1995;89:248–54.
60. Cui L, Yan G, Sattabongkot J, Cao Y, Chen B, Chen X, et al. Malaria in the greater Mekong subregion: heterogeneity and complexity. Acta Trop. 2012;121(3):227–39.
61. Sinka ME, Bangs MJ, Manguin S, Chareonviriyaphap T, Patil AP, Temperley WH, et al. The dominant Anopheles vectors of human malaria in the Asia-Pacific region: occurrence data, distribution maps and bionomic precis. Parasit Vectors. 2011;4:89.
62. Cook J, Grignard L, Al-Eryani S, Al-Selwei M, Mnzava A, Al-Yarie H, et al. High heterogeneity of malaria transmission and a large sub-patent and diverse reservoir of infection in Wusab as Safil district, Republic of Yemen. Malar J. 2016;15:193.
63. Okell LC, Ghani AC, Lyons E, Drakeley CJ. Submicroscopic infection in Plasmodium falciparum-endemic populations: a systematic review and meta-analysis. J Infect Dis. 2009;200(10):1509–17.
64. Okell LC, Bousema T, Griffin JT, Ouedraogo AL, Ghani AC, Drakeley CJ. Factors determining the occurrence of submicroscopic malaria infections and their relevance for control. Nat Commun. 2012;3:1237.
65. Lindblade KA, Steinhardt L, Samuels A, Kachur SP, Slutsker L. The silent threat: asymptomatic parasitemia and malaria transmission. Expert Rev Anti-Infect Ther. 2013;11(6):623–39.

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Table 5.S1: Crude associations between the risk factors and the prevalence of infections due to *P. falciparum* and *P. falciparum* as detected by PCR and LM.

	Prevalence							
	P. falciparum				*P. vivax*			
	PCR		LM		PCR		LM	
	OR/Odds [CI_{95}]	p-value	OR/Odds [CI_{95}]	p-value	OR/Odds [CI_{95}]	p-value	OR/Odds [CI_{95}]	p-value
Covariates								
Areas of residence								
[b]Ilahita 1,2,3,4,6,7	0.05 [0.04 - 0.06]	<0.001	0.01 [0.005 - 0.015]	<0.001	0.07 [0.04 - 0.10]	<0.001	0.03 [0.02 - 0.05]	<0.001
Balanga & Balif	1.04 [0.59 - 1.85]	0.885	1.46 [0.33 - 6.48]	0.618	0.99 [0.52 - 1.88]	0.983	0.61 [0.25 - 1.50]	0.284
Kamanokor & Ilahita 5	3.04 [1.87 - 4.92]	<0.001	5.75 [2.56 - 12.9]	<0.001	9.36 [5.54 - 15.8]	<0.001	12.0 [6.03 - 23.9]	<0.001
Sunuhu 1&2	8.49 [6.14 - 11.8]	<0.001	19.7 [10.6 - 36.8]	<0.001	12.6 [8.11 - 19.6]	<0.001	11.7 [6.23 - 21.9]	<0.001
	[a]Overall p<0.0001		[a]Overall p<0.0001		[a]Overall p<0.0001		[a]Overall p<0.0001	
Age in years (linear)	1.24 [1.09 - 1.41]	0.001	1.13 [0.94 - 1.36]	0.183	1.11 [0.98 - 1.26]	0.109	0.96 [0.84 - 1.10]	0.554
Haemoglobin level	0.72 [0.64 - 0.80]	<0.001	0.45 [0.38 - 0.54]	<0.001	0.98 [0.90 - 1.07]	0.655	0.94 [0.83 - 1.07]	0.331
Haemoglobin categories								
[b]≥0 grams/dL	0.10 [0.08 - 0.12]	<0.001	0.02 [0.01 - 0.03]	<0.001	0.25 [0.20 - 0.30]	<0.001	0.11 [0.09 - 0.14]	<0.001
9 – 9.9 grams/dL	1.29 [1.04 - 1.60]	0.022	2.02 [1.36 - 3.00]	<0.001	0.99 [0.86 - 1.13]	0.832	1.00 [0.81 - 1.23]	0.975
≤ 9 grams/dL	2.11 [1.64 - 2.72]	<0.001	5.70 [3.54 - 9.19]	<0.001	1.18 [0.96 - 1.44]	0.114	1.29 [1.05 - 1.84]	0.023
	[a]Overall p<0.0001		[a]Overall p<0.0001		[a]Overall p=0.1777		[a]Overall p=0.0344	
Active detection of infection (ADI) timepoint								

[b]Enrolment	0.23 [0.18 - 0.29]	<0.001	0.12 [0.08 - 0.16]	<0.001	0.30 [0.24 - 0.38]	<0.001	0.17 [0.13 - 0.22]	<0.001
Week 4 / Interval 1	0.42 [0.31 - 0.57]	<0.001	0.51 [0.34 - 0.75]	0.001	0.89 [0.73 - 1.08]	0.222	1.02 [0.76 - 1.37]	0.88
Week 8 / Interval 2	0.42 [0.31 - 0.58]	<0.001	0.32 [0.19 - 0.53]	<0.001	0.90 [0.74 - 1.10]	0.307	1.05 [0.78 - 1.42]	0.749
Week 12 / Interval 3	0.33 [0.23 - 0.48]	<0.001	0.19 [0.10 - 0.38]	<0.001	0.78 [0.62 - 0.97]	0.025	0.88 [0.64 - 1.22]	0.458
Week 16 / Interval 4	0.39 [0.27 - 0.56]	<0.001	0.31 [0.18 - 0.53]	<0.001	0.75 [0.60 - 0.93]	0.009	0.71 [0.51 - 0.99]	0.040
Week 20 / Interval 5	0.46 [0.30 - 0.71]	<0.001	0.25 [0.13 - 0.49]	<0.001	0.82 [0.61 - 1.10]	0.178	0.52 [0.36 - 0.77]	0.001
Week 24 / Interval 6	0.36 [0.24 - 0.54]	<0.001	0.31 [0.17 - 0.57]	<0.001	0.93 [0.72 - 1.20]	0.569	0.53 [0.35 - 0.81]	0.003
Week 28 / Interval 7	0.38 [0.26 - 0.57]	<0.001	0.40 [0.24 - 0.69]	0.001	0.78 [0.60 - 1.02]	0.071	0.82 [0.57 - 1.18]	0.295
Week 32 / Interval 8	0.42 [0.29 - 0.61]	<0.001	0.29 [0.16 - 0.53]	<0.001	0.72 [0.55 - 0.95]	0.018	0.65 [0.43 - 0.96]	0.033
Week 36 / Interval 9	0.54 [0.38 - 0.76]	<0.001	0.46 [0.28 - 0.75]	0.002	0.72 [0.55 - 0.95]	0.018	0.54 [0.36 - 0.80]	0.002
Week 40 / Interval 10	2.10 [1.59 - 2.78]	<0.001	0.77 [0.49 - 1.19]	0.231	0.99 [0.77 - 1.28]	0.954	0.39 [0.25 - 0.60]	<0.001
	[a]Overall p<0.0001		[a]Overall p<0.0001		[a]Overall p<0.0001		[a]Overall p<0.0001	
Bed net use	0.58 [0.27 - 1.29]	0.182	0.64 [0.13 - 3.06]	0.578	0.80 [0.45 - 1.40]	0.431	1.08 [0.38 - 3.01]	0.898
Febrile illness	2.28 [1.66 - 3.15]	<0.001	4.60 [3.09 - 6.83]	<0.001	1.07 [0.78 - 1.47]	0.663	1.27 [0.83 - 1.95]	0.265
History of febrile illness	2.97 [1.57 - 5.63]	0.001	3.57 [1.42 - 8.99]	0.007	1.68 [1.06 - 2.66]	0.028	1.87 [0.86 - 4.07]	0.116
Enlarged spleen	2.25 [1.23 - 4.11]	0.009	3.28 [1.55 - 6.93]	0.002	1.82 [1.07 - 3.11]	0.028	2.11 [1.04 - 4.27]	0.038
Recent antimalarial use	1.59 [0.87 - 2.90]	0.133	1.36 [0.47 - 3.98]	0.570	0.36 [0.15 - 0.85]	0.021	0.39 [0.10 - 1.56]	0.181

Abbrevitaion: PCR = polymerase chain reaction; LM = light microscopy; OR = odds ratio; CI_{95} = 95% confidence interval. Estimates of crude associations estimated by GEE for prevalence. [a]Overall significance level for the variable estimated using wald chi-squared test; bComparison group; For multilevel variables, comparison group estimates are presented as odds or incidence rate.

Table 5.S2: Crude associations between the risk factors and the prevalence, clinical incidence and molecular force of blood-stage infections.

	Clinical incidence				$_{mol}$FOB			
	P. falciparum		*P. vivax*		*P. falciparum*		*P. vivax*	
Covariates	IRR/IR [CI_{95}]	p-value	IRR/IR [CI_{95}]	p-value	IRR/IR [CI_{95}]	p-value	IRR/IR [CI_{95}]	p-value
Areas of residence								
[b]Ilahita 1,2,3,4,6,7	0.05 [0.02 - 0.11]	<0.001	0.06 [0.03 - 0.13]	<0.001	1.09 [0.78 - 1.53]	0.622	0.65 [0.44 - 0.96]	0.030
Balanga & Balif	0.56 [0.11 - 2.77]	0.476	0.96 [0.27 - 3.41]	0.946	1.15 [0.66 - 2.00]	0.618	1.45 [0.79 - 2.65]	0.234
Kamanokor & Ilahita 5	5.03 [1.86 - 13.6]	0.001	8.73 [3.44 - 22.2]	<0.001	1.67 [0.97 - 2.87]	0.062	7.46 [4.67 - 11.9]	<0.001
Sunuhu 1&2	9.59 [4.05 - 22.7]	<0.001	5.53 [2.25 - 13.6]	<0.001	3.28 [2.21 - 4.85]	<0.001	8.29 [5.35 - 12.8]	<0.001
	[a]Overall p<0.0001		[a]Overall p<0.0001		[a]Overall p<0.0001		[a]Overall p<0.0001	
[c]Age in years (linear)	1.27 [1.01 - 1.59]	0.044	1.01 [0.78 - 1.32]	0.925	1.21 [1.07 - 1.36]	0.003	1.22 [1.08 - 1.38]	0.001
Haemoglobin level	0.38 [0.26 - 0.55]	<0.001	0.27 [0.17 - 0.43]	<0.001	0.96 [0.83 - 1.11]	0.571	0.86 [0.77 - 0.96]	0.008
Haemoglobin categories								
[b]≥0 grams/dL	0.16 [0.10 - 0.26]	<0.001	0.10 [0.06 - 0.19]	<0.001	1.60 [1.22 - 2.10]	0.001	2.15 [1.73 - 2.67]	<0.001
9 – 9.9 grams/dL	1.09 [0.58 - 2.07]	0.782	1.84 [0.83 - 4.07]	0.132	1.18 [0.83 - 1.67]	0.359	1.15 [0.95 - 1.41]	0.159
≤ 9 grams/dL	1.48 [0.73 - 3.01]	0.278	3.63 [1.58 - 8.33]	0.002	1.29 [0.85 - 1.95]	0.229	1.33 [1.00 - 1.78]	0.048
	[a]Overall p=0.5469		[a]Overall p=0.0093		[a]Overall p=0.4532		[a]Overall p=0.1244	
Active detection of infection timepoint								
[b]Enrolment					Not applicable			
Week 4 / Interval 1	Cannot be tested due to aggregated data							
Week 8 / Interval 2					0.69 [0.34 - 1.41]	0.309	0.82 [0.56 - 1.20]	0.306
Week 12 / Interval 3					0.18 [0.07 - 0.46]	<0.001	0.50 [0.34 - 0.73]	<0.001
Week 16 / Interval 4					0.62 [0.30 - 1.29]	0.202	0.72 [0.49 - 1.05]	0.091

Week 20 / Interval 5					2.10 [1.08 - 4.07]	0.029	1.13 [0.78 - 1.61]	0.52
Week 24 / Interval 6					0.96 [0.51 - 1.80]	0.889	0.69 [0.47 - 1.01]	0.057
Week 28 / Interval 7					0.92 [0.48 - 1.78]	0.802	0.62 [0.41 - 0.93]	0.021
Week 32 / Interval 8					1.04 [0.55 - 1.95]	0.905	0.77 [0.53 - 1.11]	0.165
Week 36 / Interval 9					5.85 [3.54 - 9.68]	<0.001	0.71 [0.47 - 1.07]	0.100
Week 40 / Interval 10					[a]Overall p<0.0001		[a]Overall p=0.0002	
Bed net use	0.01 [0.00003 - 4.85]	0.148	0.05 [0.00001 - 152.5]	0.459	Data not converging			
Febrile illness	In the definition of clinical episode				1.12 [0.63 - 1.98]	0.693	1.25 [0.85 - 1.82]	0.253
History of febrile illness	Cannot be tested due to aggregated data				1.15 [0.20 - 6.46]	0.877	0.94 [0.29 - 3.10]	0.922
Enlarged spleen								
Recent antimalarial use					4.89 [3.05 - 7.84]	<0.001	1.14 [0.49 - 2.63]	0.758
$_{mol}$FOB	1.20 (1.12 - 1.28)	<0.001	1.27 (1.19 - 1.36)	<0.001	Not applicable			

Abbrevitaion: IRR = incidence rate ratio; IR = incidence rate; CI_{95} = 95% confidence interval; $_{mol}$FOB = molecular force of blood-stage infections; Estimates of crude associations estimated by GEE for molecular force of blood-stage infections and negative binomial regression model used for clinical malaria episodes. Recent antimalarial use was not tested in the model for clinical malaria episodes due to aggregated clinical data. [a]Overall significance level for the variable estimated using wald chi-squared test; [b]Comparison group; For multilevel variables, comparison group estimates are presented as odds or incidence rate. [c]Age in years at enrolment was used for clinical malaria incidence while at the start of interval was used for $_{mol}$FOB.

Table 5.S3: Key predictors of the prevalence of infections due to *P. falciparum* and *P. vivax* as diagnosed by light microscopy in 2013

	P. falciparum				*P. vivax*			
	Observed positive (%; n=4359)	AOR	CI_{95}	p-value	Observed positive (%; n=4359)	AOR	CI_{95}	p-value
Areas of residence								
Ilahita 1-4, 7	0.8	Reference			2.7	Reference		
Balanga & Balif	1.1	1.69	(0.41 - 6.99)	0.469	1.7	0.77	(0.30 – 2.01)	0.595
Kamanokor & Ilahita 5	4.7	4.14	(1.67 - 10.3)	0.002	25.4	15.1	(7.52 – 30.4)	<0.001
Sunuhu 1&2	14.4	18.0	(8.94 - 36.4)	<0.001	25.0	16.0	(8.44 – 30.3)	<0.001
			[a]Overall p<0.0001				[a]Overall p<0.0001	
Age		1.48	(1.21 - 1.81)	<0.001		2.59	(1.14 – 1.51)	<0.001
ACD time-points								
Enrolment	10.4	Reference			14.8	Reference		
Week 4	5.4	0.46	(0.27 - 0.78)	0.004	15.1	0.99	(0.69 - 1.42)	0.949
Week 8	3.6	0.25	(0.13 - 0.48)	<0.001	15.2	1.02	(0.71 - 1.46)	0.925
Week 12	2.2	0.13	(0.05 - 0.30)	<0.001	13.2	0.80	(0.53 - 1.19)	0.269
Week 16	3.4	0.21	(0.11 - 0.40)	<0.001	10.5	0.61	(0.41 - 0.91)	0.016
Week 20	3.0	0.17	(0.07 - 0.39)	<0.001	8.5	0.40	(0.26 - 0.63)	<0.001
Week 24	3.5	0.20	(0.10 - 0.43)	<0.001	8.4	0.40	(0.24 - 0.67)	<0.001
Week 28	4.0	0.31	(0.15 - 0.62)	0.001	11.8	0.68	(0.43 - 1.10)	0.117
Week 32	3.3	0.19	(0.09 - 0.42)	<0.001	10.1	0.49	(0.30 - 0.80)	0.004
Week 36	5.0	0.38	(0.20 - 0.74)	0.004	8.5	0.39	(0.24 - 0.62)	<0.001
Week 40	8.1	0.63	(0.33 - 1.21)	0.166	6.1	0.26	(0.15 - 0.44)	<0.001
			[a]Overall p<0.0001				[a]Overall p<0.0001	

Haemoglobin level		0.49	(0.41 - 0.58)	<0.001				
Recent antimalarial use	8.9				8.9	0.29	(0.09 - 0.90)	0.032
Enlarged spleen	22.7				36.4			
Febrile illness	18.3	3.29	(2.12 - 5.12)	<0.001	18.8			
2 weeks history of febrile illness[b]	16.7				26.2			

Abbreviation: AOR = adjusted odds ratio; CI_{95} = 95% confidence interval Multivariate GEE model-based estimates of the risk of infection detected at each monthly active detection of infection visits via backward selection of significant risk factors. [a]Overall significance level for the variable estimated using wald chi-squared test; [b]Excluding febrile illness at the time of visit; AOR: multivariate adjusted odds ratio; CI_{95}: 95% confidence interval.

6 Chapter 6: General Discussion

6.1 Summary of key findings

This thesis has focussed on understanding the factors that influence growth in utero and early childhood among young Papua New Guinean children. Specifically, to investigate the direct and indirect associations between selected risk factors, birthweight and LBW, the impact of infectious diseases on stunting, wasting and underweight and the changing burden of malaria in the most at-risk age group.

Although a multitude of factors have been associated with LBW (Kramer 1987), in this thesis, we selectively investigated the association of birthweight and LBW with *modifiable* risk factors including maternal undernutrition, anaemia, iron deficiency, peripheral malaria infection, IPTp intervention and inflammation. These factors have been identified in previous studies and previous analyses of this same dataset to be key in causing LBW in PNG (Brabin and Piper 1997, Allen, Raiko et al. 1998, Mueller 2002, Stanisic, Moore et al. 2015, Unger, Ome-Kaius et al. 2015, Fowkes, Moore et al. 2018). The nutritional status of pregnant women was observed to be a key determinant of foetal birthweight and risk of LBW. Low MUAC at enrolment was directly associated with a reduction in mean birthweight and an increased risk of LBW, independent of effects of MUAC mediated through other factors. There was a tendency for iron deficiency to be directly associated with an increase in mean birthweight, but with no effect on rates of LBW. Maternal anaemia on the other hand was directly associated with an increased risk of LBW but did not influence birthweight. IPTP intervention with SPAZ during pregnancy was directly associated with increased mean birthweight and reduced propensity for LBW independent of its effects through anaemia and peripheral malaria infection during the study. Inflammation as indicated by high CRP levels was a direct predictor of LBW but not birthweight while no associations were observed for peripheral microscopically detected malaria infection at first antenatal care visit and birthweight and LBW. We did not observe any indirect associations for any of these selected factors in our analyses.

Secondary analysis of data from a trial of IPTi in PNG children revealed that stunting was the most common form of undernutrition observed among these young children. At 15 months of age, 49.1% of the children were stunted and 43.8% of these stunted children were classified as severely stunted. Similar proportions were observed at 27 months of age (stunted: 47.1%; severely stunted: 42.4%). The overall prevalence of stunting and severe stunting in the study however was 52.9% and 25.0%, respectively. Whereas the overall prevalence of underweight

and wasting were 16.1% and 6.1% respectively, the severe forms of the two were rarely observed in the study (severe underweight: 3%; severe wasting: 1%). Acute illness episodes of malaria, pneumonia and diarrhoea each appeared to have differential impacts on child undernutrition. Acute episodes of pneumonia and diarrhoea were associated with increased odds of underweight and their negative effect on weight-for-age Z-score was observed to last up to 3 months. Malaria on the other hand had a delayed negative association with weight-for-age Z-score as well as a tendency to be associated with increased odds of stunting.

In-depth analysis of the impact of improved malaria control on the burden of malaria infections and illness among children aged 1 - 5 years demonstrated that standard malaria control interventions have differential impact on rates of *P. falciparum* and *P. vivax* infections in young children but not clinical malaria episodes. Within a few months of the first population-wide distribution of LLINs into the study area, there was an immediate reduction in the prevalence and incidence of *P. falciparum* but not *P. vivax* infections. The decline in *P. vivax* infections was only evident in a cohort study conducted after 5 years of sustained control in the study area. Contrary to the pattern of decline observed for infections, the burden of *P. vivax* clinical episodes dropped immediately following the first LLIN distribution. The pattern of decline in the incidence of clinical episodes due to *P. falciparum* was similar to that observed of the infections. During the time of significantly reduced transmission in 2013, the two key predictors of infection and clinical episodes were individual level of exposure to new infections as measured by $_{mol}$FOB and the place of residence. In this same time period, there was marked spatial clustering of infections and illness and a high proportion of the infections detected were low-density and asymptomatic.

6.2 Direct and indirect associations of low birthweight

Although a multitude of factors have been implicated as causes of LBW, it is often less clear as to whether they are causal determinants or just the markers or associated factors representing a true underlying causal determinant. Differentiating between these factors requires adequately controlling for appropriate confounding factors or use of analytical approaches that can allow distinguishing between the two. It is challenging to collect data on all the factors that can potentially influence birthweight or risk of LBW in one study and thus adequately control for confounding. While direct associations can be determined using standard analytical approaches such as linear and logistic regressions, these methods lack the ability to determine both direct

and indirect associations thus the observed associations are considered direct independent associations on the assumption that potential confounding was adequately accounted for. Failure to adequately control for confounding can result in associations that could lead to misinformed policies and strategies. For instance, if insufficient caloric intake was a true causal determinant of LBW, and if anaemia was acting as a marker of poor maternal nutritional status, failure to adequately control for calorie intake may lead investigators to conclude that there is an association between anaemia and LBW. Consequently, routine iron supplementation upon detection of anaemia during pregnancy may have no impact on foetal growth as anaemia has no independent effect on foetal growth (Kramer 1987). Distinguishing between the associated factors and causal determinants should be priority research areas to guide the development and implementation of strategies and interventions that are targeted at alleviating the burden of LBW. There is very limited published data on this subject.

In the meta-analysis undertaken by Kramer in 1987 (Kramer 1987), the modifiable factors identified to have direct causal association with intrauterine growth included maternal height, pre-pregnancy weight, paternal height and weight, maternal birthweight, parity, history of prior LBW, gestational weight gain, general morbidity and episodic illness, malaria and maternal habits (cigarette smoking, alcohol consumption and tobacco chewing). For gestational duration, pre-pregnancy weight, prior history of prematurity or miscarriage, in utero exposure to diethylstilboestrol and cigarette smoking were identified to have direct causal effects. Similar observations of direct associations especially between maternal nutrition, infections or inflammation and birthweight were also observed in a recent study by Ashorn *et al,* which also elucidated the indirect pathways through which some of these factors influenced newborn size. A joint effect of various risk factors including maternal infections, inflammation, nutrition and constitutional factors (e.g. parity) on newborn size (Ashorn, Hallamaa et al. 2018) providing evidence for a more holistic approach where multiple exposures could be concurrently addressed with interventions to achieve a maximum effect on LBW. Supporting this concept is the observation made in our primary analysis that despite the low prevalence of malaria observed in the study, IPTp with SPAZ reduced LBW by about 30% suggesting that the intervention not only addressed not only malaria but also other additional factors that influenced LBW (Unger, Ome-Kaius et al. 2015).

Mechanisms through which IPTp may be operating to improve birthweight and reduce LBW were investigated in this thesis. IPTp improved birthweight and reduced LBW by mechanisms

independent of the currently investigated risk factors which included peripheral blood malaria infection and maternal haemoglobin concentration. However, it was shown in the main trial analysis that IPTp reduced peripheral malaria at delivery as well as active placental malaria (Unger, Ome-Kaius et al. 2015) indicating the clearance of malaria infections by IPTp. This indicated that IPTp with SPAZ did indeed reduce malaria. However, given the low overall malaria burden, the main impact on birthweight seemed not to be due to its effect on malaria. The low prevalence of malaria detected during the study may therefore have prevented us from seeing the effect of SPAZ IPTp on birth outcomes because the malaria-attributable fraction of LBW was too small. It was also shown that IPTp was associated with reduction in PTD and *N. gonorrhoeae* indicating a clearance of infections associated with PTD, especially infections of the reproductive tract (RTIs) (Unger, Ome-Kaius et al. 2015). STIs/RTIs are key predictors of adverse birth outcomes (Mullick, Watson-Jones et al. 2005, Chico, Hack et al. 2013, Romero, Dey et al. 2014) and azithromycin has been shown in studies to be effective in treating these infections (Chico, Hack et al. 2013). The positive impact of IPTp with SPAZ may therefore be in part be due to clearance of the STIs/RTIs thus improving birth outcomes.

In separate analyses of this same dataset, it was shown that IPTp was associated with an increase in gestational weight gain, which in itself is a predictor of birthweight (Medicine and Council 2009, Unger, Wangnapi et al. 2016), and with a reduction in inflammation biomarkers at delivery (Unger, Hansa et al. 2019). Whether these benefits are due to SP, or AZ, or the combination, is not known, but there has been interest in AZ-based combination treatments for use as IPTp to address the dual burden of malaria and STIs (Chico, Hack et al. 2013). With malaria parasites developing resistance to SP in many settings, there has been an increasing urgency to find alternative treatment regimens and AZ-based combination treatments are being considered as potential candidates to replace SP (Chico, Hack et al. 2013, Moore, Benjamin et al. 2019). They could be developed into an antenatal intervention to be used in many LMICs to address not only malaria and STIs but also the poor gestational weight gain that was frequently observed in our study, and thus to improve birth outcomes. Additionally, it may have beneficial impact on the mother's and the infant's nutritional status post-delivery, and those benefits may even last up to 5 years of age (Luntamo, Kulmala et al. 2013, Unger, Wangnapi et al. 2016, Roca, Camara et al. 2018, Hallamaa, Cheung et al. 2019). In the recent study by Hallamaa *et al*, the mean values of the anthropometric indices (weight, MUAC and head circumference) among infants of women receiving monthly SP plus two doses of AZ as

IPTp were higher compared to monthly SP alone indicating that SP alone was not enough to create long-lasting effect on child growth (Hallamaa, Cheung et al. 2019).

The concern with implementing such an azithromycin-based intervention on a large scale is the development of antimicrobial resistance to organisms such as *Streptococcus pneumoniae*, *E coli* and *Staphylococcus aureus* (O'Brien, Emerson et al. 2019) which could pose a major challenge in the management of diseases caused by these infections such as pneumonia, diarrhoea, meningitis and sepsis (Bergman, Huikko et al. 2006). Of note, macrolides are not typically used against diarrhoea caused by *E. coli*. In a separate sub-analysis of this same dataset, IPTp with SPAZ was associated with a small but significant increase in macrolide-resistant pneumococci (Unger, Aho et al. 2015). Azithromycin is a long-acting macrolide and it is believed that such macrolides can select for resistance more effectively than other macrolides. Moreover, increasing treatment frequency has been linked to increased selection for resistance especially in *Streptococcus pneumoniae* (Baquero 1999, O'Brien, Emerson et al. 2019). There is however some evidence that suggests that resistance may reduce after removal of antibiotic pressure (Haug, Lakew et al. 2010). Frequency of azithromycin use and the impact of selection of resistance by AZ on response to management of diseases such as pneumococcal meningitis, pneumonia and sepsis are key factors to consider when considering AZ-based regimens as IPTp interventions.

Nutritional status of women both before and during pregnancy plays a crucial role in optimising pregnancy outcomes (Kramer 1987, Mason, Saldanha et al. 2012, Ashorn, Hallamaa et al. 2018). The observation of the negative impact of maternal undernutrition on birthweight and LBW in this thesis re-emphasises the importance of nutrition and provides support that inclusion of nutritional interventions as part of antenatal care is important as they are likely to have a direct impact on the nutritional status of the mother. Besides weight and height, simple to measure indicators such as MUAC should also be routinely measured as part of antenatal care to identify women of suboptimal nutritional status for timely interventions. Dietary advice for diet improvements as well as general health education on infections and other determinants of undernutrition, balanced protein-energy supplementation and high protein supplementation are some of the nutritional interventions that could be beneficial to such women (Imdad and Bhutta 2012, Bhutta, Das et al. 2013).

Somewhat counterintuitively, maternal anaemia, i.e., haemoglobin < 11g/dL (WHO Vitamin and Mineral Nutrition Information System (VMNIS)) at enrolment (which occurred mostly during the second trimester) was observed in this analysis to be a direct predictor of *reduced* odds of LBW which contradicts the general consensus that anaemia is a risk factor for LBW (Levy, Fraser et al. 2005, Rahman, Abe et al. 2016, Figueiredo, Gomes-Filho et al. 2018, Jung, Rahman et al. 2019). Studies however have also demonstrated better birth outcomes with haemoglobin levels of 85-110g/l which by current WHO definitions (WHO Vitamin and Mineral Nutrition Information System (VMNIS)) and used in our analysis would be classified as mild to moderate anaemia (Steer, Alam et al. 1995, Steer 2000, Dewey and Oaks 2017). It is particularly very low levels of haemoglobin indicative of severe anaemia (haemoglobin < 7.0 g/dL) (WHO Vitamin and Mineral Nutrition Information System (VMNIS)) that are associated with increased risks of LBW (Kozuki, Lee et al. 2012). Furthermore, the association between anaemia and adverse birth outcomes may only be evident if the haemoglobin is measured in the first trimester of pregnancy (Dewey and Oaks 2017). For most of the women in PNG and elsewhere in LMICs, women present late, often in their second to third trimester to antenatal care (Simkhada, Teijlingen et al. 2008, Kisuule, Kaye et al. 2013) which presents a major challenge in identifying anaemic women earlier for timely interventions.

The conflicting evidence in the literature about the association between anaemia and LBW raises the important question as to what level of haemoglobin can one consider optimal and what cut-offs can be used define anaemia that may worsen pregnancy outcomes. Clarifying this is important considering low haemoglobin as defined by WHO, i.e., haemoglobin < 110 g/l (WHO Vitamin and Mineral Nutrition Information System (VMNIS)) is in many settings an indication for iron supplementation. WHO recommends daily oral iron and folic acid supplementation as part of the antenatal care to prevent maternal anaemia, puerperal sepsis, LBW and PTD (WHO). A recent study in the malaria endemic setting of PNG showed that iron deficiency was associated with better birth outcomes independent of its effects on malaria (Fowkes, Moore et al. 2018) raising concerns about the routine iron supplementation. Although not statistically significant, we also observed in this analysis an increase in mean birthweight among women who were iron deficient, also independent of its effect on malaria. Studies however show that iron supplementation can be beneficial only if the women were initially iron deficient (Mwangi, Roth et al. 2015, Verhoef, Mwangi et al. 2019) which Verhoef *et al* (Verhoef, Mwangi et al. 2019) indicated to be the underlying explanation for the observation made in the study by Fowkes *et al* (Fowkes, Moore et al. 2018). Due to the lack of quality data

on iron supplementation and the indications for it (e.g. haemoglobin <11 g/dL) in our study, we are unable to draw any firm conclusions as to whether our observations of positive impact of iron deficiency on birthweight were due to iron supplementation in iron deficient women. However, to note that two-thirds of the women were iron deficient and 69% of women were anaemic with the latter likely to have been identified for iron supplementation. Women who are iron replete taking iron supplementation may be at risk of adverse birth outcomes due to haemoconcentration and subsequent increased blood viscosity, oxidative stress and impaired systemic response to infections and associated inflammation (Dewey and Oaks 2017, Verhoef, Mwangi et al. 2019). To ensure that only women who are iron deficient can receive iron supplementation will require iron deficiency screening alongside haemoglobin level measurements. Research that can clearly indicate as to what level of haemoglobin can one consider it to be due to iron deficiency will be important for resource poor settings like PNG where routine testing for iron deficiency is likely to be challenging.

In spite of the widespread recognition of the importance of LBW globally, many LMICs including PNG lack reliable data on the magnitude and geographical distribution, which also presents a major challenge to monitor the progress of interventions that are implemented in the country to alleviate the burden of LBW (UNICEF 2016). Improvement in data collection and reporting should be the focus of the PNG government as this would be important to advise as to what interventions work and guide development and implementation of interventions in the country.

6.3 Infectious causes of child undernutrition

Suboptimal growth in utero may continue during early childhood causing growth faltering (Arifeen, Black et al. 2000, Christian 2009, Christian, Lee et al. 2013). Children born LBW are considered more prone to growth faltering during early childhood (Marks, Reichman et al. 2006). Additional causes of child undernutrition are diverse and include factors such as suboptimal breastfeeding, inappropriate weaning and child feeding practises, low quality and inappropriate complementary foods, and environmental factors including infections (Checkley, Buckley et al. 2008, Prost 2009). In chapter 4, we specifically investigated the impact of acute malaria, pneumonia and diarrhoeal illness episodes on child growth in a cohort of young children. Despite frequently co-occurring in PNG, little is known about the impact of these infectious illnesses on growth faltering.

In a cohort of 1-3 year old children, stunting was the most common form of undernutrition observed, which is supported by previous evidence from PNG revealing a high burden of stunting (WHO , Gibson, Heywood et al. 1991, Keeble and Keeble 2006, Wand, Lote et al. 2012). The overall prevalence of severe stunting in this cohort was 25%, which is higher than the national prevalence of 17.6% based on the 2005 national data (UNICEF 2016). Severely stunted children have significantly increased risk of dying (Olofin, McDonald et al. 2013) but given short stature is very common in PNG, they will likely not be identified for treatment. Health workers are also less likely to identify these children given height is not routinely measured as part of child growth monitoring as seen in many LMICs (Dewey and Begum 2011). Any form of child undernutrition at any degree of severity have been shown to heighten the risks of child morbidities and mortality due to common childhood infectious diseases including respiratory infections, malaria and diarrhoea (Smith 1991, Caulfield, de Onis et al. 2004, Olofin, McDonald et al. 2013). Childhood infectious diseases may also further exacerbate any pre-existing poor nutritional status of the child (Smith 1991). In this thesis, acute episodes of pneumonia and diarrhoea negatively impacted weight with the effects lasting up to 3 months while the effect of malaria on weight, although more modest lasted up to 6 or more months.

Observations made in this thesis highlight major concerns for the country. First, failure to recognise and identify stunting in any degree of severity could translate into large proportion of unproductive human capacity and adversely affect societal and national development. Second, failure to identify severely stunted children could result in high rates of child mortality thus reduction in population. Third, infectious diseases including malaria, pneumonia and diarrhoea which are highly prevalent in the country play a role in adversely impacting nutritional status of young children and these effects may be long-lasting. These observations however also provide evidence that could be capitalised on to improve nutritional status of young children in PNG. It is very clear from this analysis and many previous studies that stunting is significantly more common in children than any other form of child undernutrition in the country. This calls for concerted and targeted strategies to tackle stunting. PNG is a poor country faced with multiple problems and to adequately address stunting or any other form of undernutrition will be challenging. However, starting small with simple additions to routine childcare such as routine measurement of child length and height, educating health workers, parents and community at large to recognise stunting as a health problem and collecting quality

data on the burden and distribution of undernutrition will be vital in moving towards reducing stunting in the country. Although more research is needed on this subject, there has been some indication that MUAC may identify stunting (Emergency Nutrition Network (ENN) 2014). Given it is simple to measure and that it is already routinely implemented to identify severe acute wasting, routine measuring of MUAC may provide an alternative to height measurements, where the latter are not practical for logistics reasons, for example in village clinics. Routine measurement of length/height and MUAC will be key to monitoring linear growth of the child allowing early detection of stunting with timely interventions.

It has long been recognised that quality and not quantity of food intake was the problem in PNG (A. P. C. Oomen 1971, Gibson, Heywood et al. 1991, Mueller and Smith 1999, Mueller, Vounatsou et al. 2001). The persistence of the high burden of child undernutrition, particularly that of stunting where linear growth is dependent on protein and other essential nutrients, suggests that quality of diet remains a major underlying problem in the country. While promoting nutritious diets through education and community awareness may be achieved, their impact on improving nutrition status of children or undernutrition in general is likely to be minimal given the vast majority of the population in PNG are not well educated, unemployed and living in rural settings (UNICEF 2016) and will likely not have the means to continuously afford a nutritious meal. Underlying challenges such as education, employment and food insecurity among other factors will need to be addressed before significant progress can be made. PNG government however should commit to the implementation of strategies and evidence-based nutrition-specific interventions such as high protein supplementation or control of infectious diseases (Bhutta, Ahmed et al. 2008, Bhutta, Das et al. 2013, Bhutta, Das et al. 2013, da Silva Lopes, Ota et al. 2017) to achieve immediate effects which could eventually translate into long-term positive outcomes (Hoddinott, Maluccio et al. 2008, Martorell, Melgar et al. 2010, Martorell and Zongrone 2012).

The observed adverse association between the infectious diseases and weight-for-age and underweight is an indication that malaria, pneumonia and diarrhoea play a part in contributing to the high burden of undernutrition in the country. These observations provide supporting evidence that infection control should form an important component in the drive for reducing child undernutrition in the country. Some of the effective strategies against these infections such as the scale-up of malaria control measures (LLINs, test-and-treat, AL), vaccines against infections (e.g. *Streptococcus pneumoniae* and *Haemophilus influenzae*) and water, sanitation

and hygiene (WaSH) program among others are already being implemented in the country. These interventions however are not reaching every child in PNG for reasons such as logistic difficulties, failing health systems and lack of sufficient financial among many other factors (PNG Department of Health 2010). Strengthening these infection control strategies is urgently needed to prevent the infection-associated morbidities as well as undernutrition. The long-term effects of the three illnesses on weight-for-age highlights that close monitoring of nutritional status of children following an episode will be important. This can allow for early detection of growth faltering and timely interventions to allow catch-up of lost growth. Malaria for instance was observed to have effects lasting several months, and interventions such as IPTi for malaria prevention may be worth considering in a PNG setting. While IPTi for malaria was previously investigated in PNG and showed promising results for prevention of malaria (Senn, Rarau et al. 2012), research will be needed to investigate its impact on malaria and undernutrition in this setting.

6.4 Malaria in young children

Malaria affects a child's health, growth and development from the time of conception through to childhood. During pregnancy, malaria infection could result in maternal anaemia and placental malaria through which nutrient supply to the foetus as well as the intrauterine environment could be compromised leading to IUGR and preterm delivery resulting in LBW (Brabin and Piper 1997, Allen, Raiko et al. 1998). Malaria in pregnancy may adversely influence childhood growth independent of LBW (Kalanda, van Buuren et al. 2005, Walther, Miles et al. 2010), lead to an increased risk of malaria-related morbidities (Schwarz, Adegnika et al. 2008, Asante, Owusu-Agyei et al. 2013) and anaemia (Desai, ter Kuile et al. 2007, Boel, Rijken et al. 2012) during infancy. Between 2004 and 2014, PNG observed a dramatic decline in malaria infection prevalence and clinical incidence as evidenced in the nationwide surveys (Hetzel, Morris et al. 2015, Hetzel, Pulford et al. 2017) and our analysis (chapter 5; (Ome-Kaius, Kattenberg et al. 2019)). This indicates that strong and sustained malaria control leads to few clinical episodes of malaria during early childhood and hence lowers the risk of negative effects on child growth and development. Similarly, the risk of malaria during pregnancy and its associated risk of adverse birth outcomes is also reduced.

The analysis presented in Chapter 5 confirms that the key biological differences between *P. falciparum* and *P. vivax* renders them differentially susceptible to standard control tools such

as LLINs and case management. *P. vivax* takes longer to respond to the control interventions which clearly is due to the reservoir of hypnozoites and this poses a major challenge for elimination. Furthermore, with significant reduction in the overall burden of malaria, the remaining infections and clinical episodes are becoming clustered in high risk areas and populations surrounded by areas with little or no transmission and it can be challenging to identify such areas. Moreover, the infections are mostly of low density, asymptomatic and submicroscopic. Further reduction in malaria will thus require a shift in the focus of programs to address these issues.

Data from PNG indicates that sustained implementation of nationwide distribution of LLINs and strengthened case management can reduce the burden of infections and illness due to both *P. falciparum* and *P. vivax.* The interventions should therefore be maintained. However, in order to accelerate the reduction in the burden of *P. vivax* infections, there is still the need to strengthen the implementation of *P. vivax* radical cure to maximise the impact of control in PNG. Although primaquine radical cure is part of malaria treatment protocol in co-endemic countries such as PNG, due to its associated toxicity in glucose-6-phospate dehydrogenase (G6PD) deficient individuals and the lack of G6PD testing, it is often not prescribed (John, Douglas et al. 2012). Additionally, the current 14-day regimen of 0.25mg/kg/day primaquine treatment is often associated with low compliance (John, Douglas et al. 2012) and limited efficacy against the Chesson strain of vivax endemic in PNG (Ehrman, Ellis et al. 1945, Baird and Hoffman 2004, Baird 2009). To accelerate control of *P. vivax* in co-endemic settings and further reduce the burden, these issues must be addressed.

The high burden of low-density and asymptomatic malaria infections observed in specific geographic areas results in a reservoir of parasites that cannot be easily and routinely detected by the currently implemented methods of detection (LM and RDTs) and thus evade treatment. If these potential hotspot areas are not identified and targeted with additional interventions, they are likely to be the areas where residual transmission will persist and act as a source of infection for the rest of the community or for seasonal increases in clinical malaria episodes (Bousema, Griffin et al. 2012, Bejon, Williams et al. 2014). As the malaria control program considers a more stratified approach to malaria control in PNG, identifying and targeting these areas of residual transmission may become an important priority. Asymptomatic parasite carriage, reported fever, drug use and serological responses to malaria specific antigens are some of the approaches used in identifying hotspots of malaria transmission in other settings

(Bousema, Griffin et al. 2012) and may warrant considering in the PNG context when some low burden areas of the country shift from control phase to elimination.

6.5 Further research

The investigations that form the basis of this thesis utilised several datasets that were not specifically designed to address additional questions relating to causes of LBW and undernutrition. Further research into the mechanisms that may be involved in mediating the impact of IPTp on LBW is required. Applying SEM in the existing data set to further investigate this may not be appropriate considering the limited data points for most of the key variables such as the gestation weight gain, STIs and gestation age as confirmed by ultrasonography. Future studies are therefore needed to explore in more detail the mechanisms by which SP and AZ operate to improve pregnancy outcomes, beyond their effects as antimalarials. SEM analysis should be considered in future analysis that investigate the causes of adverse birth outcomes as they can provide important information that will be key to development of interventions as in whether or not to address a single cause or to concurrently address multiple factors to achieve a maximum effect from an intervention. The association observed for anaemia and LBW contradicts the general consensus of the association between anaemia and LBW and is an area that warrants further research, particularly to clarify the optimal haemoglobin levels and cut-offs used for anaemia. Detailed analysis of the association between anaemia, iron deficiency and adverse birth outcomes are currently being investigated by our group.

MUAC is an easy to measure indicator of nutritional status. It could be used to identify pregnant women of suboptimal nutritional status to allow for early detection and timely intervention to which may have a positive impact on birth outcomes. Similarly, there has been indication that MUAC may identify stunting in childhood (Emergency Nutrition Network (ENN) 2014) but data has been limited. Research is needed to investigate the utility of MUAC as part of routine antenatal care and routine child health care and the associated benefits of birth outcomes and early child growth, stunting in particular. If this is feasible and MUAC performs well in identifying suboptimal nutritional status that may influence risk of poor birth outcomes or stunting, it could be recommended for inclusion into antenatal and child health care.

Aspects of the relationship between infectious diseases and child growth that remain to be clarified and will require further research include the lag effects of infectious diseases on stunting and wasting. We have clearly shown that acute episodes of malaria, pneumonia and diarrhoea have a delayed effect on weight-for-age Z-score. Lack of serial data on height-for-age precluded assessment of the lag effects of these infections on linear growth and weight-for-height Z-score. A large study with serial measurements from birth to after 24 months of age will allow a full assessment of the effects of these infections on child growth. This will provide a complete picture of the pattern of child growth and how infections may contribute to altering child growth from birth to after 24 months in PNG.

Lastly, the impact of malaria control interventions on transmission are a function of diverse social and ecological settings leading to differences in mosquito abundance, mosquito behaviour and human-mosquito interaction. While improvements in the quality of housing have occurred over the past decade in many urban areas of PNG, housing for PNG's rural majority remains largely dependent on bush material. Higher quality of housing and socio-economic status was associated with reduced risk of malaria in previous studies in PNG (Hetzel, Pulford et al. 2017); however, such data is not available from the child cohorts analysed. Further work understanding the dynamic, complex and responsive ecological niches driving ongoing malaria transmission in certain areas of PNG will inform the development of targeted control and elimination.

6.6 Concluding remarks

Although progress is being made in reducing the global burden of infectious diseases and child undernutrition including LBW, stunting, wasting and underweight, it is proving stubbornly difficult to reduce rates to desired levels. Although the underlying reasons are multifactorial, this thesis focussed on understanding the factors that influence the risks of LBW and the association between infectious diseases and early child growth. The findings of the thesis indicate that child growth in utero was independently influenced by maternal undernourishment, anaemia and IPTp with SPAZ. During early childhood, suboptimal growth expressed as stunting, wasting and underweight were differentially influenced by acute malaria, pneumonia and diarrhoea. Children suffering from acute episodes of pneumonia and diarrhoea had higher odds of being underweight as well as experiencing weight faltering for up to 3 months following an episode. In contrast, children suffering a malaria episode

experienced weight faltering several months later. Of these infections, malaria poses a major threat on child's life from when in utero through early childhood. The currently implemented control measures reduced the burden of malaria in young children but the impact on the predominant species in this age group, *P. vivax* was delayed highlighting the need to strengthen implementation of *P. vivax* radical cure to maximise the impact of control in PNG.

Reference List

A. P. C. Oomen, H. (1971). Ecology of human nutrition in New Guinea.

Abera, Z., D. Ejara and S. Gebremedhin (2019). "Nutritional and non-nutritional factors associated with low birth weight in Sawula Town, Gamo Gofa Zone, Southern Ethiopia." BMC Res Notes **12**(1): 540.

Abeysena, C., P. Jayawardana and A. S. R. de (2010). "Maternal haemoglobin level at booking visit and its effect on adverse pregnancy outcome." Aust N Z J Obstet Gynaecol **50**(5): 423-427.

Abrams, E. T. and S. R. Meshnick (2009). "Malaria during pregnancy in endemic areas: a lens for examining maternal-fetal conflict." Am J Hum Biol **21**(5): 643-650.

Abu-Saad, K. and D. Fraser (2010). "Maternal nutrition and birth outcomes." Epidemiol Rev **32**: 5-25.

Adair, L. S. (2014). "Long-term consequences of nutrition and growth in early childhood and possible preventive interventions." Nestle Nutr Inst Workshop Ser **78**: 111-120.

Ahmed, A. M., T. Ahmed, S. K. Roy, N. Alam and M. I. Hossain (2012). "Determinants of undernutrition in children under 2 years of age from rural Bangladesh." Indian Pediatr **49**(10): 821-824.

Alexandre, M. A., S. G. Benzecry, A. M. Siqueira, S. Vitor-Silva, G. C. Melo, W. M. Monteiro, H. P. Leite, M. V. Lacerda and M. Alecrim (2015). "The association between nutritional status and malaria in children from a rural community in the Amazonian region: a longitudinal study." PLoS Negl Trop Dis **9**(4): e0003743.

Ali, D., K. K. Saha, P. H. Nguyen, M. T. Diressie, M. T. Ruel, P. Menon and R. Rawat (2013). "Household food insecurity is associated with higher child undernutrition in Bangladesh, Ethiopia, and Vietnam, but the effect is not mediated by child dietary diversity." J Nutr **143**(12): 2015-2021.

Allen, L. H., J. M. Peerson and G. Maternal Micronutrient Supplementation Study (2009). "Impact of multiple micronutrient versus iron-folic acid supplements on maternal anemia and micronutrient status in pregnancy." Food Nutr Bull **30**(4 Suppl): S527-532.

Allen, S. J., A. O'Donnell, N. D. Alexander and J. B. Clegg (1996). "Severe malaria in children in Papua New Guinea." QJM **89**(10): 779-788.

Allen, S. J., A. Raiko, A. O'Donnell, N. D. Alexander and J. B. Clegg (1998). "Causes of preterm delivery and intrauterine growth retardation in a malaria endemic region of Papua New Guinea." Arch Dis Child Fetal Neonatal Ed **79**(2): F135-140.

Amos, A. B., J. Wapi and C. A. Klufio (1993). "Longitudinal fetal biometry of normal pregnant Melanesian Papua New Guineans to construct standards of reference for Papua New Guinea." P N G Med J **36**(3): 219-227.

Ansell, J., K. A. Hamilton, M. Pinder, G. E. Walraven and S. W. Lindsay (2002). "Short-range attractiveness of pregnant women to Anopheles gambiae mosquitoes." Trans R Soc Trop Med Hyg **96**(2): 113-116.

Arifeen, S. E., R. E. Black, L. E. Caulfield, G. Antelman, A. H. Baqui, Q. Nahar, S. Alamgir and H. Mahmud (2000). "Infant growth patterns in the slums of Dhaka in relation to birth weight, intrauterine growth retardation, and prematurity." Am J Clin Nutr **72**(4): 1010-1017.

Armstrong, B. (2006). "Models for the relationship between ambient temperature and daily mortality." Epidemiology **17**(6): 624-631.

Arnold, L., W. Hoy and Z. Wang (2016). "Low birthweight increases risk for cardiovascular disease hospitalisations in a remote Indigenous Australian community--a prospective cohort study." Aust N Z J Public Health **40 Suppl 1**: S102-106.

Asante, K. P., S. Owusu-Agyei, M. Cairns, D. Dodoo, E. A. Boamah, R. Gyasi, G. Adjei, B. Gyan, A. Agyeman-Budu, T. Dodoo, E. Mahama, N. Amoako, D. K. Dosoo, K. Koram, B. Greenwood and D. Chandramohan (2013). "Placental malaria and the risk of malaria in infants in a high malaria transmission area in ghana: a prospective cohort study." J Infect Dis **208**(9): 1504-1513.

Ashorn, P., L. Hallamaa, L. H. Allen, U. Ashorn, U. Chandrasiri, M. Deitchler, R. Doyle, U. Harjunmaa, J. M. Jorgensen, S. Kamiza, N. Klein, K. Maleta, M. Nkhoma, B. M. Oaks, B. Poelman, S. J. Rogerson, C. P. Stewart, M. Zeilani and K. G. Dewey (2018). "Co-causation of reduced newborn size by maternal undernutrition, infections, and inflammation." Matern Child Nutr.

Asparouhov, T. and B. Muthen. (2010). "Weighted least squares estimation with missing data. Technical appendix." Retrieved 02 - Feb-2019, 2020.

Assis, A. M., M. L. Barreto, L. M. Santos, R. Fiaccone and G. S. da Silva Gomes (2005). "Growth faltering in childhood related to diarrhea: a longitudinal community based study." Eur J Clin Nutr **59**(11): 1317-1323.

Badfar, G., M. Shohani, A. Soleymani and M. Azami (2019). "Maternal anemia during pregnancy and small for gestational age: a systematic review and meta-analysis." J Matern Fetal Neonatal Med **32**(10): 1728-1734.

Bailey, K. V. and J. Whiteman (1963). "Dietary Studies in the Chimbu (New Guinea Highlands)." Trop Geogr Med **15**: 377-388.

Baird, J. K. (2009). "Resistance to therapies for infection by Plasmodium vivax." Clin Microbiol Rev **22**(3): 508-534.

Baird, J. K. and S. L. Hoffman (2004). "Primaquine therapy for malaria." Clin Infect Dis **39**(9): 1336-1345.

Baquero, F. (1999). "Evolving resistance patterns of Streptococcus pneumoniae: a link with long-acting macrolide consumption?" J Chemother **11 Suppl 1**: 35-43.

Bardaji, A., B. Sigauque, S. Sanz, M. Maixenchs, J. Ordi, J. J. Aponte, S. Mabunda, P. L. Alonso and C. Menendez (2011). "Impact of malaria at the end of pregnancy on infant mortality and morbidity." J Infect Dis **203**(5): 691-699.

Barker, D. J., P. D. Gluckman, K. M. Godfrey, J. E. Harding, J. A. Owens and J. S. Robinson (1993). "Fetal nutrition and cardiovascular disease in adult life." Lancet **341**(8850): 938-941.

Barker, D. J. and C. N. Martyn (1992). "The maternal and fetal origins of cardiovascular disease." J Epidemiol Community Health **46**(1): 8-11.

Barker, D. J. and C. Osmond (1986). "Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales." Lancet **1**(8489): 1077-1081.

Barker, D. J., C. Osmond and C. M. Law (1989). "The intrauterine and early postnatal origins of cardiovascular disease and chronic bronchitis." J Epidemiol Community Health **43**(3): 237-240.

Barker, D. J., P. D. Winter, C. Osmond, B. Margetts and S. J. Simmonds (1989). "Weight in infancy and death from ischaemic heart disease." Lancet **2**(8663): 577-580.

Barros, F. C., S. R. Huttly, C. G. Victora, B. R. Kirkwood and J. P. Vaughan (1992). "Comparison of the causes and consequences of prematurity and intrauterine growth retardation: a longitudinal study in southern Brazil." Pediatrics **90**(2 Pt 1): 238-244.

Baschat, A. A. (2004). "Pathophysiology of Fetal Growth Restriction: Implications for Diagnosis and Surveillance." Obstetrical & Gynecological Survey **59**(8): 617-627.

Bate, C. A., J. Taverne and J. H. Playfair (1989). "Soluble malarial antigens are toxic and induce the production of tumour necrosis factor in vivo." Immunology **66**(4): 600-605.

Batiro, B., T. Demissie, Y. Halala and A. A. Anjulo (2017). "Determinants of stunting among children aged 6-59 months at Kindo Didaye woreda, Wolaita Zone, Southern Ethiopia: Unmatched case control study." PLoS One **12**(12): e0189106.

Bauld, R., G. R. Sutherland and A. D. Bain (1974). "Chromosome studies in investigation of stillbirths and neonatal deaths." Arch Dis Child **49**(10): 782-788.

Becker, S., R. E. Black and K. H. Brown (1991). "Relative effects of diarrhea, fever, and dietary energy intake on weight gain in rural Bangladeshi children." Am J Clin Nutr **53**(6): 1499-1503.

Beebe, N. W., T. Russell, T. R. Burkot and R. D. Cooper (2015). "Anopheles punctulatus group: evolution, distribution, and control." Annu Rev Entomol **60**: 335-350.

Beeson, J. G., N. Amin, M. Kanjala and S. J. Rogerson (2002). "Selective accumulation of mature asexual stages of Plasmodium falciparum-infected erythrocytes in the placenta." Infect Immun **70**(10): 5412-5415.

Beeson, J. G., G. V. Brown, M. E. Molyneux, C. Mhango, F. Dzinjalamala and S. J. Rogerson (1999). "Plasmodium falciparum isolates from infected pregnant women and children are associated with distinct adhesive and antigenic properties." J Infect Dis **180**(2): 464-472.

Bejon, P., T. N. Williams, A. Liljander, A. M. Noor, J. Wambua, E. Ogada, A. Olotu, F. H. Osier, S. I. Hay, A. Farnert and K. Marsh (2010). "Stable and unstable malaria hotspots in longitudinal cohort studies in Kenya." PLoS Med **7**(7): e1000304.

Bejon, P., T. N. Williams, C. Nyundo, S. I. Hay, D. Benz, P. W. Gething, M. Otiende, J. Peshu, M. Bashraheil, B. Greenhouse, T. Bousema, E. Bauni, K. Marsh, D. L. Smith and S. Borrmann (2014). "A micro-epidemiological analysis of febrile malaria in Coastal Kenya showing hotspots within hotspots." Elife **3**: e02130.

Belkacemi, L., D. M. Nelson, M. Desai and M. G. Ross (2010). "Maternal undernutrition influences placental-fetal development." Biol Reprod **83**(3): 325-331.

Bentler, P. M. (1990). "Comparative fit indexes in structural equation models." Psychological Bulletin **107**: 238.

Berglund, S. K., B. Kristrom, M. Bjorn, J. Lindberg, B. Westrup, M. Norman and M. Domellof (2016). "Marginally low birthweight increases the risk of underweight and short stature at three and a half years of age." Acta Paediatr **105**(6): 610-617.

Bergman, M., S. Huikko, P. Huovinen, P. Paakkari, H. Seppala and R. Finnish Study Group for Antimicrobial (2006). "Macrolide and azithromycin use are linked to increased macrolide resistance in Streptococcus pneumoniae." Antimicrob Agents Chemother **50**(11): 3646-3650.

Berical, A. C., D. Harris, C. S. Dela Cruz and J. D. Possick (2016). "Pneumococcal Vaccination Strategies. An Update and Perspective." Ann Am Thorac Soc **13**(6): 933-944.

Betuela, I., S. Maraga, M. W. Hetzel, T. Tandrapah, A. Sie, S. Yala, J. Kundi, P. Siba, J. C. Reeder and I. Mueller (2012). "Epidemiology of malaria in the Papua New Guinean highlands." Trop Med Int Health **17**(10): 1181-1191.

Betuela, I., A. Rosanas-Urgell, B. Kiniboro, D. I. Stanisic, L. Samol, E. de Lazzari, H. A. Del Portillo, P. Siba, P. L. Alonso, Q. Bassat and I. Mueller (2012). "Relapses contribute significantly to the risk of Plasmodium vivax infection and disease in Papua New Guinean children 1-5 years of age." J Infect Dis **206**(11): 1771-1780.

Beutler, B., I. W. Milsark and A. C. Cerami (1985). "Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin." Science **229**(4716): 869-871.

Bharati, P., M. Pal, M. Bandyopadhyay, A. Bhakta, S. Chakraborty and P. Bharati (2011). "Prevalence and causes of low birth weight in India." Malays J Nutr **17**(3): 301-313.

Bhutta, Z. A. (2006). "Effect of infections and environmental factors on growth and nutritional status in developing countries." J Pediatr Gastroenterol Nutr **43 Suppl 3**: S13-21.

Bhutta, Z. A., T. Ahmed, R. E. Black, S. Cousens, K. Dewey, E. Giugliani, B. A. Haider, B. Kirkwood, S. S. Morris, H. P. Sachdev and M. Shekar (2008). "What works? Interventions for maternal and child undernutrition and survival." Lancet **371**(9610): 417-440.

Bhutta, Z. A., J. K. Das, A. Rizvi, M. F. Gaffey, N. Walker, S. Horton, P. Webb, A. Lartey, R. E. Black, G. Lancet Nutrition Interventions Review, Maternal and G. Child Nutrition

Study (2013). "Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost?" Lancet **382**(9890): 452-477.

Bhutta, Z. A., J. K. Das, A. Rizvi, M. F. Gaffey, N. Walker, S. Horton, P. Webb, A. Lartey, R. E. Black, G. Lancet Nutrition Interventions Review, Maternal and G. Child Nutrition Study (2013a). "Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost?" Lancet **382**(9890): 452-477.

Bhutta, Z. A., J. K. Das, N. Walker, A. Rizvi, H. Campbell, I. Rudan, R. E. Black, D. Lancet and G. Pneumonia Interventions Study (2013). "Interventions to address deaths from childhood pneumonia and diarrhoea equitably: what works and at what cost?" Lancet **381**(9875): 1417-1429.

Black, M., S. P. Walker, L. C. Fernald, C. T. Andersen, A. M. DiGirolamo, C. Lu, D. C. McCoy, G. Fink, Y. R. Shawar, J. Shiffman, A. E. Devercelli, Q. T. Wodon, E. Vargas-Baron, S. Grantham-McGregor and for the Lancet Early Childhood Development Series Steering Committee (2016). "Advancing Early Childhood Development: from Science to Scale 1; Early childhood development coming of age: science through the life course." Lancet **16**.

Black, R. E. (1991). "Would control of childhood infectious diseases reduce malnutrition?" Acta Paediatr Scand Suppl **374**: 133-140.

Black, R. E., L. H. Allen, Z. A. Bhutta, L. E. Caulfield, M. de Onis, M. Ezzati, C. Mathers, J. Rivera, Maternal and G. Child Undernutrition Study (2008). "Maternal and child undernutrition: global and regional exposures and health consequences." Lancet **371**(9608): 243-260.

Black, R. E., K. H. Brown and S. Becker (1984). "Effects of diarrhea associated with specific enteropathogens on the growth of children in rural Bangladesh." Pediatrics **73**(6): 799-805.

Black, R. E., K. H. Brown, S. Becker, A. R. Alim and M. H. Merson (1982). "Contamination of weaning foods and transmission of enterotoxigenic Escherichia coli diarrhoea in children in rural Bangladesh." Trans R Soc Trop Med Hyg **76**(2): 259-264.

Black, R. E., S. Cousens, H. L. Johnson, J. E. Lawn, I. Rudan, D. G. Bassani, P. Jha, H. Campbell, C. F. Walker, R. Cibulskis, T. Eisele, L. Liu, C. Mathers, W. H. O. Child Health Epidemiology Reference Group of and Unicef (2010). "Global, regional, and national causes of child mortality in 2008: a systematic analysis." Lancet **375**(9730): 1969-1987.

Black, R. E., C. G. Victora, S. P. Walker, Z. A. Bhutta, P. Christian, M. de Onis, M. Ezzati, S. Grantham-McGregor, J. Katz, R. Martorell, R. Uauy, Maternal and G. Child Nutrition Study (2013). "Maternal and child undernutrition and overweight in low-income and middle-income countries." Lancet **382**(9890): 427-451.

Bleicher, A. V., H. W. Unger, S. J. Rogerson and E. H. Aitken (2018). "A sandwich enzyme-linked immunosorbent assay for the quantitation of human plasma ferritin." MethodsX **5**: 648-651.

Blencowe, H., S. Cousens, D. Chou, M. Z. Oestergaard, L. Say, A. B. Moller, M. Kinney, J. Lawn and G. Born Too Soon Preterm Birth Action (2013). "Born too soon: the global epidemiology of 15 million preterm births." Reproductive health **10 Suppl 1**(Suppl 1): S2-S2.

Blencowe, H., S. Cousens, M. Z. Oestergaard, D. Chou, A. B. Moller, R. Narwal, A. Adler, C. Vera Garcia, S. Rohde, L. Say and J. E. Lawn (2012). "National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications." Lancet **379**(9832): 2162-2172.

Blencowe, H., J. Krasevec, M. de Onis, R. E. Black, X. An, G. A. Stevens, E. Borghi, C. Hayashi, D. Estevez, L. Cegolon, S. Shiekh, V. Ponce Hardy, J. E. Lawn and S. Cousens (2019). "National, regional, and worldwide estimates of low birthweight in 2015, with trends from 2000: a systematic analysis." Lancet Glob Health **7**(7): e849-e860.

Bloss, E., F. Wainaina and R. C. Bailey (2004). "Prevalence and predictors of underweight, stunting, and wasting among children aged 5 and under in western Kenya." J Trop Pediatr **50**(5): 260-270.

Boel, M. E., M. J. Rijken, B. J. Brabin, F. Nosten and R. McGready (2012). "The epidemiology of postpartum malaria: a systematic review." Malar J **11**: 114.

Boeuf, P., E. H. Aitken, U. Chandrasiri, C. L. Chua, B. McInerney, L. McQuade, M. Duffy, M. Molyneux, G. Brown, J. Glazier and S. J. Rogerson (2013). "Plasmodium falciparum malaria elicits inflammatory responses that dysregulate placental amino acid transport." PLoS Pathog **9**(2): e1003153.

Borgella, S., N. Fievet, B. T. Huynh, S. Ibitokou, G. Hounguevou, J. Affedjou, J. C. Sagbo, P. Houngbegnon, B. Guezo-Mevo, A. Massougbodji, A. J. Luty, M. Cot and P. Deloron (2013). "Impact of pregnancy-associated malaria on infant malaria infection in southern Benin." PLoS One **8**(11): e80624.

Bousema, T., C. Drakeley, S. Gesase, R. Hashim, S. Magesa, F. Mosha, S. Otieno, I. Carneiro, J. Cox, E. Msuya, I. Kleinschmidt, C. Maxwell, B. Greenwood, E. Riley, R. Sauerwein, D. Chandramohan and R. Gosling (2010). "Identification of hot spots of malaria transmission for targeted malaria control." J Infect Dis **201**(11): 1764-1774.

Bousema, T., J. T. Griffin, R. W. Sauerwein, D. L. Smith, T. S. Churcher, W. Takken, A. Ghani, C. Drakeley and R. Gosling (2012). "Hitting hotspots: spatial targeting of malaria for control and elimination." PLoS Med **9**(1): e1001165.

Brabin, B. and P. M. Johnson (2005). "Placental malaria and pre-eclampsia through the looking glass backwards?" Journal of Reproductive Immunology **65**: 1-15.

Brabin, B. and C. Piper (1997). "Anaemia- and malaria-attributable low birthweight in two populations in Papua New Guinea." Ann Hum Biol **24**(6): 547-555.

Brabin, B., H. Tinto and S. A. Roberts (2019). "Testing an infection model to explain excess risk of preterm birth with long-term iron supplementation in a malaria endemic area." Malar J **18**(1): 374.

Brabin, B. J., M. Ginny, J. Sapau, K. Galme and J. Paino (1990). "Consequences of maternal anaemia on outcome of pregnancy in a malaria endemic area in Papua New Guinea." Annals of Tropical Medicine & Parasitology **84**(1): 11-24.

Brabin, B. J., C. Romagosa, S. Abdelgalil, C. Menendez, F. H. Verhoeff, R. McGready, K. A. Fletcher, S. Owens, U. D'Alessandro, F. Nosten, P. R. Fischer and J. Ordi (2004). "The sick placenta-the role of malaria." Placenta **25**(5): 359-378.

Bradley-Moore, A. M., B. M. Greenwood, A. K. Bradley, B. R. Kirkwood and H. M. Gilles (1985). "Malaria chemoprophylaxis with chloroquine in young Nigerian children. III. Its effects on nutrition." Ann Trop Med Parasitol **79**: 575-584.

Brasseur, P., M. Badiane, M. Cisse, P. Agnamey, M. T. Vaillant and P. L. Olliaro (2011). "Changing patterns of malaria during 1996-2010 in an area of moderate transmission in southern Senegal." Malar J **10**: 203.

Bresnahan, K. A. and S. A. Tanumihardjo (2014). "Undernutrition, the acute phase response to infection, and its effects on micronutrient status indicators." Adv Nutr **5**(6): 702-711.

Briand, V., J. Saal, C. Ghafari, B. T. Huynh, N. Fievet, C. Schmiegelow, A. Massougbodji, P. Deloron, J. Zeitlin and M. Cot (2016). "Fetal Growth Restriction Is Associated With Malaria in Pregnancy: A Prospective Longitudinal Study in Benin." J Infect Dis **214**(3): 417-425.

Briend, A. (1990). "Is diarrhoea a major cause of malnutrition among the under-fives in developing countries? A review of available evidence." Eur J Clin Nutr **44**(9): 611-628.

Briend, A., K. Z. Hasan, K. M. Aziz and B. A. Hoque (1989). "Are diarrhoea control programmes likely to reduce childhood malnutrition? Observations from rural Bangladesh." Lancet **2**(8658): 319-322.

Briend, A., K. Z. Hasan, K. M. Aziz and B. A. Hoque (1990). "Diarrhoea and catch-up growth." Lancet **335**(8698): 1157-1158.

Briend, A., T. Khara and C. Dolan (2015). "Wasting and stunting--similarities and differences: policy and programmatic implications." Food Nutr Bull **36**(1 Suppl): S15-23.

Brodsky, D. and H. Christou (2004). "Current concepts in intrauterine growth restriction." J Intensive Care Med **19**(6): 307-319.

Brosens, I., R. Pijnenborg, L. Vercruysse and R. Romero (2011). "The "Great Obstetrical Syndromes" are associated with disorders of deep placentation." Am J Obstet Gynecol **204**(3): 193-201.

Browne, E. N., G. H. Maude and F. N. Binka (2001). "The impact of insecticide-treated bednets on malaria and anaemia in pregnancy in Kassena-Nankana district, Ghana: a randomized controlled trial." Trop Med Int Health **6**(9): 667-676.

Buisman, L. R., E. Van de Poel, O. O'Donnell and E. K. A. van Doorslaer (2019). "What explains the fall in child stunting in Sub-Saharan Africa?" SSM Popul Health **8**: 100384.

Bukenya, G. B., T. Barnes and N. Nwokolo (1991). "Low birthweight and acute childhood diarrhoea: evidence of their association in an urban settlement of Papua New Guinea." Ann Trop Paediatr **11**(4): 357-362.

Bukenya, G. B. and N. Nwokolo (1991). "Compound hygiene, presence of standpipe and the risk of childhood diarrhoea in an urban settlement of Papua New Guinea." Int J Epidemiol **20**(2): 534-539.

Burkot, T. R., P. M. Graves, R. Paru, R. A. Wirtz and P. F. Heywood (1988). "Human malaria transmission studies in the Anopheles punctulatus complex in Papua New Guinea: sporozoite rates, inoculation rates, and sporozoite densities." Am J Trop Med Hyg **39**(2): 135-144.

Burton, G. J. and E. Jauniaux (2018). "Pathophysiology of placental-derived fetal growth restriction." American Journal of Obstetrics and Gynecology **218**(2, Supplement): S745-S761.

Carlin, A. and Z. Alfirevic (2008). "Physiological changes of pregnancy and monitoring." Best Pract Res Clin Obstet Gynaecol **22**(5): 801-823.

Carswell, F., A. O. Hughes, R. I. Palmer, J. Higginson, P. S. Harland and R. H. Meakins (1981). "Nutritional status, globulin titers, and parasitic infections of two populations of Tanzanian school children." Am J Clin Nutr **34**(7): 1292-1299.

Cattani, J. A., J. L. Tulloch, H. Vrbova, D. Jolley, F. D. Gibson, J. S. Moir, P. F. Heywood, M. P. Alpers, A. Stevenson and R. Clancy (1986). "The epidemiology of malaria in a population surrounding Madang, Papua New Guinea." Am J Trop Med Hyg **35**(1): 3-15.

Caulfield, L. E., M. de Onis, M. Blossner and R. E. Black (2004). "Undernutrition as an underlying cause of child deaths associated with diarrhea, pneumonia, malaria, and measles." American Journal of Clinical Nutrition **80**(1): 193-198.

Ceesay, S. J., C. Casals-Pascual, J. Erskine, S. E. Anya, N. O. Duah, A. J. C. Fulford, S. S. S. Sesay, I. Abubakar, S. Dunyo, O. Sey, A. Palmer, M. Fofana, T. Corrah, K. A. Bojang, H. C. Whittle, B. M. Greenwood and D. J. Conway (2008). "Changes in malaria indices between 1999 and 2007 in The Gambia: a retrospective analysis." Lancet **372**(9649): 1545-1554.

Chandra, R. K. (2007). "Nutrition and the immune system." Proceedings of the Nutrition Society **52**(1): 77-84.

Chandrasiri, U. P., C. L. Chua, A. J. Umbers, E. Chaluluka, J. D. Glazier, S. J. Rogerson and P. Boeuf (2014). "Insight into the pathogenesis of fetal growth restriction in placental malaria: decreased placental glucose transporter isoform 1 expression." J Infect Dis **209**(10): 1663-1667.

Chang, S. C., K. O. O'Brien, M. S. Nathanson, J. Mancini and F. R. Witter (2003). "Hemoglobin concentrations influence birth outcomes in pregnant African-American adolescents." J Nutr **133**(7): 2348-2355.

Checkley, W., G. Buckley, R. H. Gilman, A. M. Assis, R. L. Guerrant, S. S. Morris, K. Molbak, P. Valentiner-Branth, C. F. Lanata, R. E. Black, M. Childhood and N. Infection (2008). "Multi-country analysis of the effects of diarrhoea on childhood stunting." Int J Epidemiol **37**(4): 816-830.

Checkley, W., L. D. Epstein, R. H. Gilman, L. Cabrera and R. E. Black (2003). "Effects of acute diarrhea on linear growth in Peruvian children." Am J Epidemiol **157**(2): 166-175.

Chico, R. M., B. B. Hack, M. J. Newport, E. Ngulube and D. Chandramohan (2013). "On the pathway to better birth outcomes? A systematic review of azithromycin and curable sexually transmitted infections." Expert Rev Anti Infect Ther **11**(12): 1303-1332.

Christian, P. (2009). "Prenatal origins of undernutrition." Nestle Nutr Workshop Ser Pediatr Program **63**: 59-73; discussion 74-57, 259-268.

Christian, P., S. E. Lee, M. Donahue Angel, L. S. Adair, S. E. Arifeen, P. Ashorn, F. C. Barros, C. H. Fall, W. W. Fawzi, W. Hao, G. Hu, J. H. Humphrey, L. Huybregts, C. V. Joglekar, S. K. Kariuki, P. Kolsteren, G. V. Krishnaveni, E. Liu, R. Martorell, D. Osrin, L. A. Persson, U. Ramakrishnan, L. Richter, D. Roberfroid, A. Sania, F. O. Ter Kuile, J. Tielsch, C. G. Victora, C. S. Yajnik, H. Yan, L. Zeng and R. E. Black (2013). "Risk of childhood undernutrition related to small-for-gestational age and preterm birth in low- and middle-income countries." Int J Epidemiol **42**(5): 1340-1355.

Clark, I. A., A. C. Budd, L. M. Alleva and W. B. Cowden (2006). "Human malarial disease: a consequence of inflammatory cytokine release." Malar J **5**: 85.

Clark, I. A. and L. Schofield (2000). "Pathogenesis of malaria." Parasitol Today **16**(10): 451-454.

Clark, I. A., J. L. Virelizier, E. A. Carswell and P. R. Wood (1981). "Possible importance of macrophage-derived mediators in acute malaria." Infect Immun **32**(3): 1058-1066.

Cole, T. J. and J. M. Parkin (1977). "Infection and its effect on the growth of young children: a comparison of The Gambia and Uganda." Trans R Soc Trop Med Hyg **71**(3): 196-198.

Collaborators, G. B. D. D. D. (2017). "Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study 2015." The Lancet. Infectious diseases **17**(9): 909-948.

Conroy, A. L., K. L. Silver, K. Zhong, M. Rennie, P. Ward, J. V. Sarma, M. E. Molyneux, J. Sled, J. F. Fletcher, S. Rogerson and K. C. Kain (2013). "Complement activation and the resulting placental vascular insufficiency drives fetal growth restriction associated with placental malaria." Cell Host Microbe **13**(2): 215-226.

Cooper, R. D., D. G. Waterson, S. P. Frances, N. W. Beebe, B. Pluess and A. W. Sweeney (2009). "Malaria vectors of Papua New Guinea." Int J Parasitol **39**(13): 1495-1501.

Coura, J. R., M. Suarez-Mutis and S. Ladeia-Andrade (2006). "A new challenge for malaria control in Brazil: asymptomatic Plasmodium infection--a review." Mem Inst Oswaldo Cruz **101**(3): 229-237.

Cromer, D., K. J. Evans, L. Schofield and M. P. Davenport (2006). "Preferential invasion of reticulocytes during late-stage Plasmodium berghei infection accounts for reduced circulating reticulocyte levels." Int J Parasitol **36**(13): 1389-1397.

da Silva Lopes, K., E. Ota, P. Shakya, A. Dagvadorj, O. O. Balogun, J. P. Pena-Rosas, L. M. De-Regil and R. Mori (2017). "Effects of nutrition interventions during pregnancy on low birth weight: an overview of systematic reviews." BMJ Glob Health **2**(3): e000389.

Danaei, G., K. G. Andrews, C. R. Sudfeld, G. Fink, D. C. McCoy, E. Peet, A. Sania, M. C. Smith Fawzi, M. Ezzati and W. W. Fawzi (2016). "Risk Factors for Childhood Stunting in 137 Developing Countries: A Comparative Risk Assessment Analysis at Global, Regional, and Country Levels." PLoS Med **13**(11): e1002164.

Das, D., R. F. Grais, E. A. Okiro, K. Stepniewska, R. Mansoor, S. van der Kam, D. J. Terlouw, J. Tarning, K. I. Barnes and P. J. Guerin (2018). "Complex interactions between malaria and malnutrition: a systematic literature review." BMC Med **16**(1): 186.

de Onis, M. and F. Branca (2016). "Childhood stunting: a global perspective." Matern Child Nutr **12 Suppl 1**: 12-26.

Deen, J. L., G. E. Walraven and L. von Seidlein (2002). "Increased risk for malaria in chronically malnourished children under 5 years of age in rural Gambia." J Trop Pediatr **48**(2): 78-83.

Desai, M., J. Gutman, S. M. Taylor, R. E. Wiegand, C. Khairallah, K. Kayentao, P. Ouma, S. O. Coulibaly, L. Kalilani, K. E. Mace, E. Arinaitwe, D. P. Mathanga, O. Doumbo, K. Otieno, D. Edgar, E. Chaluluka, M. Kamuliwo, V. Ades, J. Skarbinski, Y. P. Shi, P. Magnussen, S. Meshnick and F. O. Ter Kuile (2016). "Impact of Sulfadoxine-Pyrimethamine Resistance on Effectiveness of Intermittent Preventive Therapy for Malaria in Pregnancy at Clearing Infections and Preventing Low Birth Weight." Clin Infect Dis **62**(3): 323-333.

Desai, M., J. Hill, S. Fernandes, P. Walker, C. Pell, J. Gutman, K. Kayentao, R. Gonzalez, J. Webster, B. Greenwood, M. Cot and F. O. ter Kuile (2018). "Prevention of malaria in pregnancy." The Lancet Infectious Diseases **18**(4): e119-e132.

Desai, M., F. O. ter Kuile, F. Nosten, R. McGready, K. Asamoa, B. Brabin and R. D. Newman (2007). "Epidemiology and burden of malaria in pregnancy." The Lancet Infectious Diseases **7**(2): 93-104.

Dewey, K. G. and K. Begum (2011). "Long-term consequences of stunting in early life." Matern Child Nutr **7 Suppl 3**: 5-18.

Dewey, K. G. and B. M. Oaks (2017). "U-shaped curve for risk associated with maternal hemoglobin, iron status, or iron supplementation." Am J Clin Nutr **106**(Suppl 6): 1694S-1702S.

Dolan, G., F. O. ter Kuile, V. Jacoutot, N. J. White, C. Luxemburger, L. Malankirii, T. Chongsuphajaisiddhi and F. Nosten (1993). "Bed nets for the prevention of malaria and anaemia in pregnancy." Trans R Soc Trop Med Hyg **87**(6): 620-626.

Edmond, K., S. Scott, V. Korczak, C. Ward, C. Sanderson, E. Theodoratou, A. Clark, U. Griffiths, I. Rudan and H. Campbell (2012). "Long term sequelae from childhood pneumonia; systematic review and meta-analysis." PLoS One **7**(2): e31239.

Ehrman, F. C., J. M. Ellis and M. D. Young (1945). "Plasmodium Vivax Chesson Strain." Science **101**(2624): 377.

Eisele, T. P., D. Larsen and R. W. Steketee (2010). "Protective efficacy of interventions for preventing malaria mortality in children in Plasmodium falciparum endemic areas." Int J Epidemiol **39 Suppl 1**: i88-101.

Emergency Nutrition Network (ENN). (2014). "Review of the links between wasting and stunting." Retrieved 25-01-2017, 2017, from https://www.ennonline.net/fex/50/relationshipwastingstunting.

Emerson, E., A. Savage and G. Llewellyn (2020). "Prevalence of underweight, wasting and stunting among young children with a significant cognitive delay in 47 low-income and middle-income countries." J Intellect Disabil Res **64**(2): 93-102.

Ernst, G. D., L. L. de Jonge, A. Hofman, J. Lindemans, H. Russcher, E. A. Steegers and V. W. Jaddoe (2011). "C-reactive protein levels in early pregnancy, fetal growth patterns, and the risk for neonatal complications: the Generation R Study." Am J Obstet Gynecol **205**(2): 132 e131-112.

Ernst, K. C., S. O. Adoka, D. O. Kowuor, M. L. Wilson and C. C. John (2006). "Malaria hotspot areas in a highland Kenya site are consistent in epidemic and non-epidemic years and are associated with ecological factors." Malar J **5**: 78.

Espinoza, J. L. (2019). "Malaria Resurgence in the Americas: An Underestimated Threat." Pathogens (Basel, Switzerland) **8**(1): 11.

Fall, C. H., C. S. Yajnik, S. Rao, A. A. Davies, N. Brown and H. J. Farrant (2003). "Micronutrients and fetal growth." J Nutr **133**(5 Suppl 2): 1747S-1756S.

Faye, C. M., S. Fonn and J. Levin (2019). "Factors associated with recovery from stunting among under-five children in two Nairobi informal settlements." PLoS One **14**(4): e0215488.

Fekadu, Y., A. Mesfin, D. Haile and B. J. Stoecker (2015). "Factors associated with nutritional status of infants and young children in Somali Region, Ethiopia: a cross- sectional study." BMC Public Health **15**: 846.

Ferro-Luzzi, A., N. G. Norgan and J. V. Durnin (1975). "Food intake, its relationship to body weight and age, and its apparent nutritional adequacy in New Guinean children." Am J Clin Nutr **28**(12): 1443-1453.

Fiedler, U. and H. G. Augustin (2006). "Angiopoietins: a link between angiogenesis and inflammation." Trends Immunol **27**(12): 552-558.

Fievet, N., M. Moussa, G. Tami, B. Maubert, M. Cot, P. Deloron and G. Chaouat (2001). "Plasmodium falciparum induces a Th1/Th2 disequilibrium, favoring the Th1-type pathway, in the human placenta." J Infect Dis **183**(10): 1530-1534.

Figueiredo, A., I. S. Gomes-Filho, R. B. Silva, P. P. S. Pereira, F. Mata, A. O. Lyrio, E. S. Souza, S. S. Cruz and M. G. Pereira (2018). "Maternal Anemia and Low Birth Weight: A Systematic Review and Meta-Analysis." Nutrients **10**(5).

Fowkes, F., K. Moore, D. H. Opi, J. Simpson, F. Langham, D. I. Stanisic, A. Ura, C. L. King, P. Siba, I. Mueller, S. Rogerson and J. Beeson (2018). "Iron deficiency during pregnancy is associated with a reduced risk of adverse birth outcomes in a malaria-endemic area in a longitudinal cohort study." BMC Medicine **16**.

Fowkes, F. J., P. Boeuf and J. G. Beeson (2016). "Immunity to malaria in an era of declining malaria transmission." Parasitology **143**(2): 139-153.

Fried, M. and P. E. Duffy (1996). "Adherence of Plasmodium falciparum to chondroitin sulfate A in the human placenta." Science **272**(5267): 1502-1504.

Fried, M., J. D. Kurtis, B. Swihart, S. Pond-Tor, A. Barry, Y. Sidibe, S. Gaoussou, M. Traore, S. Keita, A. Mahamar, O. Attaher, A. B. Dembele, K. B. Cisse, B. S. Diarra, M. B. Kanoute, A. Dicko and P. E. Duffy (2017). "Systemic Inflammatory Response to Malaria During Pregnancy Is Associated With Pregnancy Loss and Preterm Delivery." Clin Infect Dis **65**(10): 1729-1735.

Fried, M., R. O. Muga, A. O. Misore and P. E. Duffy (1998). "Malaria elicits type 1 cytokines in the human placenta: IFN-gamma and TNF-alpha associated with pregnancy outcomes." J Immunol **160**(5): 2523-2530.

Friedman, J. F., A. M. Kwena, L. B. Mirel, S. K. Kariuki, D. J. Terlouw, P. A. Phillips-Howard, W. A. Hawley, B. L. Nahlen, Y. P. Shi and F. O. ter Kuile (2005). "Malaria and nutritional status among pre-school children: results from cross-sectional surveys in western Kenya." Am J Trop Med Hyg **73**(4): 698-704.

G. B. D. Child Mortality Collaborators (2016). "Global, regional, national, and selected subnational levels of stillbirths, neonatal, infant, and under-5 mortality, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015." Lancet **388**(10053): 1725-1774.

G. B. D. Mortality and Causes of Death Collaborators (2016). "Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015." Lancet **388**(10053): 1459-1544.

Galatas, B., Q. Bassat and A. Mayor (2016). "Malaria Parasites in the Asymptomatic: Looking for the Hay in the Haystack." Trends Parasitol **32**(4): 296-308.

Gamble, C., J. P. Ekwaru and F. O. ter Kuile (2006). "Insecticide-treated nets for preventing malaria in pregnancy." Cochrane Database Syst Rev(2): CD003755.

Garenne, M., D. Willie, B. Maire, O. Fontaine, R. Eeckels, A. Briend and J. Van den Broeck (2009). "Incidence and duration of severe wasting in two African populations." Public Health Nutr **12**(11): 1974-1982.

Gari, T., E. Loha, W. Deressa, T. Solomon and B. Lindtjorn (2018). "Malaria increased the risk of stunting and wasting among young children in Ethiopia: Results of a cohort study." PLoS One **13**(1): e0190983.

Garn, S. M., S. A. Ridella, A. S. Petzold and F. Falkner (1981). "Maternal hematologic levels and pregnancy outcomes." Semin Perinatol **5**(2): 155-162.

Gasparrini, A. (2011). "Distributed Lag Linear and Non-Linear Models in R: The Package dlnm." J Stat Softw **43**(8): 1-20.

Gasparrini, A., B. Armstrong and M. G. Kenward (2010). "Distributed lag non-linear models." Stat Med **29**(21): 2224-2234.

Geelhoed, D., F. Agadzi, L. Visser, E. Ablordeppey, K. Asare, P. O'Rourke, J. S. Van Leeuwen and J. Van Roosmalen (2006). "Maternal and fetal outcome after severe anemia in pregnancy in rural Ghana." Acta Obstet Gynecol Scand **85**(1): 49-55.

Genton, B., F. al-Yaman, H. P. Beck, J. Hii, S. Mellor, A. Narara, N. Gibson, T. Smith and M. P. Alpers (1995). "The epidemiology of malaria in the Wosera area, East Sepik Province, Papua New Guinea, in preparation for vaccine trials. I. Malariometric indices and immunity." Ann Trop Med Parasitol **89**(4): 359-376.

Genton, B., F. Al-Yaman, M. Ginny, J. Taraika and M. P. Alpers (1998). "Relation of anthropometry to malaria morbidity and immunity in Papua New Guinean children." Am J Clin Nutr **68**(3): 734-741.

Genton, B., V. D'Acremont, L. Rare, K. Baea, J. C. Reeder, M. P. Alpers and I. Muller (2008). "Plasmodium vivax and mixed infections are associated with severe malaria in children: a prospective cohort study from Papua New Guinea." PLoS Med **5**(6): e127.

Gibson, R. S., A. Heywood, C. Yaman, A. Sohlstrom, L. U. Thompson and P. Heywood (1991). "Growth in children from the Wosera subdistrict, Papua New Guinea, in relation to energy and protein intakes and zinc status." Am J Clin Nutr **53**(3): 782-789.

Gill, C. J., M. Young, K. Schroder, L. Carvajal-Velez, M. McNabb, S. Aboubaker, S. Qazi and Z. A. Bhutta (2013). "Bottlenecks, barriers, and solutions: results from multicountry consultations focused on reduction of childhood pneumonia and diarrhoea deaths." The Lancet **381**(9876): 1487-1498.

Gillrie, M. R., K. Lee, D. C. Gowda, S. P. Davis, M. Monestier, L. Cui, T. T. Hien, N. P. Day and M. Ho (2012). "Plasmodium falciparum histones induce endothelial proinflammatory response and barrier dysfunction." Am J Pathol **180**(3): 1028-1039.

Golden, M. H. (1994). "Is complete catch-up possible for stunted malnourished children?" Eur J Clin Nutr **48 Suppl 1**: S58-70; discussion S71.

Gonzalez, R., E. Sevene, G. Jagoe, L. Slutsker and C. Menendez (2016). "A Public Health Paradox: The Women Most Vulnerable to Malaria Are the Least Protected." PLoS Med **13**(5): e1002014.

Goris, J. M., N. Zomerdijk and V. J. Temple (2017). "Nutritional status and dietary diversity of Kamea in Gulf Province, Papua New Guineas." Asia Pac J Clin Nutr **26**(4): 665-670.

Grantham-McGregor, S., Y. B. Cheung, S. Cueto, P. Glewwe, L. Richter and B. Strupp (2007). "Developmental potential in the first 5 years for children in developing countries." The Lancet **369**(9555): 60-70.

Greenhill, A., W. Pomat, T. Duke, W. Lagani, D. Mokela and D. Lehmann (2010). "The role of biomedical research in the fight against pneumonia in Papua New Guinea." P N G Med J **53**(1-2): 1-4.

Greenwood, B. M. (1989). "The microepidemiology of malaria and its importance to malaria control." Trans R Soc Trop Med Hyg **83 Suppl**: 25-29.

Gregory, R. D. and T. M. Blackburn (1991). "Parasite prevalence and host sample size." Parasitol Today **7**(11): 316-318.

Griffin, J. T., N. M. Ferguson and A. C. Ghani (2014). "Estimates of the changing age-burden of Plasmodium falciparum malaria disease in sub-Saharan Africa." Nat Commun **5**: 3136.

Griffin, J. T., T. D. Hollingsworth, H. Reyburn, C. J. Drakeley, E. M. Riley and A. C. Ghani (2015). "Gradual acquisition of immunity to severe malaria with increasing exposure." Proc Biol Sci **282**(1801): 20142657.

Grimwood, K. and A. B. Chang (2015). "Long-term effects of pneumonia in young children." Pneumonia **6**: 101-114.

Gupta, S., R. W. Snow, C. A. Donnelly, K. Marsh and C. Newbold (1999). "Immunity to non-cerebral severe malaria is acquired after one or two infections." Nat Med **5**(3): 340-343.

Haider, B. A., I. Olofin, M. Wang, D. Spiegelman, M. Ezzati, W. W. Fawzi and G. Nutrition Impact Model Study (2013). "Anaemia, prenatal iron use, and risk of adverse pregnancy outcomes: systematic review and meta-analysis." BMJ **346**: f3443.

Hallamaa, L., Y. B. Cheung, M. Luntamo, U. Ashorn, T. Kulmala, C. Mangani and P. Ashorn (2019). "The impact of maternal antenatal treatment with two doses of azithromycin and monthly sulphadoxine-pyrimethamine on child weight, mid-upper arm circumference and head circumference: A randomized controlled trial." PLoS One **14**(5): e0216536.

Hamalainen, H., K. Hakkarainen and S. Heinonen (2003). "Anaemia in the first but not in the second or third trimester is a risk factor for low birth weight." Clin Nutr **22**(3): 271-275.

Harding, J. E. (2001). "The nutritional basis of the fetal origins of adult disease." Int J Epidemiol **30**(1): 15-23.

Harris, I., W. W. Sharrock, L. M. Bain, K. A. Gray, A. Bobogare, L. Boaz, K. Lilley, D. Krause, A. Vallely, M. L. Johnson, M. L. Gatton, G. D. Shanks and Q. Cheng (2010). "A large proportion of asymptomatic Plasmodium infections with low and sub-microscopic parasite densities in the low transmission setting of Temotu Province, Solomon Islands: challenges for malaria diagnostics in an elimination setting." Malar J **9**: 254.

Haug, S., T. Lakew, G. Habtemariam, W. Alemayehu, V. Cevallos, Z. Zhou, J. House, K. Ray, T. Porco, T. Rutar, J. Keenan, T. M. Lietman and B. D. Gaynor (2010). "The decline of pneumococcal resistance after cessation of mass antibiotic distributions for trachoma." Clin Infect Dis **51**(5): 571-574.

Hay, S. I., D. L. Smith and R. W. Snow (2008). "Measuring malaria endemicity from intense to interrupted transmission." Lancet Infect Dis **8**(6): 369-378.

Hetzel, M. W., A. A. Choudhury, J. Pulford, Y. Ura, M. Whittaker, P. M. Siba and I. Mueller (2014). "Progress in mosquito net coverage in Papua New Guinea." Malar J **13**: 242.

Hetzel, M. W., H. Morris, N. Tarongka, C. Barnadas, J. Pulford, L. Makita, P. M. Siba and I. Mueller (2015). "Prevalence of malaria across Papua New Guinea after initial roll-out of insecticide-treated mosquito nets." Trop Med Int Health **20**(12): 1745-1755.

Hetzel, M. W., J. Pulford, H. N. Gouda, A. Hodge, P. Siba and I. Mueller (2014). Papua New Guinea & The Global Fund Round 8 Malaria Control Programme Evaluation 2009 - 2014: Report on The Papua New Guinea National Malaria Control Program: Primary outcome & impact indicators, 2009-2014, Papua New Guinea Institute of Medical Research.

Hetzel, M. W., J. Pulford, Y. Ura, S. Jamea-Maiasa, A. Tandrapah, N. Tarongka, L. Lorry, L. J. Robinson, K. Lilley, L. Makita, P. M. Siba and I. Mueller (2017). "Insecticide-treated nets and malaria prevalence, Papua New Guinea, 2008-2014." Bull World Health Organ **95**(10): 695-705B.

Hetzel, M. W., O. P. M. Saweri, J. J. Kuadima, I. Smith, Y. Ura, A. Tandrapah, S. Jamea-Maiasa, P. Siba and J. Pulford (2018). Papua New Guinea Indicator Survery 2016 - 2017: Malarai Prevention, infection, and Treatment, Papua New Guinea Institute of Medical Research, Goroka.

Heywood, P. F. (1983). "Growth and Nutrition in Papua-New-Guinea." Journal of Human Evolution **12**(1): 133-143.

Hill, J., J. Hoyt, F. Achieng, P. Ouma, A. L'Lanziva, S. Kariuki, M. Desai and J. Webster (2016). "User and Provider Acceptability of Intermittent Screening and Treatment and Intermittent Preventive Treatment with Dihydroartemisinin-Piperaquine to Prevent Malaria in Pregnancy in Western Kenya." PLoS One **11**(3): e0150259.

Hoddinott, J., J. R. Behrman, J. A. Maluccio, P. Melgar, A. R. Quisumbing, M. Ramirez-Zea, A. D. Stein, K. M. Yount and R. Martorell (2013). "Adult consequences of growth failure in early childhood." Am J Clin Nutr **98**(5): 1170-1178.

Hoddinott, J., J. A. Maluccio, J. R. Behrman, R. Flores and R. Martorell (2008). "Effect of a nutrition intervention during early childhood on economic productivity in Guatemalan adults." Lancet **371**(9610): 411-416.

Hofmann, N. E., S. Karl, R. Wampfler, B. Kiniboro, A. Teliki, J. Iga, A. Waltmann, I. Betuela, I. Felger, L. J. Robinson and I. Mueller (2017). "The complex relationship of exposure to new Plasmodium infections and incidence of clinical malaria in Papua New Guinea." Elife **6**.

Hou, X. (2016). "Stagnant Stunting Rate despite Rapid Economic Growth-An Analysis of Cross Sectional Survey Data of Undernutrition among Children under Five in Papua New Guinea." AIMS Public Health **3**(1): 25-39.

Huynh, B. T., N. Fievet, G. Gbaguidi, S. Dechavanne, S. Borgella, B. Guezo-Mevo, A. Massougbodji, N. T. Ndam, P. Deloron and M. Cot (2011). "Influence of the timing of malaria infection during pregnancy on birth weight and on maternal anemia in Benin." Am J Trop Med Hyg **85**(2): 214-220.

Hyman, R. W., M. Fukushima, H. Jiang, E. Fung, L. Rand, B. Johnson, K. C. Vo, A. B. Caughey, J. F. Hilton, R. W. Davis and L. C. Giudice (2014). "Diversity of the vaginal microbiome correlates with preterm birth." Reproductive sciences (Thousand Oaks, Calif.) **21**(1): 32-40.

Hytten, F. (1985). "Blood volume changes in normal pregnancy." Clin Haematol **14**(3): 601-612.

Imdad, A. and Z. A. Bhutta (2012). "Maternal nutrition and birth outcomes: effect of balanced protein-energy supplementation." Paediatr Perinat Epidemiol **26 Suppl 1**: 178-190.

Institute of Medicine (2000). From Neurons to Neighbourhoods: The Science of Early Childhood Development Washington, DC., The National Academies Press.

Isanaka, S., T. Roederer, A. Djibo, F. J. Luquero, N. Nombela, P. J. Guerin and R. F. Grais (2010). "Reducing wasting in young children with preventive supplementation: a cohort study in Niger." Pediatrics **126**(2): e442-450.

Jakubowski, A. A., E. S. Casper, J. L. Gabrilove, M. A. Templeton, S. A. Sherwin and H. F. Oettgen (1989). "Phase I trial of intramuscularly administered tumor necrosis factor in patients with advanced cancer." J Clin Oncol **7**(3): 298-303.

Jehan, I., S. Zaidi, S. Rizvi, N. Mobeen, E. M. McClure, B. Munoz, O. Pasha, L. L. Wright and R. L. Goldenberg (2010). "Dating gestational age by last menstrual period, symphysis-fundal height, and ultrasound in urban Pakistan." Int J Gynaecol Obstet **110**(3): 231-234.

John, G. K., N. M. Douglas, L. von Seidlein, F. Nosten, J. K. Baird, N. J. White and R. N. Price (2012). "Primaquine radical cure of Plasmodium vivax: a critical review of the literature." Malar J **11**: 280.

Jung, J., M. M. Rahman, M. S. Rahman, K. T. Swe, M. R. Islam, M. O. Rahman and S. Akter (2019). "Effects of hemoglobin levels during pregnancy on adverse maternal and infant outcomes: a systematic review and meta-analysis." Ann N Y Acad Sci **1450**(1): 69-82.

Kalanda, B. F., S. van Buuren, F. H. Verhoeff and B. J. Brabin (2005). "Catch-up growth in Malawian babies, a longitudinal study of normal and low birthweight babies born in a malarious endemic area." Early Hum Dev **81**(10): 841-850.

Kalanda, B. F., F. H. Verhoeff, L. Chimsuku, G. Harper and B. J. Brabin (2006). "Adverse Birth Outcomes in a Malarious Area." Epidemiology and Infection **134**(3): 659-666.

Kang, H., B. Kreuels, O. Adjei, R. Krumkamp, J. May and D. S. Small (2013). "The causal effect of malaria on stunting: a Mendelian randomization and matching approach." Int J Epidemiol **42**(5): 1390-1398.

Kapisi, J., A. Kakuru, P. Jagannathan, M. K. Muhindo, P. Natureeba, P. Awori, M. Nakalembe, R. Ssekitoleko, P. Olwoch, J. Ategeka, P. Nayebare, T. D. Clark, G. Rizzuto, A. Muehlenbachs, D. V. Havlir, M. R. Kamya, G. Dorsey and S. L. Gaw (2017). "Relationships between infection with Plasmodium falciparum during pregnancy, measures of placental malaria, and adverse birth outcomes." Malar J **16**(1): 400.

Kar, B. R., S. L. Rao and B. A. Chandramouli (2008). "Cognitive development in children with chronic protein energy malnutrition." Behav Brain Funct **4**: 31.

Karinen, L., A. Pouta, A. Bloigu, P. Koskela, M. Paldanius, M. Leinonen, P. Saikku, M. R. Jervelin and A. L. Hartikainen (2005). "Serum C-reactive protein and Chlamydia trachomatis antibodies in preterm delivery." Obstet Gynecol **106**(1): 73-80.

Karunaweera, N. D., G. E. Grau, P. Gamage, R. Carter and K. N. Mendis (1992). "Dynamics of fever and serum levels of tumor necrosis factor are closely associated during clinical paroxysms in Plasmodium vivax malaria." Proc Natl Acad Sci U S A **89**(8): 3200-3203.

Katona, P. and J. Katona-Apte (2008). "The interaction between nutrition and infection." Clin Infect Dis **46**(10): 1582-1588.

Katz, J., A. C. Lee, N. Kozuki, J. E. Lawn, S. Cousens, H. Blencowe, M. Ezzati, Z. A. Bhutta, T. Marchant, B. A. Willey, L. Adair, F. Barros, A. H. Baqui, P. Christian, W. Fawzi, R. Gonzalez, J. Humphrey, L. Huybregts, P. Kolsteren, A. Mongkolchati, L. C. Mullany, R. Ndyomugyenyi, J. K. Nien, D. Osrin, D. Roberfroid, A. Sania, C. Schmiegelow, M. F. Silveira, J. Tielsch, A. Vaidya, S. C. Velaphi, C. G. Victora, D. Watson-Jones, R. E. Black and C. S.-f.-G.-A.-P. B. W. Group (2013). "Mortality risk in preterm and small-for-gestational-age infants in low-income and middle-income countries: a pooled country analysis." Lancet **382**(9890): 417-425.

Kazura, J. W., P. M. Siba, I. Betuela and I. Mueller (2012). "Research challenges and gaps in malaria knowledge in Papua New Guinea." Acta Trop **121**(3): 274-280.

Keeble, R. and J. Keeble (2006). "Nutritional study of the 1-4 year old population of the Lower Jimi Valley, Western Highlands Province, Papua New Guinea." P N G Med J **49**(3-4): 156-161.

Keusch, G. T. (2003). "The history of nutrition: malnutrition, infection and immunity." J Nutr **133**(1): 336S-340S.

Keven, J. B., C. N. Henry-Halldin, E. K. Thomsen, I. Mueller, P. M. Siba, P. A. Zimmerman and L. J. Reimer (2010). "Pyrethroid susceptibility in natural populations of the Anopheles punctulatus group (Diptera: Culicidae) in Papua New Guinea." Am J Trop Med Hyg **83**(6): 1259-1261.

Khalil, I. A., C. Troeger, P. C. Rao, B. F. Blacker, A. Brown, T. G. Brewer, D. V. Colombara, E. L. De Hostos, C. Engmann, R. L. Guerrant, R. Haque, E. R. Houpt, G. Kang, P. S. Korpe, K. L. Kotloff, A. A. M. Lima, W. A. Petri, J. A. Platts-Mills, D. A. Shoultz, M. H. Forouzanfar, S. I. Hay, R. C. Reiner and A. H. Mokdad (2018). "Morbidity, mortality, and long-term consequences associated with diarrhoea from Cryptosporidium infection in children younger than 5 years: a meta-analyses study." The Lancet Global Health **6**(7): e758-e768.

Khantikul, N., P. Butraporn, H. S. Kim, S. Leemingsawat, M. A. Tempongko and W. Suwonkerd (2009). "Adherence to antimalarial drug therapy among vivax malaria patients in northern Thailand." J Health Popul Nutr **27**(1): 4-13.

Kim, D. and A. Saada (2013). "The social determinants of infant mortality and birth outcomes in Western developed nations: a cross-country systematic review." Int J Environ Res Public Health **10**(6): 2296-2335.

Kisuule, I., D. K. Kaye, F. Najjuka, S. K. Ssematimba, A. Arinda, G. Nakitende and L. Otim (2013). "Timing and reasons for coming late for the first antenatal care visit by pregnant women at Mulago hospital, Kampala Uganda." BMC Pregnancy Childbirth **13**: 121.

Klebanoff, M. A., P. H. Shiono, H. W. Berendes and G. G. Rhoads (1989). "Facts and artifacts about anemia and preterm delivery." JAMA **262**(4): 511-515.

Kline, R. B. (2015). Principles and Practice of Structural Equation Modeling, Fourth Edition. New York, UNITED STATES, Guilford Publications.

Klufio, C. A., A. B. Amoa, L. Augerea and F. Wurr (1997). "A case-control study of singleton low birthweight babies at the Port Moresby General Hospital." P N G Med J **40**(3-4): 136.

Koepfli, C., K. L. Colborn, B. Kiniboro, E. Lin, T. P. Speed, P. M. Siba, I. Felger and I. Mueller (2013). "A high force of plasmodium vivax blood-stage infection drives the rapid acquisition of immunity in papua new guinean children." PLoS Negl Trop Dis **7**(9): e2403.

Koepfli, C., M. Ome-Kaius, S. Jally, E. Malau, S. Maripal, J. Ginny, L. Timinao, J. H. Kattenberg, T. Obadia, M. White, P. Rarau, N. Senn, A. E. Barry, J. W. Kazura, I. Mueller and L. J. Robinson (2017). "Sustained Malaria Control Over an 8-Year Period in Papua New Guinea: The Challenge of Low-Density Asymptomatic Plasmodium Infections." J Infect Dis **216**(11): 1434-1443.

Kogan, M. D. (1995). "Social causes of low birth weight." J R Soc Med **88**(11): 611-615.

Koimbu, G., C. Czeher, M. Katusele, M. Sakur, L. Kilepak, A. Tandrapah, M. W. Hetzel, J. Pulford, L. Robinson and S. Karl (2018). "Status of Insecticide Resistance in Papua New Guinea: An Update from Nation-Wide Monitoring of Anopheles Mosquitoes." Am J Trop Med Hyg **98**(1): 162-165.

Kotloff, K. L., J. P. Nataro, W. C. Blackwelder, D. Nasrin, T. H. Farag, S. Panchalingam, Y. Wu, S. O. Sow, D. Sur, R. F. Breiman, A. S. G. Faruque, A. K. M. Zaidi, D. Saha, P. L. Alonso, B. Tamboura, D. Sanogo, U. Onwuchekwa, B. Manna, T. Ramamurthy, S. Kanungo, J. B. Ochieng, R. Omore, J. O. Oundo, A. Hossain, S. K. Das, S. Ahmed, S. Qureshi, F. Quadri, R. A. Adegbola, M. Antonio, M. J. Hossain, A. Akinsola, I. Mandomando, T. Nhampossa, S. Acácio, K. Biswas, C. E. O'Reilly, E. D. Mintz, L. Y. Berkeley, K. Muhsen, H. Sommerfelt, R. M. Robins-Browne and M. M. Levine (2013). "Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study." The Lancet **382**(9888): 209-222.

Kozuki, N., A. C. Lee, J. Katz and G. Child Health Epidemiology Reference (2012). "Moderate to severe, but not mild, maternal anemia is associated with increased risk of small-for-gestational-age outcomes." J Nutr **142**(2): 358-362.

Kramer, M. S. (1987). "Determinants of low birth weight: methodological assessment and meta-analysis." Bull World Health Organ **65**(5): 663-737.

Kramer, M. S. (2003). "The epidemiology of adverse pregnancy outcomes: an overview." J Nutr **133**(5 Suppl 2): 1592s-1596s.

Laman, M., L. Manning, A. R. Greenhill, T. Mare, A. Michael, S. Shem, J. Vince, W. Lagani, I. Hwaiwhanje, P. M. Siba, I. Mueller and T. M. Davis (2012). "Predictors of acute bacterial meningitis in children from a malaria-endemic area of Papua New Guinea." Am J Trop Med Hyg **86**(2): 240-245.

Lao, T. T. and T. C. Pun (1996). "Anaemia in pregnancy--is the current definition meaningful?" Eur J Obstet Gynecol Reprod Biol **68**(1-2): 53-58.

Lasky, R. E., R. E. Klein, C. Yarbrough, P. L. Engle, A. Lechtig and R. Martorell (1981). "The relationship between physical growth and infant behavioral development in rural Guatemala." Child Dev **52**(1): 219-226.

Lawn, J. E., H. Blencowe, S. Oza, D. You, A. C. Lee, P. Waiswa, M. Lalli, Z. Bhutta, A. J. Barros, P. Christian, C. Mathers, S. N. Cousens and G. Lancet Every Newborn Study (2014). "Every Newborn: progress, priorities, and potential beyond survival." Lancet **384**(9938): 189-205.

Lee, G., P. Yori, M. P. Olortegui, W. Pan, L. Caulfield, R. H. Gilman, J. W. Sanders, H. S. Delgado and M. Kosek (2012). "Comparative effects of vivax malaria, fever and diarrhoea on child growth." Int J Epidemiol **41**(2): 531-539.

Lee, M. S. J., K. Maruyama, Y. Fujita, A. Konishi, P. M. Lelliott, S. Itagaki, T. Horii, J.-w. Lin, S. M. Khan, E. Kuroda, S. Akira, K. J. Ishii and C. Coban (2017). "Plasmodium products persist in the bone marrow and promote chronic bone loss." Science Immunology **2**(12): eaam8093.

Lehmann, D. (1992). "Epidemiology of acute respiratory tract infections, especially those due to Haemophilus influenzae, in Papua New Guinean children." J Infect Dis **165 Suppl 1**: S20-25.

Levy, A., D. Fraser, M. Katz, M. Mazor and E. Sheiner (2005). "Maternal anemia during pregnancy is an independent risk factor for low birthweight and preterm delivery." Eur J Obstet Gynecol Reprod Biol **122**(2): 182-186.

Liang, K. Y. and S. L. Zeger (1986). "Longitudinal Data-Analysis Using Generalized Linear-Models." Biometrika **73**(1): 13-22.

Lin, C.-C. and J. Santolaya-Forgas (1998). "Current concepts of fetal growth restriction: part I. Causes, classification, and pathophysiology." Obstetrics & Gynecology **92**(6): 1044-1055.

Lin, E., B. Kiniboro, L. Gray, S. Dobbie, L. Robinson, A. Laumaea, S. Schopflin, D. Stanisic, I. Betuela, M. Blood-Zikursh, P. Siba, I. Felger, L. Schofield, P. Zimmerman and I. Mueller (2010). "Differential patterns of infection and disease with P. falciparum and P. vivax in young Papua New Guinean children." PLoS One **5**(2): e9047.

Lindsay, S., J. Ansell, C. Selman, V. Cox, K. Hamilton and G. Walraven (2000). "Effect of pregnancy on exposure to malaria mosquitoes." Lancet **355**(9219): 1972.

Liu, L., H. L. Johnson, S. Cousens, J. Perin, S. Scott, J. Lawn, I. Rudan, H. Campbell, R. Cibulskis, M. Li, C. Mathers, R. Black and W. H. O. Child Health Epidemiology Reference Group of (2012). "Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000." Lancet **379**: 2151-2161.

Lu, Z. M. (1991). "The Relationship Between Maternal Hematocrit and Pregnancy Outcome." Obstetrics & Gynecology **77**(2).

Lucas, A. (1998). "The Effects of Childhood Diet on Adult Health and Disease." The Journal of Nutrition.

Lufele, E., A. Umbers, J. Ordi, M. Ome-Kaius, R. Wangnapi, H. Unger, N. Tarongka, P. Siba, I. Mueller, L. Robinson and S. Rogerson (2017). "Risk factors and pregnancy outcomes associated with placental malaria in a prospective cohort of Papua New Guinean women." Malar J **16**(1): 427.

Luntamo, M., T. Kulmala, Y. B. Cheung, K. Maleta and P. Ashorn (2013). "The effect of antenatal monthly sulphadoxine-pyrimethamine, alone or with azithromycin, on foetal and neonatal growth faltering in Malawi: a randomised controlled trial." Trop Med Int Health **18**(4): 386-397.

Luzolo, A. L. and D. M. Ngoyi (2019). "Cerebral malaria." Brain Res Bull **145**: 53-58.

Madianos, P. N., Y. A. Bobetsis and S. Offenbacher (2013). "Adverse pregnancy outcomes (APOs) and periodontal disease: pathogenic mechanisms." J Periodontol **84**(4 Suppl): S170-180.

Mahendroo, M. (2012). "Cervical remodeling in term and preterm birth: insights from an animal model." Reproduction **143**(4): 429-438.

Maleta, K., S. Virtanen, M. Espo, T. Kulmala and P. Ashorn (2003). "Timing of growth faltering in rural Malawi." Arch Dis Child **88**(7): 574-578.

Malhotra, I., A. Dent, P. Mungai, A. Wamachi, J. H. Ouma, D. L. Narum, E. Muchiri, D. J. Tisch and C. L. King (2009). "Can prenatal malaria exposure produce an immune tolerant phenotype? A prospective birth cohort study in Kenya." PLoS Med **6**(7): e1000116.

Malhotra, M., J. B. Sharma, S. Batra, S. Sharma, N. S. Murthy and R. Arora (2002). "Maternal and perinatal outcome in varying degrees of anemia." Int J Gynaecol Obstet **79**(2): 93-100.

Manning, L., M. Laman, I. Law, C. Bona, S. Aipit, D. Teine, J. Warrell, A. Rosanas-Urgell, E. Lin, B. Kiniboro, J. Vince, I. Hwaiwhanje, H. Karunajeewa, P. Michon, P. Siba, I. Mueller and T. M. Davis (2011). "Features and prognosis of severe malaria caused by Plasmodium falciparum, Plasmodium vivax and mixed Plasmodium species in Papua New Guinean children." PLoS One **6**(12): e29203.

Manning, L., M. Laman, A. Rosanas-Urgell, P. Michon, S. Aipit, C. Bona, P. Siba, I. Mueller and T. M. Davis (2012). "Severe anemia in Papua New Guinean children from a malaria-endemic area: a case-control etiologic study." PLoS Negl Trop Dis **6**(12): e1972.

March of Dimes, P., Save teh Children, World Health Organization (2012). "Born Too Soon: The Global Action Report on Preterm Birth." World Health Organization, Geneva.

Marks, K. A., B. Reichman, A. Lusky and E. Zmora (2006). "Fetal growth and postnatal growth failure in very-low-birthweight infants." Acta Paediatr **95**(2): 236-242.

Marsden, P. D. (1964). "The Sukuta Project. A Longitudinal Study of Health in Gambian Children from Birth to 18 Months of Age." Trans R Soc Trop Med Hyg **58**: 455-489.

Marsh, K. (1992). "Malaria--a neglected disease?" Parasitology **104 Suppl**: S53-69.

Marsh, K. and S. Kinyanjui (2006). "Immune effector mechanisms in malaria." Parasite Immunol **28**(1-2): 51-60.

Martorell, R., J. P. Habicht, C. Yarbrough, A. Lechtig, R. E. Klein and K. A. Western (1975). "Acute morbidity and physical growth in rural Guatemalan children." Am J Dis Child **129**(11): 1296-1301.

Martorell, R., L. K. Khan and D. G. Schroeder (1994). "Reversibility of stunting: epidemiological findings in children from developing countries." Eur J Clin Nutr **48 Suppl 1**: S45-57.

Martorell, R., P. Melgar, J. A. Maluccio, A. D. Stein and J. A. Rivera (2010). "The nutrition intervention improved adult human capital and economic productivity." J Nutr **140**(2): 411-414.

Martorell, R. and A. Zongrone (2012). "Intergenerational influences on child growth and undernutrition." Paediatr Perinat Epidemiol **26 Suppl 1**: 302-314.

Mason, J. B., L. S. Saldanha and R. Martorell (2012). "The importance of maternal undernutrition for maternal, neonatal, and child health outcomes: an editorial." Food Nutr Bull **33**(2 Suppl): S3-5.

Mata, L. (1990). "Infection, malnutrition and growth." J Gastroenterol Hepatol **5 Suppl 1**: 32-44.

Mata, L. J., J. J. Urrutia, C. Albertazzi, O. Pellecer and E. Arellano (1972). "Influence of recurrent infections on nutrition and growth of children in Guatemala." Am J Clin Nutr **25**(11): 1267-1275.

Mayor, A., A. Bardaji, I. Felger, C. L. King, P. Cistero, C. Dobano, D. I. Stanisic, P. Siba, M. Wahlgren, H. del Portillo, I. Mueller, C. Menendez, J. Ordi and S. Rogerson (2012). "Placental infection with Plasmodium vivax: a histopathological and molecular study." J Infect Dis **206**(12): 1904-1910.

McAllister, D. A., L. Liu, T. Shi, Y. Chu, C. Reed, J. Burrows, D. Adeloye, I. Rudan, R. E. Black, H. Campbell and H. Nair (2019). "Global, regional, and national estimates of pneumonia morbidity and mortality in children younger than 5 years between 2000 and 2015: a systematic analysis." The Lancet Global Health **7**(1): e47-e57.

McDonald, C. M., I. Olofin, S. Flaxman, W. W. Fawzi, D. Spiegelman, L. E. Caulfield, R. E. Black, M. Ezzati, G. Danaei and S. Nutrition Impact Model (2013). "The effect of multiple anthropometric deficits on child mortality: meta-analysis of individual data in 10 prospective studies from developing countries." Am J Clin Nutr **97**(4): 896-901.

McGready, R., B. B. Davison, K. Stepniewska, T. Cho, H. Shee, A. Brockman, R. Udomsangpetch, S. Looareesuwan, N. J. White, S. R. Meshnick and F. Nosten (2004). "The effects of Plasmodium falciparum and P. vivax infections on placental histopathology in an area of low malaria transmission." Am J Trop Med Hyg **70**(4): 398-407.

McGready, R., S. J. Lee, J. Wiladphaingern, E. A. Ashley, M. J. Rijken, M. Boel, J. A. Simpson, M. K. Paw, M. Pimanpanarak, O. Mu, P. Singhasivanon, N. J. White and F. H. Nosten (2012). "Adverse effects of falciparum and vivax malaria and the safety of antimalarial treatment in early pregnancy: a population-based study." Lancet Infect Dis **12**(5): 388-396.

McGregor, I. A. (1982). "Malaria: nutritional implications." Rev Infect Dis **4**(4): 798-804.

McNamara, D. T., L. J. Kasehagen, B. T. Grimberg, J. Cole-Tobian, W. E. Collins and P. A. Zimmerman (2006). "Diagnosing infection levels of four human malaria parasite species by a polymerase chain reaction/ligase detection reaction fluorescent microsphere-based assay." Am J Trop Med Hyg **74**(3): 413-421.

McNamara, D. T., J. M. Thomson, L. J. Kasehagen and P. A. Zimmerman (2004). "Development of a multiplex PCR-ligase detection reaction assay for diagnosis of infection by the four parasite species causing malaria in humans." J Clin Microbiol **42**(6): 2403-2410.

Medicine, I. o. and N. R. Council (2009). Weight Gain During Pregnancy: Reexamining the Guidelines. Washington, DC, The National Academies Press.

Mehlotra, R. K., K. Lorry, W. Kastens, S. M. Miller, M. P. Alpers, M. Bockarie, J. W. Kazura and P. A. Zimmerman (2000). "Random distribution of mixed species malaria infections in Papua New Guinea." Am J Trop Med Hyg **62**(2): 225-231.

Menendez, C., A. F. Fleming and P. Alonso (2000). "Malaria-related Anaemia." Paraitol Today **16**(11).

Merialdi, M., G. Carroli, J. Villar, E. Abalos, A. M. Gulmezoglu, R. Kulier and M. de Onis (2003). "Nutritional interventions during pregnancy for the prevention or treatment of impaired fetal growth: An overview of randomized controlled trials." Journal of Nutrition **133**(5): 1626s-1631s.

Michon, P., J. L. Cole-Tobian, E. Dabod, S. Schoepflin, J. Igu, M. Susapu, N. Tarongka, P. A. Zimmerman, J. C. Reeder, J. G. Beeson, L. Schofield, C. L. King and I. Mueller (2007). "The risk of malarial infections and disease in Papua New Guinean children." Am J Trop Med Hyg **76**(6): 997-1008.

Mitchell, G. H., T. J. Hadley, M. H. McGinniss, F. W. Klotz and L. H. Miller (1986). "Invasion of erythrocytes by Plasmodium falciparum malaria parasites: evidence for receptor heterogeneity and two receptors." Blood **67**(5): 1519-1521.

Mockenhaupt, F. P., S. Ehrhardt, J. Burkhardt, S. Y. Bosomtwe, S. Laryea, S. D. Anemana, R. N. Otchwemah, J. P. Cramer, E. Dietz, S. Gellert and U. Bienzle (2004). "Manifestation and outcome of severe malaria in children in northern Ghana." Am J Trop Med Hyg **71**(2): 167-172.

Moffat, T. (2003). "Diarrhea, respiratory infections, protozoan gastrointestinal parasites, and child growth in Kathmandu, Nepal." Am J Phys Anthropol **122**(1): 85-97.

Mohsena, M., C. G. Mascie-Taylor and R. Goto (2010). "Association between socio-economic status and childhood undernutrition in Bangladesh; a comparison of possession score and poverty index." Public Health Nutr **13**(10): 1498-1504.

Moore, B. R., J. M. Benjamin, R. Tobe, M. Ome-Kaius, G. Yadi, B. Kasian, C. Kong, L. J. Robinson, M. Laman, I. Mueller, S. Rogerson and T. M. E. Davis (2019). "A Randomized Open-Label Evaluation of the Antimalarial Prophylactic Efficacy of Azithromycin-Piperaquine versus Sulfadoxine-Pyrimethamine in Pregnant Papua New Guinean Women." Antimicrob Agents Chemother **63**(10).

Moore, G. E., Z. Ali, R. U. Khan, S. Blunt, P. R. Bennett and J. I. Vaughan (1997). "The incidence of uniparental disomy associated with intrauterine growth retardation in a cohort of thirty-five severely affected babies." Am J Obstet Gynecol **176**(2): 294-299.

Moore, K. A., F. J. I. Fowkes, J. Wiladphaingern, N. S. Wai, M. K. Paw, M. Pimanpanarak, V. I. Carrara, J. Raksuansak, J. A. Simpson, N. J. White, F. Nosten and R. McGready (2017). "Mediation of the effect of malaria in pregnancy on stillbirth and neonatal death in an area of low transmission: observational data analysis." BMC Med **15**(1): 98.

Moore, K. A., J. A. Simpson, M. J. L. Scoullar, R. McGready and F. J. I. Fowkes (2017). "Quantification of the association between malaria in pregnancy and stillbirth: a systematic review and meta-analysis." The Lancet Global Health **5**(11): e1101-e1112.

Moore, S. R., A. A. Lima, M. R. Conaway, J. B. Schorling, A. M. Soares and R. L. Guerrant (2001). "Early childhood diarrhoea and helminthiases associate with long-term linear growth faltering." Int J Epidemiol **30**(6): 1457-1464.

Moormann, A. M., A. D. Sullivan, R. A. Rochford, S. W. Chensue, P. J. Bock, T. Nyirenda and S. R. Meshnick (1999). "Malaria and pregnancy: placental cytokine expression and its relationship to intrauterine growth retardation." J Infect Dis **180**(6): 1987-1993.

Moradi, S., A. Mirzababaei, H. Mohammadi, S. P. Moosavian, A. Arab, B. Jannat and K. Mirzaei (2019). "Food insecurity and the risk of undernutrition complications among children and adolescents: A systematic review and meta-analysis." Nutrition **62**: 52-60.

Motarjemi, Y., F. Kaferstein, G. Moy and F. Quevedo (1993). "Contaminated weaning food: a major risk factor for diarrhoea and associated malnutrition." Bull World Health Organ **71**(1): 79-92.

Moy, R. J., C. M. T. F. de, R. G. Choto, A. S. McNeish and I. W. Booth (1994). "Diarrhoea and growth faltering in rural Zimbabwe." Eur J Clin Nutr **48**(11): 810-821.

Mueller, I. (2002). "Regional patterns of birthwts in PNG in relation to diet, environment and socio-economic factors."

Mueller, I., M. R. Galinski, J. K. Baird, J. M. Carlton, D. K. Kochar, P. L. Alonso and H. A. del Portillo (2009). "Key gaps in the knowledge of Plasmodium vivax, a neglected human malaria parasite." Lancet Infect Dis **9**(9): 555-566.

Mueller, I., S. Rogerson, G. D. Mola and J. C. Reeder (2008). "A review of the current state of malaria among pregnant women in Papua New Guinea." P N G Med J **51**(1-2): 12-16.

Mueller, I., S. Schoepflin, T. A. Smith, K. L. Benton, M. T. Bretscher, E. Lin, B. Kiniboro, P. A. Zimmerman, T. P. Speed, P. Siba and I. Felger (2012). "Force of infection is key to understanding the epidemiology of Plasmodium falciparum malaria in Papua New Guinean children." Proc Natl Acad Sci U S A **109**(25): 10030-10035.

Mueller, I. and T. A. Smith (1999). "Patterns of child growth in Papua New Guinea and their relation to environmental, dietary and socioeconomic factors--further analyses of the 1982-1983 Papua New Guinea National Nutrition Survey." P N G Med J **42**(3-4): 94-113.

Mueller, I., J. Tulloch, J. Marfurt, R. Hide and J. C. Reeder (2005). "Malaria control in Papua New Guinea results in complex epidemiological changes." P N G Med J **48**(3-4): 151-157.

Mueller, I., P. Vounatsou, B. J. Allen and T. Smith (2001). "Spatial patterns of child growth in Papua New Guinea and their relation to environment, diet, socio-economic status and subsistence activities." Ann Hum Biol **28**(3): 263-280.

Mueller, I., S. Widmer, D. Michel, S. Maraga, D. T. McNamara, B. Kiniboro, A. Sie, T. A. Smith and P. A. Zimmerman (2009). "High sensitivity detection of Plasmodium species reveals positive correlations between infections of different species, shifts in age distribution and reduced local variation in Papua New Guinea." Malar J **8**: 41.

Muller, I., M. Bockarie, M. Alpers and T. Smith (2003). "The epidemiology of malaria in Papua New Guinea." Trends Parasitol **19**(6): 253-259.

Mullick, S., D. Watson-Jones, M. Beksinska and D. Mabey (2005). "Sexually transmitted infections in pregnancy: prevalence, impact on pregnancy outcomes, and approach to treatment in developing countries." Sex Transm Infect **81**(4): 294-302.

Murphy, J. F., J. O'Riordan, R. G. Newcombe, E. C. Coles and J. F. Pearson (1986). "Relation of haemoglobin levels in first and second trimesters to outcome of pregnancy." Lancet **1**(8488): 992-995.

Muthen, L. K. and B. O. Muthen (1998-2017). Mplus User's Guide. Eighth Edition. Los Angeles, CA: Muthen & Muthen.

Mwangi, M. N., J. M. Roth, M. R. Smit, L. Trijsburg, A. M. Mwangi, A. Y. Demir, J. P. Wielders, P. F. Mens, J. J. Verweij, S. E. Cox, A. M. Prentice, I. D. Brouwer, H. F. Savelkoul, P. E. Andang'o and H. Verhoef (2015). "Effect of Daily Antenatal Iron Supplementation on Plasmodium Infection in Kenyan Women: A Randomized Clinical Trial." JAMA **314**(10): 1009-1020.

Myatt, M., T. Khara and S. Collins (2006). "A review of methods to detect cases of severely malnourished children in the community for their admission into community-based therapeutic care programs." Food Nutr Bull **27**(3 Suppl): S7-23.

Namaste, S. M., G. J. Aaron, R. Varadhan, J. M. Peerson, P. S. Suchdev and B. W. Group (2017). "Methodologic approach for the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project." Am J Clin Nutr **106**(Suppl 1): 333S-347S.

Namaste, S. M., F. Rohner, J. Huang, N. L. Bhushan, R. Flores-Ayala, R. Kupka, Z. Mei, R. Rawat, A. M. Williams, D. J. Raiten, C. A. Northrop-Clewes and P. S. Suchdev (2017). "Adjusting ferritin concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project." Am J Clin Nutr **106**(Suppl 1): 359S-371S.

Negrato, C. A. and M. B. Gomes (2013). "Low birth weight: causes and consequences." Diabetol Metab Syndr **5**: 49.

Ngai, M., A. M. Weckman, C. Erice, C. R. McDonald, L. S. Cahill, J. G. Sled and K. C. Kain (2020). "Malaria in Pregnancy and Adverse Birth Outcomes: New Mechanisms and Therapeutic Opportunities." Trends Parasitol **36**(2): 127-137.

Nguitragool, W., S. Karl, M. White, C. Koepfli, I. Felger, P. Singhasivanon, I. Mueller and J. Sattabongkot (2019). "Highly heterogeneous residual malaria risk in western Thailand." Int J Parasitol **49**(6): 455-462.

Nosten, F., R. McGready, J. A. Simpson, K. L. Thwai, S. Balkan, T. Cho, L. Hkirijaroen, S. Looareesuwan and N. J. White (1999). "Effects of Plasmodium vivax malaria in pregnancy." Lancet **354**(9178): 546-549.

Nosten, F., F. ter Kuile, L. Maelankirri, B. Decludt and N. J. White (1991). "Malaria during pregnancy in an area of unstable endemicity." Trans R Soc Trop Med Hyg **85**(4): 424-429.

Nyakeriga, A. M., M. Troye-Blomberg, A. K. Chemtai, K. Marsh and T. N. Williams (2004). "Malaria and nutritional status in children living on the coast of Kenya." Am J Clin Nutr **80**(6): 1604-1610.

O'Brien, K. S., P. Emerson, P. J. Hooper, A. L. Reingold, E. G. Dennis, J. D. Keenan, T. M. Lietman and C. E. Oldenburg (2019). "Antimicrobial resistance following mass azithromycin distribution for trachoma: a systematic review." The Lancet Infectious Diseases **19**(1): e14-e25.

Okah, F. A., J. Cai and G. L. Hoff (2005). "Term-gestation low birth weight and health-compromising behaviors during pregnancy." Obstet Gynecol **105**(3): 543-550.

Olofin, I., C. M. McDonald, M. Ezzati, S. Flaxman, R. E. Black, W. W. Fawzi, L. E. Caulfield, G. Danaei and S. Nutrition Impact Model (2013). "Associations of suboptimal growth with all-cause and cause-specific mortality in children under five years: a pooled analysis of ten prospective studies." PLoS One **8**(5): e64636.

Ome-Kaius, M., J. H. Kattenberg, S. Zaloumis, M. Siba, B. Kiniboro, S. Jally, Z. Razook, D. Mantila, D. Sui, J. Ginny, A. Rosanas-Urgell, S. Karl, T. Obadia, A. Barry, S. J. Rogerson, M. Laman, D. Tisch, I. Felger, J. W. Kazura, I. Mueller and L. J. Robinson (2019). "Differential impact of malaria control interventions on P. falciparum and P. vivax infections in young Papua New Guinean children." BMC Med **17**(1): 220.

Ordi, J., M. R. Ismail, P. J. Ventura, E. Kahigwa, R. Hirt, A. Cardesa, P. L. Alonso and C. Menendez (1998). "Massive chronic intervillositis of the placenta associated with malaria infection." Am J Surg Pathol **22**(8): 1006-1011.

Ouedraogo, S., G. K. Koura, M. M. Accrombessi, F. Bodeau-Livinec, A. Massougbodji and M. Cot (2012). "Maternal anemia at first antenatal visit: prevalence and risk factors in a malaria-endemic area in Benin." Am J Trop Med Hyg **87**(3): 418-424.

Owoaje, E., O. Onifade and A. Desmennu (2014). "Family and socioeconomic risk factors for undernutrition among children aged 6 to 23 Months in Ibadan, Nigeria." Pan Afr Med J **17**: 161.

Paediatric Society of Papua New Guinea. (2017). "Child Morbidity and Mortality: Annual Report 2017." Retrieved 15june2019, 2019, from https://pngpaediatricsociety.org/wp-content/uploads/2013/05/2017-Annual-Child-Morbidity-and-Mortality-Report.pdf.

Pasvol, G., D. J. Weatherall and R. J. M. Wilson (1980). "The Increased Susceptibility of Young Red Cells to Invasion by the Malarial Parasite Plasmodium falciparum." British Journal of Haematology **45**(2): 285-295.

Phillips, R. E. and G. Pasvol (1992). "Anaemia of Plasmodium falciparum malaria." Baillieres Clin Haematol **5**(2): 315-330.

PNG Department of Health (2010). "PNG National Health Plan: 2011 - 2020; Volume 1 Policies and Strategies."

PNG Department of National Planning and Monitoring. "The Papua New Guinea Water, Sanitation and Hygiene (WaSH) Policy 2015." Retrieved 10jul2019, from https://png-data.sprep.org/dataset/papua-new-guinea-water-sanitation-and-hygiene-wash-policy-2015.

PNG National Department of Health and Paediatric Society of Papua New Guinea (2015). Papua New Guinea Child Health Policy and Plan 2009-2020

PNG Paediatric Society (2006). Standard Treatment for Common Illness of Children in Papua New Guinea: A manual for nurses, community health workers, health extension officers, and doctors.

PNG Paediatric Society and PNG Department of Health. (2018). "Child Morbidity and Mortality: Ninth Annual Report." Retrieved 10 Aug 2019, 2019, from https://pngpaediatricsociety.org/wp-content/uploads/2019/06/2018-Annual-Child-Morbidity-and-Mortality-Report.pdf.

PNG, P. s. o. Standard Treatment for Common Illness of Children in Papua New Guinea: A manual for nurses, community health workers, health extension officers, and doctors; Eighth Edition 2006.

Poespoprodjo, J. R., W. Fobia, E. Kenangalem, D. A. Lampah, N. Warikar, A. Seal, R. McGready, P. Sugiarto, E. Tjitra, N. M. Anstey and R. N. Price (2008). "Adverse pregnancy outcomes in an area where multidrug-resistant plasmodium vivax and Plasmodium falciparum infections are endemic." Clinical infectious diseases : an official publication of the Infectious Diseases Society of America **46**(9): 1374-1381.

Pomat, W. S., A. R. Greenhill and D. Lehmann (2010). "Pneumonia in Papua New Guinea: lessons learnt for the way forward." P N G Med J **53**(3-4): 89-93.

Prendergast, A. J. and J. H. Humphrey (2014). "The stunting syndrome in developing countries." Paediatr Int Child Health **34**(4): 250-265.

Prost, M. A. (2009). "Postnatal origins of undernutrition." Nestle Nutr Workshop Ser Pediatr Program **63**: 79-92; discussion 92-74, 259-268.

Pulford, J., S. F. Kurumop, Y. Ura, P. M. Siba, I. Mueller and M. W. Hetzel (2013). "Malaria case management in Papua New Guinea following the introduction of a revised treatment protocol." Malar J **12**: 433.

Racicot, K., I. Cardenas, V. Wunsche, P. Aldo, S. Guller, R. E. Means, R. Romero and G. Mor (2013). "Viral infection of the pregnant cervix predisposes to ascending bacterial infection." J Immunol **191**(2): 934-941.

Radeva-Petrova, D., K. Kayentao, F. O. ter Kuile, D. Sinclair and P. Garner (2014). "Drugs for preventing malaria in pregnant women in endemic areas: any drug regimen versus placebo or no treatment." Cochrane Database Syst Rev(10): CD000169.

Raghupathy, R. (1997). "Th1-type immunity is incompatible with successful pregnancy." Immunol Today **18**: 478-482.

Rahman, M. M., S. K. Abe, M. S. Rahman, M. Kanda, S. Narita, V. Bilano, E. Ota, S. Gilmour and K. Shibuya (2016). "Maternal anemia and risk of adverse birth and health outcomes in low- and middle-income countries: systematic review and meta-analysis." Am J Clin Nutr **103**(2): 495-504.

Rahman, M. S., M. A. Rahman, M. Maniruzzaman and M. H. Howlader (2019). "Prevalence of undernutrition in Bangladeshi children." J Biosoc Sci: 1-14.

Rahmati, S., A. Delpishe, M. Azami, M. R. Hafezi Ahmadi and K. Sayehmiri (2017). "Maternal Anemia during pregnancy and infant low birth weight: A systematic review and Meta-analysis." Int J Reprod Biomed (Yazd) **15**(3): 125-134.

Ramakrishnan, U., R. Martorell, D. G. Schroeder and R. Flores (1999). "Role of intergenerational effects on linear growth." J Nutr **129**(2S Suppl): 544S-549S.

Rasmussen, S. and P. Oian (1993). "First- and second-trimester hemoglobin levels. Relation to birth weight and gestational age." Acta Obstet Gynecol Scand **72**(4): 246-251.

Ratjen, F., L. Saiman, N. Mayer-Hamblett, L. C. Lands, M. Kloster, V. Thompson, P. Emmett, B. Marshall, F. Accurso, S. Sagel and M. Anstead (2012). "Effect of azithromycin on systemic markers of inflammation in patients with cystic fibrosis uninfected with Pseudomonas aeruginosa." Chest **142**(5): 1259-1266.

Rebelo, F., M. M. Schlussel, J. S. Vaz, A. B. Franco-Sena, T. J. Pinto, F. I. Bastos, A. R. Adegboye and G. Kac (2013). "C-reactive protein and later preeclampsia: systematic review and meta-analysis taking into account the weight status." J Hypertens **31**(1): 16-26.

Reimer, L. J., E. K. Thomsen, G. Koimbu, J. B. Keven, I. Mueller, P. M. Siba, J. W. Kazura, M. W. Hetzel and P. A. Zimmerman (2016). "Malaria transmission dynamics surrounding the first nationwide long-lasting insecticidal net distribution in Papua New Guinea." Malar J **15**: 25.

Renthal, N. E., K. C. Williams and C. R. Mendelson (2013). "MicroRNAs--mediators of myometrial contractility during pregnancy and labour." Nat Rev Endocrinol **9**(7): 391-401.

Richard, S. A., R. E. Black and W. Checkley (2012). "Revisiting the relationship of weight and height in early childhood." Adv Nutr **3**(2): 250-254.

Richard, S. A., R. E. Black, R. H. Gilman, R. L. Guerrant, G. Kang, C. F. Lanata, K. Molbak, Z. A. Rasmussen, R. B. Sack, P. Valentiner-Branth, W. Checkley, M. Childhood and N.

Infection (2013). "Diarrhea in early childhood: short-term association with weight and long-term association with length." Am J Epidemiol **178**(7): 1129-1138.

Rijken, M. J., A. M. De Livera, S. J. Lee, M. E. Boel, S. Rungwilailaekhiri, J. Wiladphaingern, M. K. Paw, M. Pimanpanarak, S. Pukrittayakamee, J. A. Simpson, F. Nosten and R. McGready (2014). "Quantifying Low Birth Weight, Preterm Birth and Small-for-Gestational-Age Effects of Malaria in Pregnancy: A Population Cohort Study." PLOS ONE **9**(7): e100247.

Rijken, M. J., R. McGready, M. E. Boel, R. Poespoprodjo, N. Singh, D. Syafruddin, S. Rogerson and F. Nosten (2012). "Malaria in pregnancy in the Asia-Pacific region." The Lancet Infectious Diseases **12**(1): 75-88.

Rijken, M. J., J. A. Rijken, A. T. Papageorghiou, S. H. Kennedy, G. H. Visser, F. Nosten and R. McGready (2011). "Malaria in pregnancy: the difficulties in measuring birthweight." BJOG **118**(6): 671-678.

Riley, I. D. (2010). "Pneumonia research in Papua New Guinea: 1967-1986." P N G Med J **53**(3-4): 106-118.

Robinson, L. J., R. Wampfler, I. Betuela, S. Karl, M. T. White, C. S. Li Wai Suen, N. E. Hofmann, B. Kinboro, A. Waltmann, J. Brewster, L. Lorry, N. Tarongka, L. Samol, M. Silkey, Q. Bassat, P. M. Siba, L. Schofield, I. Felger and I. Mueller (2015). "Strategies for understanding and reducing the Plasmodium vivax and Plasmodium ovale hypnozoite reservoir in Papua New Guinean children: a randomised placebo-controlled trial and mathematical model." PLoS Med **12**(10): e1001891.

Roca, A., B. Camara, C. Oluwalana, K. Lette, C. Bottomley and U. D'Alessandro (2018). "Long-lasting effect of oral azithromycin taken by women during labour on infant nutrition: Follow-up cohort of a randomized clinical trial in western Gambia." PLOS ONE **13**(10): e0206348.

Rogerson, S. J. and P. Boeuf (2007). "New approaches to pathogenesis of malaria in pregnancy." Parasitology **134**(Pt 13): 1883-1893.

Rogerson, S. J., H. C. Brown, E. Pollina, E. T. Abrams, E. Tadesse, V. M. Lema and M. E. Molyneux (2003). "Placental tumor necrosis factor alpha but not gamma interferon is associated with placental malaria and low birth weight in Malawian women." Infect Immun **71**(1): 267-270.

Rogerson, S. J., M. Desai, A. Mayor, E. Sicuri, S. M. Taylor and A. M. van Eijk (2018). "Burden, pathology, and costs of malaria in pregnancy: new developments for an old problem." Lancet Infect Dis **18**(4): e107-e118.

Rogerson, S. J., L. Hviid, P. E. Duffy, R. F. G. Leke and D. W. Taylor (2007). "Malaria in pregnancy: pathogenesis and immunity." The Lancet Infectious Diseases **7**(2): 105-117.

Rogerson, S. J., E. Pollina, A. Getachew, E. Tadesse, V. M. Lema and M. E. Molyneux (2003). "Placental monocyte infiltrates in response to Plasmodium falciparum malaria infection and their association with adverse pregnancy outcomes." Am J Trop Med Hyg **68**(1): 115-119.

Romero, R., S. K. Dey and S. J. Fisher (2014). "Preterm labor: one syndrome, many causes." Science **345**(6198): 760-765.

Romero, R., R. Gomez, T. Chaiworapongsa, G. Conoscenti, J. C. Kim and Y. M. Kim (2001). "The role of infection in preterm labour and delivery." Paediatr Perinat Epidemiol **15 Suppl 2**: 41-56.

Romero, R., S. S. Hassan, P. Gajer, A. L. Tarca, D. W. Fadrosh, J. Bieda, P. Chaemsaithong, J. Miranda, T. Chaiworapongsa and J. Ravel (2014). "The vaginal microbiota of pregnant women who subsequently have spontaneous preterm labor and delivery and those with a normal delivery at term." Microbiome **2**(1): 18.

Romero, R. and M. Mazor (1988). "Infection and preterm labor." Clin Obstet Gynecol **31**(3): 553-584.

Rowland, M. G., T. J. Cole and R. G. Whitehead (1977). "A quantitative study into the role of infection in determining nutritional status in Gambian village children." Br J Nutr **37**(3): 441-450.

Rowland, M. G., S. G. Rowland and T. J. Cole (1988). "Impact of infection on the growth of children from 0 to 2 years in an urban West African community." Am J Clin Nutr **47**(1): 134-138.

Rudan, I., K. L. O'Brien, H. Nair, L. Liu, E. Theodoratou, S. Qazi, I. Luksic, C. L. Fischer Walker, R. E. Black, H. Campbell and G. Child Health Epidemiology Reference (2013). "Epidemiology and etiology of childhood pneumonia in 2010: estimates of incidence, severe morbidity, mortality, underlying risk factors and causative pathogens for 192 countries." J Glob Health **3**(1): 010401.

Saito, M., V. Briand, A. M. Min and R. McGready (2020). "Deleterious effects of malaria in pregnancy on the developing fetus: a review on prevention and treatment with antimalarial drugs." Lancet Child Adolesc Health **4**(10): 761-774.

Sama, W., S. Owusu-Agyei, I. Felger, K. Dietz and T. Smith (2006). "Age and seasonal variation in the transition rates and detectability of Plasmodium falciparum malaria." Parasitology **132**(Pt 1): 13-21.

Samiak, L. and T. I. Emeto (2017). "Vaccination and nutritional status of children in Karawari, East Sepik Province, Papua New Guinea." PLoS One **12**(11): e0187796.

Sato, A. P., E. Fujimori and S. C. Szarfarc (2014). "[Hemoglobin curves during pregnancy before and after fortification of flours with iron]." Rev Esc Enferm USP **48**(3): 409-414.

Sattabongkot, J., C. Suansomjit, W. Nguitragool, J. Sirichaisinthop, S. Warit, M. Tiensuwan and S. Buates (2018). "Prevalence of asymptomatic Plasmodium infections with sub-microscopic parasite densities in the northwestern border of Thailand: a potential threat to malaria elimination." Malar J **17**(1): 329.

Save the Children. "Short Changed: The Human and Economic Cost of Child Undernutrtion in Papua New Guinea; June 2017." Retrieved 18june2019, 2019, from https://www.savethechildren.org.au/getmedia/565e0352-6a4f-46c1-bea8-331acd1b4c8c/png-nutrition-report.pdf.aspx.

Sayers, S. M., P. A. L. Lancaster and C. L. Whitehead (2017). Fetal Growth Restriction: Causes and Outcomes. International Encyclopedia of Public Health**:** 132-142.

Schaible, U. E. and S. H. E. Kaufmann (2007). "Malnutrition and infection: complex mechanisms and global impacts." PLoS medicine **4**(5): e115-e115.

Schofield, L. and F. Hackett (1993). "Signal transduction in host cells by a glycosylphosphatidylinositol toxin of malaria parasites." J Exp Med **177**(1): 145-153.

Schumacker, R. E. and R. G. Lomax (2010). A beginner's guide to structural equation modelling. New York, Taylor & Francis Group.

Schwarz, N. G., A. A. Adegnika, L. P. Breitling, J. Gabor, S. T. Agnandji, R. D. Newman, B. Lell, S. Issifou, M. Yazdanbakhsh, A. J. Luty, P. G. Kremsner and M. P. Grobusch (2008). "Placental malaria increases malaria risk in the first 30 months of life." Clin Infect Dis **47**(8): 1017-1025.

Scrimshaw, N. S. (2003). "Historical Concepts of Interactions, Synergism and Antagonism between Nutrition and Infection." American Society for Nutritional Sciences.

Scrimshaw, N. S., C. E. Taylor and J. E. Gordon (1959). "Interactions of nutrition and infection." Am J Med Sci **237**(3): 367-403.

Scrimshaw, N. S., C. E. Taylor and J. E. Gordon (1968). "Interactions of nutrition and infection." Monogr Ser World Health Organ **57**: 3-329.

Senn, N., P. Rarau, M. Salib, D. Manong, P. Siba, S. Rogerson, I. Mueller and B. Genton (2014). "Use of antibiotics within the IMCI guidelines in outpatient settings in Papua New Guinean children: an observational and effectiveness study." PLoS One **9**(3): e90990.

Senn, N., P. Rarau, D. I. Stanisic, L. Robinson, C. Barnadas, D. Manong, M. Salib, J. Iga, N. Tarongka, S. Ley, A. Rosanas-Urgell, J. J. Aponte, P. A. Zimmerman, J. G. Beeson, L. Schofield, P. Siba, S. J. Rogerson, J. C. Reeder and I. Mueller (2012). "Intermittent preventive treatment for malaria in Papua New Guinean infants exposed to Plasmodium falciparum and P. vivax: a randomized controlled trial." PLoS Med **9**(3): e1001195.

Shankar, H., N. Kumar, R. Sandhir, M. P. Singh, S. Mittal, T. Adhikari, M. Tarique, P. Kaur, M. S. Radhika, A. Kumar and D. N. Rao (2019). "Association of dietary intake below recommendations and micronutrient deficiencies during pregnancy and low birthweight." J Perinat Med.

Sharp, P. T. and P. Harvey (1980). "Malaria and growth stunting in young children of the highlands of Papua New Guinea." P N G Med J **23**(3): 132-140.

Shava, E., S. Moyo, R. Zash, M. Diseko, E. N. Dintwa, L. Mupfumi, J. Mabuta, G. Mayondi, J. Y. Chen, S. Lockman, M. Mmalane, J. Makhema and R. Shapiro (2019). "Brief Report: High Rates of Adverse Birth Outcomes in HIV and Syphilis Coinfected Women in Botswana." J Acquir Immune Defic Syndr **81**(5): e135-e140.

Shonkoff, J. P., A. S. Garner, C. Committee on Psychosocial Aspects of, H. Family, A. Committee on Early Childhood, C. Dependent, D. Section on and P. Behavioral (2012). "The lifelong effects of early childhood adversity and toxic stress." Pediatrics **129**(1): e232-246.

Shrimpton, R., C. G. Victora, M. de Onis, R. C. Lima, M. Blossner and G. Clugston (2001). "Worldwide timing of growth faltering: implications for nutritional interventions." Pediatrics **107**(5): E75.

Shulman, C. E., E. K. Dorman, A. O. Talisuna, B. S. Lowe, C. Nevill, R. W. Snow, H. Jilo, N. Peshu, J. N. Bulmer, S. Graham and K. Marsh (1998). "A community randomized controlled trial of insecticide-treated bednets for the prevention of malaria and anaemia among primigravid women on the Kenyan coast." Trop Med Int Health **3**(3): 197-204.

Simkhada, B., E. R. Teijlingen, M. Porter and P. Simkhada (2008). "Factors affecting the utilization of antenatal care in developing countries: systematic review of the literature." J Adv Nurs **61**(3): 244-260.

Singh, N., M. M. Shukla and V. P. Sharma (1999). "Epidemiology of malaria in pregnancy in central India." Bull World Health Organ **77**(7): 567-572.

Skranes, J. and G. C. Lohaugen (2016). "Reduction in general intelligence and executive function persists into adulthood among very preterm or very low birthweight children." Evid Based Ment Health.

Smith, T., H. P. Beck, A. Kitua, S. Mwankusye, I. Felger, N. Fraser-Hurt, A. Irion, P. Alonso, T. Teuscher and M. Tanner (1999). "Epidemiology of multiple Plasmodium falciparum infections - 4. Age dependence of the multiplicity of Plasmodium falciparum infections and of other malariological indices in an area of high endemicity." Transactions of the Royal Society of Tropical Medicine and Hygiene **93**: S15-S20.

Smith, T. and P. Vounatsou (2003). "Estimation of infection and recovery rates for highly polymorphic parasites when detectability is imperfect, using hidden Markov models." Stat Med **22**(10): 1709-1724.

Smith, T. A., Lehmann, D, Coakley, C, Spooner, V, Alpers, M.P (1991). "Relationships between growth and acute lower-respiratory infections in children aged<5 y in a highland population of Papua New Guinea." Am J Clin Nutr **53**: 963-970.

Soma-Pillay, P., C. Nelson-Piercy, H. Tolppanen and A. Mebazaa (2016). "Physiological changes in pregnancy." Cardiovasc J Afr **27**(2): 89-94.

Souza, R. M., R. Ataide, J. G. Dombrowski, V. Ippolito, E. H. Aitken, S. N. Valle, J. M. Alvarez, S. Epiphanio and C. R. Marinho (2013). "Placental histopathological changes associated with Plasmodium vivax infection during pregnancy." PLoS Negl Trop Dis **7**(2): e2071.

Stanisic, D. I., K. A. Moore, F. Baiwog, A. Ura, C. Clapham, C. L. King, P. M. Siba, J. G. Beeson, I. Mueller, F. J. Fowkes and S. J. Rogerson (2015). "Risk factors for malaria and adverse birth outcomes in a prospective cohort of pregnant women resident in a high malaria transmission area of Papua New Guinea." Trans R Soc Trop Med Hyg **109**(5): 313-324.

Steer, P., M. A. Alam, J. Wadsworth and A. Welch (1995). "Relation between Maternal Hemoglobin Concentration and Birth-Weight in Different Ethnic-Groups." British Medical Journal **310**(6978): 489-491.

Steer, P. J. (2000). "Maternal hemoglobin concentration and birth weight." Am J Clin Nutr **71**(5 Suppl): 1285S-1287S.

Stegmann, B. J. and J. C. Carey (2002). "TORCH Infections. Toxoplasmosis, Other (syphilis, varicella-zoster, parvovirus B19), Rubella, Cytomegalovirus (CMV), and Herpes infections." Curr Womens Health Rep **2**(4): 253-258.

Steiger, J. H. (1990). "Structural Model Evaluation and Modification: An Interval Estimation Approach." Multivariate Behav Res **25**(2): 173-180.

Stevens, G. A., M. M. Finucane, L. M. De-Regil, C. J. Paciorek, S. R. Flaxman, F. Branca, J. P. Pena-Rosas, Z. A. Bhutta, M. Ezzati and G. Nutrition Impact Model Study (2013). "Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995-2011: a systematic analysis of population-representative data." Lancet Glob Health **1**(1): e16-25.

Stevens, G. A., M. M. Finucane, C. J. Paciorek, S. R. Flaxman, R. A. White, A. J. Donner, M. Ezzati and G. Nutrition Impact Model Study (2012). "Trends in mild, moderate, and severe stunting and underweight, and progress towards MDG 1 in 141 developing countries: a systematic analysis of population representative data." Lancet (London, England) **380**(9844): 824-834.

Storm, J. and A. G. Craig (2014). "Pathogenesis of cerebral malaria—inflammation and cytoadherence." Frontiers in Cellular and Infection Microbiology **4**(100).

Strickland, G. T., A. Zafar-Latif, E. Fox, A. A. Khaliq and M. A. Chowdhry (1987). "Endemic malaria in four villages of the Pakistani province of Punjab." Trans R Soc Trop Med Hyg **81**(1): 36-41.

Suguitan, A. L., Jr., T. J. Cadigan, T. A. Nguyen, A. Zhou, R. J. Leke, S. Metenou, L. Thuita, R. Megnekou, J. Fogako, R. G. Leke and D. W. Taylor (2003). "Malaria-associated cytokine changes in the placenta of women with pre-term deliveries in Yaounde, Cameroon." Am J Trop Med Hyg **69**(6): 574-581.

Sulaiman, A. A., S. O. Bushara, W. M. Elmadhoun, S. K. Noor, M. Abdelkarim, I. N. Aldeen, M. M. Osman, A. O. Almobarak, H. Awadalla and M. H. Ahmed (2018). "Prevalence and determinants of undernutrition among children under 5-year-old in rural areas: A cross-sectional survey in North Sudan." J Family Med Prim Care **7**(1): 104-110.

Sylvester, B., D. B. Gasarasi, S. Aboud, D. Tarimo, S. Massawe, R. Mpembeni and G. Swedberg (2016). "Prenatal exposure to Plasmodium falciparum increases frequency and shortens time from birth to first clinical malaria episodes during the first two years of life: prospective birth cohort study." Malar J **15**(1): 379.

Takeuchi, R., S. Lawpoolsri, M. Imwong, J. Kobayashi, J. Kaewkungwal, S. Pukrittayakamee, S. Puangsa-art, N. Thanyavanich, W. Maneeboonyang, N. P. Day and P. Singhasivanon (2010). "Directly-observed therapy (DOT) for the radical 14-day primaquine treatment of Plasmodium vivax malaria on the Thai-Myanmar border." Malar J **9**: 308.

ter Kuile, F. O., M. E. Parise, F. H. Verhoeff, V. Udhayakumar, R. D. Newman, A. M. van Eijk, S. J. Rogerson and R. W. Steketee (2004). "The burden of co-infection with human

immunodeficiency virus type 1 and malaria in pregnant women in sub-saharan Africa." Am J Trop Med Hyg **71**(2 Suppl): 41-54.

ter Kuile, F. O. and S. J. Rogerson (2008). "Plasmodium vivax infection during pregnancy: an important problem in need of new solutions." Clin Infect Dis **46**(9): 1382-1384.

ter Kuile, F. O., D. J. Terlouw, S. K. Kariuki, P. A. Phillips-Howard, L. B. Mirel, W. A. Hawley, J. F. Friedman, Y. P. Shi, M. S. Kolczak, A. A. Lal, J. M. Vulule and B. L. Nahlen (2003). "Impact of permethrin-treated bed nets on malaria, anemia, and growth in infants in an area of intense perennial malaria transmission in western Kenya." Am J Trop Med Hyg **68**(4 Suppl): 68-77.

The World Bank. (2006). "Repositioning nutrition as central to development: a strategy for large-scale action." Retrieved 15 Feb 2020, 2020, from http://siteresources.worldbank.org/NUTRITION/Resources/281846-1131636806329/NutritionStrategy.pdf.

Thompson, R. A. and C. A. Nelson (2001). "Developmental Science and the Media: Early Brain Development." American Psychologist.

Thomsen, E. K., G. Koimbu, J. Pulford, S. Jamea-Maiasa, Y. Ura, J. B. Keven, P. M. Siba, I. Mueller, M. W. Hetzel and L. J. Reimer (2017). "Mosquito Behavior Change After Distribution of Bednets Results in Decreased Protection Against Malaria Exposure." J Infect Dis **215**(5): 790-797.

Thurnham, D. I., L. D. McCabe, S. Haldar, F. T. Wieringa, C. A. Northrop-Clewes and G. P. McCabe (2010). "Adjusting plasma ferritin concentrations to remove the effects of subclinical inflammation in the assessment of iron deficiency: a meta-analysis." Am J Clin Nutr **92**(3): 546-555.

Torres, A. M., K. E. Peterson, A. C. de Souza, E. J. Orav, M. Hughes and L. C. Chen (2000). "Association of diarrhoea and upper respiratory infections with weight and height gains in Bangladeshi children aged 5 to 11 years." Bull World Health Organ **78**(11): 1316-1323.

Troeger, C., D. V. Colombara, P. C. Rao, I. A. Khalil, A. Brown, T. G. Brewer, R. L. Guerrant, E. R. Houpt, K. L. Kotloff, K. Misra, W. A. Petri, Jr., J. Platts-Mills, M. S. Riddle, S. J. Swartz, M. H. Forouzanfar, R. C. Reiner, Jr., S. I. Hay and A. H. Mokdad (2018). "Global disability-adjusted life-year estimates of long-term health burden and undernutrition attributable to diarrhoeal diseases in children younger than 5 years." Lancet Glob Health **6**(3): e255-e269.

Tucker, L. R. and C. Lewis (1973). "A reliability coefficient for maximum likelihood factor analysis." Psychomtrika **38**.

Tusting, L. S., T. Bousema, D. L. Smith and C. Drakeley (2014). "Measuring changes in Plasmodium falciparum transmission: precision, accuracy and costs of metrics." Adv Parasitol **84**: 151-208.

Umbers, A. J., E. H. Aitken and S. J. Rogerson (2011). "Malaria in pregnancy: small babies, big problem." Trends Parasitol **27**(4): 168-175.

Umbers, A. J., E. H. Aitken and S. J. Rogerson (2011b). "Malaria in pregnancy: small babies, big problem." Trends Parasitol **27**(4): 168-175.

Umbers, A. J., P. Boeuf, C. Clapham, D. I. Stanisic, F. Baiwog, I. Mueller, P. Siba, C. L. King, J. G. Beeson, J. Glazier and S. J. Rogerson (2011). "Placental malaria-associated inflammation disturbs the insulin-like growth factor axis of fetal growth regulation." J Infect Dis **203**(4): 561-569.

Umbers, A. J., P. Boeuf, C. Clapham, D. I. Stanisic, F. Baiwog, I. Mueller, P. Siba, C. L. King, J. G. Beeson, J. Glazier and S. J. Rogerson (2011a). "Placental malaria-associated inflammation disturbs the insulin-like growth factor axis of fetal growth regulation." J Infect Dis **203**(4): 561-569.

Umbers, A. J., D. I. Stanisic, M. Ome, R. Wangnapi, S. Hanieh, H. W. Unger, L. J. Robinson, E. Lufele, F. Baiwog, P. M. Siba, C. L. King, J. G. Beeson, I. Mueller, J. D. Aplin, J. D. Glazier and S. J. Rogerson (2013). "Does malaria affect placental development? Evidence from in vitro models." PLoS One **8**(1): e55269.

Unger, H. W., C. Aho, M. Ome-Kaius, R. A. Wangnapi, A. J. Umbers, W. Jack, A. Lafana, A. Michael, S. Hanieh, P. Siba, I. Mueller, A. R. Greenhill and S. J. Rogerson (2015). "Impact of intermittent preventive treatment in pregnancy with azithromycin-containing regimens on maternal nasopharyngeal carriage and antibiotic sensitivity of Streptococcus pneumoniae, Haemophilus influenzae, and Staphylococcus aureus: a cross-sectional survey at delivery." J Clin Microbiol **53**(4): 1317-1323.

Unger, H. W., P. Ashorn, J. E. Cates, K. G. Dewey and S. J. Rogerson (2016). "Undernutrition and malaria in pregnancy - a dangerous dyad?" BMC Med **14**(1): 142.

Unger, H. W., A. P. Hansa, C. Buffet, W. Hasang, A. Teo, L. Randall, M. Ome-Kaius, S. Karl, A. A. Anuan, J. G. Beeson, I. Mueller, S. J. Stock and S. J. Rogerson (2019). "Sulphadoxine-pyrimethamine plus azithromycin may improve birth outcomes through impacts on inflammation and placental angiogenesis independent of malarial infection." Sci Rep **9**(1): 2260.

Unger, H. W., M. Ome-Kaius, S. Karl, D. Singirok, P. Siba, J. Walker, R. A. Wangnapi, I. Mueller and S. J. Rogerson (2015). "Factors associated with ultrasound-aided detection of suboptimal fetal growth in a malaria-endemic area in Papua New Guinea." BMC Pregnancy Childbirth **15**: 83.

Unger, H. W., M. Ome-Kaius, R. A. Wangnapi, A. J. Umbers, S. Hanieh, C. S. Suen, L. J. Robinson, A. Rosanas-Urgell, J. Wapling, E. Lufele, C. Kongs, P. Samol, D. Sui, D. Singirok, A. Bardaji, L. Schofield, C. Menendez, I. Betuela, P. Siba, I. Mueller and S. J. Rogerson (2015). "Sulphadoxine-pyrimethamine plus azithromycin for the prevention of low birthweight in Papua New Guinea: a randomised controlled trial." BMC Med **13**: 9.

Unger, H. W., R. A. Wangnapi, M. Ome-Kaius, P. Boeuf, S. Karl, I. Mueller and S. J. Rogerson (2016). "Azithromycin-containing intermittent preventive treatment in pregnancy affects gestational weight gain, an important predictor of birthweight in Papua New Guinea - an exploratory analysis." Matern Child Nutr **12**(4): 699-712.

UNICEF. "Unicef for every child: Childhood diseases." Retrieved 05 Dec 2019, 2019, from https://www.unicef.org/health/childhood-diseases#pneumonia.

UNICEF. (1991). "Conceptual Framework of Malnutrition: Causes of malnutrition." from https://www.unicef.org/sowc98/fig5.htm.

UNICEF (2015). Committing to Child Survival: A Promise Renewed, Progress Report 2015.

UNICEF. (2016). "Papua New Guinea National Nutrition Policy 2016 - 2026." Retrieved 7 March 2020, 2020, from https://www.unicef.org/png/media/1296/file/PNG-Reports-NutritionPolicy.pdf.

UNICEF and WHO. (2019). "Low birthweight estimates: Levels and trends 2000 - 2015." Retrieved 16 Aug 2019, 2019, from https://www.unicef.org/reports/UNICEF-WHO-low-birthweight-estimates-2019.

UNICEF and WHO (2019). UNICEF-WHO Low birthweight estimates: Levels and trends 2000 - 2015. Geneva: World Health Organization.

UNICEF, WHO and World Bank Group. (2019). "UNICEF-WHO-The World Bank: Joint child malnutrition estimates - levels and trends - 2019 edition." Retrieved 10 Aug 2019, 2019, from http://data.unicef.org/nutrition/low-birthweight.

United Nations. "UN Sustainable Development Goals." Retrieved 5 Dec 2019, 2019, from https://sustainabledevelopment.un.org/sdgs.

United Nations I.G.M.E (2015). Levels & Trends in Child Mortality Report 2015, United Nations Children's Fund.

United Nations Inter-Agency Group for Child Mortality Estimation (UN IGME) (2018). Levels and Trends in Child Mortality: Report 2018, Estimates developed by the United Nations Inter-agency Group for Child Mortality Estimation, United Nations Children's Fund, New York.

United Nations Inter-Agency Group for Child Mortality Estimation (UN IGME) (2019). Levels & Trends in Child Mortality: Report 2019, Estimates developed by the United Nations Inter-agency Group for Child Mortality Estimation, United Nations Children Fund, New York.

Upadhyay, R. P., G. Naik, T. S. Choudhary, R. Chowdhury, S. Taneja, N. Bhandari, J. C. Martines, R. Bahl and M. K. Bhan (2019). "Cognitive and motor outcomes in children born low birth weight: a systematic review and meta-analysis of studies from South Asia." BMC Pediatr **19**(1): 35.

van Eijk, A. M., J. Hill, D. A. Larsen, J. Webster, R. W. Steketee, T. P. Eisele and F. O. ter Kuile (2013). "Coverage of intermittent preventive treatment and insecticide-treated nets for the control of malaria during pregnancy in sub-Saharan Africa: a synthesis and meta-analysis of national survey data, 2009–11." The Lancet Infectious Diseases **13**(12): 1029-1042.

Verhoef, H., M. N. Mwangi, C. Cerami and A. M. Prentice (2019). "Antenatal iron supplementation and birth weight in conditions of high exposure to infectious diseases." BMC Med **17**(1): 146.

Victora, C. G., L. Adair, C. Fall, P. C. Hallal, R. Martorell, L. Richter, H. S. Sachdev, Maternal and G. Child Undernutrition Study (2008). "Maternal and child undernutrition: consequences for adult health and human capital." Lancet **371**(9609): 340-357.

Victora, C. G., M. de Onis, P. C. Hallal, M. Blossner and R. Shrimpton (2010). "Worldwide timing of growth faltering: revisiting implications for interventions." Pediatrics **125**(3): e473-480.

Vinit, R., L. Timinao and M. Katusele (2020). "Decreased bioefficacy of long-lasting insecticidal nets and the resurgence of malaria in Papua New Guinea. Under Review." Nature Communications.

Walker, C. L., I. Rudan, L. Liu, H. Nair, E. Theodoratou, Z. A. Bhutta, K. L. O'Brien, H. Campbell and R. E. Black (2013). "Global burden of childhood pneumonia and diarrhoea." Lancet **381**(9875): 1405-1416.

Walker, P. G., F. O. ter Kuile, T. Garske, C. Menendez and A. C. Ghani (2014). "Estimated risk of placental infection and low birthweight attributable to Plasmodium falciparum malaria in Africa in 2010: a modelling study." Lancet Glob Health **2**(8): e460-467.

Walker, S. P., S. M. Chang, A. Wright, C. Osmond and S. M. Grantham-McGregor (2015). "Early childhood stunting is associated with lower developmental levels in the subsequent generation of children." J Nutr **145**(4): 823-828.

Walther, B., D. J. Miles, S. Crozier, P. Waight, M. S. Palmero, O. Ojuola, E. Touray, M. van der Sande, H. Whittle, S. Rowland-Jones and K. L. Flanagan (2010). "Placental malaria is associated with reduced early life weight development of affected children independent of low birth weight." Malar J **9**: 16.

Waltmann, A., A. W. Darcy, I. Harris, C. Koepfli, J. Lodo, V. Vahi, D. Piziki, G. D. Shanks, A. E. Barry, M. Whittaker, J. W. Kazura and I. Mueller (2015). "High Rates of Asymptomatic, Sub-microscopic Plasmodium vivax Infection and Disappearing Plasmodium falciparum Malaria in an Area of Low Transmission in Solomon Islands." PLoS Negl Trop Dis **9**(5): e0003758.

Wand, H., N. Lote, I. Semos and P. Siba (2012). "Investigating the spatial variations of high prevalences of severe malnutrition among children in Papua New Guinea: results from geoadditive models." BMC Res Notes **5**: 288.

Wang, J. and X. Wang (2012). Structural Equation Modeling : Applications Using Mplus. Somerset, UNITED KINGDOM, John Wiley & Sons, Incorporated.

Wang, T., T. Huang, Y. Li, Y. Zheng, J. E. Manson, F. B. Hu and L. Qi (2016). "Low birthweight and risk of type 2 diabetes: a Mendelian randomisation study." Diabetologia **59**(9): 1920-1927.

Wang, Y. and S. Zhao (2010). Vascular Biology of the Placenta: Chapter 1, Introduction. San Rafael (CA), Morgan & Claypool Life Sciences.

Waterlow, J. C. (1972). "Classification and definition of protein-calorie malnutrition." Br Med J **3**(5826): 566-569.

Waterlow, J. C. (1973). "Note on the assessment and classification of protein-energy malnutrition in children." Lancet **2**(7820): 87-89.

Watkins, W. J., S. J. Kotecha and S. Kotecha (2016). "All-Cause Mortality of Low Birthweight Infants in Infancy, Childhood, and Adolescence: Population Study of England and Wales." PLoS Med **13**(5): e1002018.

Watson-Jones, D., J. Changalucha, B. Gumodoka, H. Weiss, M. Rusizoka, L. Ndeki, A. Whitehouse, R. Balira, J. Todd, D. Ngeleja, D. Ross, A. Buve, R. Hayes and D. Mabey (2002). "Syphilis in pregnancy in Tanzania. I. Impact of maternal syphilis on outcome of pregnancy." J Infect Dis **186**(7): 940-947.

Watson-Jones, D., H. A. Weiss, J. M. Changalucha, J. Todd, B. Gumodoka, J. Bulmer, R. Balira, D. Ross, K. Mugeye, R. Hayes and D. Mabey (2007). "Adverse birth outcomes in United Republic of Tanzania--impact and prevention of maternal risk factors." Bull World Health Organ **85**(1): 9-18.

Whidbey, C., M. I. Harrell, K. Burnside, L. Ngo, A. K. Becraft, L. M. Iyer, L. Aravind, J. Hitti, K. M. Adams Waldorf and L. Rajagopal (2013). "A hemolytic pigment of Group B Streptococcus allows bacterial penetration of human placenta." J Exp Med **210**(6): 1265-1281.

White, H. (1980). "A Heteroskedasticity-Consistent Covariance Matrix Estimator and a Direct Test for Heteroskedasticity." Econometrica **48**(4): 817-838.

White, M. T., S. Karl, C. Koepfli, R. J. Longley, N. E. Hofmann, R. Wampfler, I. Felger, T. Smith, W. Nguitragool, J. Sattabongkot, L. Robinson, A. Ghani and I. Mueller (2018). "Plasmodium vivax and Plasmodium falciparum infection dynamics: re-infections, recrudescences and relapses." Malar J **17**(1): 170.

WHO. "Child growth standards: Packages and Macros - igrowup Stata macro." from https://github.com/unicef-drp/igrowup_update

WHO. "NLiS Country Profile: Papua New Guinea." Retrieved 16 JUNE 2019, 2019, from http://apps.who.int/nutrition/landscape/report.aspx?iso=PNG&rid=1620.

WHO. "Updated WHO Policy Recommendation. Intermittent Preventive Treatment of malaria in pregnancy using Sulfadoxine-Pyrimethamine (IPTp-SP)." Retrieved 10 Jan 2020, 2020, from https://www.who.int/malaria/iptp_sp_updated_policy_recommendation_en_102012.pdf.

WHO. "WHO recommendations on antenatal care for a positive pregnancy experience." Retrieved 01 March 2020, 2020, from https://apps.who.int/iris/bitstream/handle/10665/250796/9789241549912-eng.pdf?sequence=1.

WHO (1995). "Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee." World Health Organ Tech Rep Ser **854**: 1-452.

WHO (2008). "Worldwide prevalence of anaemia 1993 - 2005." Geneva: World Health Organization.

WHO (2010). Nutrition Landscape Information System country profile indicators interpretation guide. Geneva: World Health Organisation.

WHO (2011 (WHO/NMH/NHD/MNM/11.2). "Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations." Vitamin and Mineral Nutrition Information System, Geneva, World Health Organization.

WHO (2014). "Global nutrition targets 2025: low birth weight policy brief (WHO/NMH/NHD/14.5)." Geneva: World Health Organization.

WHO. (2017). "Malaria in pregnant women." Retrieved 7 Jan 2020, 2020, from https://www.who.int/malaria/areas/high_risk_groups/pregnancy/en/.

WHO. (2018). "ICD-11 for Mortality and Morbidity Statistics (Version: 04/2019)." Retrieved 6 Dec 2019, 2019, from https://www.who.int/classifications/icd/en/.

WHO (2018). World Malaria Report. Geneva, World Health Organization.

WHO (2018). World Malaria Report 2018. Geneva: World Health Organization; Licence: CC BY-NC-SA 3.0 IGO.

WHO (2019). World Malaria Report 2019. Geneva: World Health Organisation; 2019. Licence: CC BY-NC-SA 3.0 IGO.

WHO and UNICEF. "Progress on drinking water, sanitation and hygiene: 2017 update and SDG baselines." Retrieved 6 Dec 2019, 2019, from https://apps.who.int/iris/bitstream/handle/10665/258617/9789241512893-eng.pdf?sequence=1.

WHO and UNICEF. (2004). "Low BIrthweight: Country, Regional and Global Estimates." Retrieved 05-12-2016, 2016, from www.who.int/entity/reproductivehealth/publications/monitoring/9280638327/en/.

WHO Multicentre Growth Reference Study Group "WHO Child Growth Standards: Length/height-for-age, Weight-for-age, Weight-for-length, Weight-for-height and Body mass index-for-age: Methods and Development. Geneva: World Health Organization, 2006."

WHO multicentre Growth Reference Study Group (2006). "WHO Child Growth Standards based on length/height, weight and age." Acta Paediatr Suppl **450**: 76-85.

WHO Multicentre Growth Reference Study Group. (2006). "WHO Child Growth Standards: Length/height-for-age, Weight-for-age, Weight-for-length, Weight-for-height and Body mass index-for-age: Methods and Development." Retrieved 5 Dec 2019, 2019, from www.who.int/childgrowth/en./.

WHO Vitamin and Mineral Nutrition Information System (VMNIS) "Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity."

WHO/UNICEF/WFP. (2014). "Global nutrition targets 2025: wasting policy brief (WHO/NMH/NHD/14.8). ." Retrieved 17 june 2019, 2019, from https://www.who.int/nutrition/publications/globaltargets2025_policybrief_wasting/en/.

Williams, T. N., K. Maitland, L. Phelps, S. Bennett, T. E. Peto, J. Viji, R. Timothy, J. B. Clegg, D. J. Weatherall and D. K. Bowden (1997). "Plasmodium vivax: a cause of malnutrition in young children." QJM **90**(12): 751-757.

Wilson, S. E., S. S. Morris, S. S. Gilbert, E. Mosites, R. Hackleman, K. L. Weum, J. Pintye, L. E. Manhart and S. E. Hawes (2013). "Scaling up access to oral rehydration solution for diarrhea: Learning from historical experience in low- and high-performing countries." J Glob Health **3**(1): 010404.

Witt, C. S., J. M. Montgomery, W. Pomat, D. Lehmann and M. P. Alpers (1990). "Detection of Streptococcus pneumoniae and Haemophilus influenzae type b antigens in the serum and urine of patients with pneumonia in Papua New Guinea: comparison of latex agglutination and counterimmunoelectrophoresis." Rev Infect Dis **12 Suppl 8**: S1001-1005.

Woolhouse, M. E., C. Dye, J. F. Etard, T. Smith, J. D. Charlwood, G. P. Garnett, P. Hagan, J. L. Hii, P. D. Ndhlovu, R. J. Quinnell, C. H. Watts, S. K. Chandiwana and R. M. Anderson (1997). "Heterogeneities in the transmission of infectious agents: implications for the design of control programs." Proc Natl Acad Sci U S A **94**(1): 338-342.

Wu, G., F. W. Bazar and T. E. Spencer (2004). "Recent Advances in Nutritional Sciences - Maternal Nutrition and Fetal Development." American Society for Nutritional Sciences.

Wu, G., B. Imhoff-Kunsch and A. W. Girard (2012). "Biological mechanisms for nutritional regulation of maternal health and fetal development." Paediatr Perinat Epidemiol **26 Suppl 1**: 4-26.

Xiong, X., P. Buekens, S. Alexander, N. Demianczuk and E. Wollast (2000). "Anemia during pregnancy and birth outcome: a meta-analysis." Am J Perinatol **17**(3): 137-146.

Xiong, X., P. Buekens, W. D. Fraser and Z. Guo (2003). "Anemia during pregnancy in a Chinese population." International Journal of Gynecology & Obstetrics **83**(2): 159-164.

Young, M. F., P. H. Nguyen, O. Y. Addo, W. Hao, H. Nguyen, H. Pham, R. Martorell and U. Ramakrishnan (2015). "The relative influence of maternal nutritional status before and during pregnancy on birth outcomes in Vietnam." Eur J Obstet Gynecol Reprod Biol **194**: 223-227.

Zeger, S. L. and K.-Y. Liang (1986). "Longitudinal Data Analysis for Discrete and Continuous Outcomes." Biometrics **42**(1): 121-130.

Zeisler, H., E. Llurba, F. Chantraine, M. Vatish, A. C. Staff, M. Sennstrom, M. Olovsson, S. P. Brennecke, H. Stepan, D. Allegranza, P. Dilba, M. Schoedl, M. Hund and S. Verlohren (2016). "Predictive Value of the sFlt-1:PlGF Ratio in Women with Suspected Preeclampsia." N Engl J Med **374**(1): 13-22.

Zhang, Q., C. V. Ananth, Z. Li and J. C. Smulian (2009). "Maternal anaemia and preterm birth: a prospective cohort study." Int J Epidemiol **38**(5): 1380-1389.

Zheng, W., K. Suzuki, T. Tanaka, M. Kohama, Z. Yamagata and G. Okinawa Child Health Study (2016). "Association between Maternal Smoking during Pregnancy and Low Birthweight: Effects by Maternal Age." PLoS One **11**(1): e0146241.