Modelling the public health impact of second-generation malaria vaccines

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Thesis Submitted for PhD Examination

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2022

Abstract

Despite significant progress in the control and elimination of malaria over the past two decades, the global burden remains high. The COVID-19 pandemic has seen malaria cases and deaths increase markedly over 2019 resulting in 241 million malaria cases and 627,000 malaria deaths worldwide in 2020, an increase of 14 million cases and 69,000 deaths. Around 47,000 of these additional deaths were linked to pandemic-related disruptions in the provision of malaria prevention, diagnosis and treatment. The need for a highly efficacious childhood malaria vaccine has never felt more pertinent and in 2021, after 30 years of research and development, the World Health Organization recommended the first ever childhood vaccine against *P. falciparum* malaria, RTS,S/AS01$_E$ (RTS,S) for widespread use.

The development of RTS,S, its deployment and continued evaluation has facilitated the synthesis of knowledge and data from across a wide range of different disciplines involved in malaria vaccine research. This depth of data has enhanced the development of mathematical modelling frameworks that combine immunological insights with epidemiological transmission models to address public health questions. These frameworks have formed a core part of the evaluation and policy recommendations surrounding RTS,S.

The work presented in this thesis builds upon these modelling frameworks to provide insights into the potential impact of alternative RTS,S vaccination approaches. The two RTS,S approaches examined in this thesis are a delayed-fractional primary series and a seasonally targeted vaccination schedule, both of which have demonstrated promising efficacy in human challenge studies and field trials respectively.

Drawing on data from the delayed-fractional RTS,S human challenge study I used a Bayesian framework to investigate immunological correlates of vaccine induced protection. I estimate that improvements to the quality, measured as antibody avidity, and not the quantity, measured as antibody titre, of the vaccine induced antibody response is critical to the increased efficacy against infection observed with this schedule.

Next, I utilised data from seasonal malaria chemoprevention and seasonal RTS,S vaccination clinical trials to fit and validate an updated efficacy profile of the drug combination Sulfadoxine-pyrimethamine plus amodiaquine (SP+AQ) used for seasonal malaria chemoprevention using a Bayesian survival analysis framework. This approach enabled me to capture uncertainty in the protection provided by seasonal malaria chemoprevention over time. I then use this updated efficacy profile along with the existing RTS,S vaccine efficacy profile to replicate trial cohorts in a transmission

model in order to validate the intervention models against clinical trial data. I found that the existing RTS,S model underestimated the protection provided by the seasonal vaccination schedule and explored several biologically motivated alterations to the model that brought results in line with those of the trial. These results combined with the trial reported antibody data suggest that efficacy improvements with this regime were not driven by increases in antibody quantity. Further model results suggest that when vaccination and chemoprevention were combined this resulted in potential synergistic interactions that enhanced the efficacy of SP+AQ in particular. This work resulted therefore in several updated versions of RTS,S and SP+AQ efficacy models that capture the current uncertainty in intervention effects.

Finally extending these updated efficacy models from the validation exercise, I used a model of malaria transmission to investigate the long-term public health impact of novel RTS,S vaccination schedules compared to the original age-based RTS,S dosing schedule in seasonal settings. I considered the impact both in the presence and absence of seasonal malaria chemoprevention. I examined impact by degree of seasonality, transmission intensity and by wider health system and operational factors. RTS,S vaccination in seasonal malaria transmission settings could be a valuable additional tool to existing seasonal interventions, with seasonal delivery maximising impact relative to an age-based approach. Decisions surrounding deployment strategies of RTS,S in such settings will need to consider the local and regional variations in seasonality, current levels of other interventions and potential achievable RTS,S coverage.

Statement of originality

I, Hayley Thompson, confirm that the work presented in this thesis is my own, under the supervision of Professor Azra Ghani, Dr Alexandra Hogan and Dr Patrick Walker. Where information has been derived from other sources, I confirm that this has been indicated in the thesis. All individuals who have shared data, made suggestions for analysis, and commented on manuscripts have been acknowledged or detailed below:

In Chapter 2 Michael White provided template code for the MCMC algorithm and the Robbins-Monro step scaler which I adapted and used in my analysis. Christian Ockenhouse at PATH kindly shared the data with me for the model fitting.

In Chapter 3 Matt Cairns shared the anonymised clinical trial data used in the model fitting of SP+AQ efficacy.

Hayley A Thompson

May 2022

Copyright declaration

Acknowledgements

Four and a half years, a pandemic and two major knee operations, what a rollercoaster. The course of this PhD has been so challenging both personally and professionally and I have to take a moment to appreciate how far I've come and all the people who have helped me reach this point.

To my supervisors thank you for all your guidance, patience (especially patience) and insightful comments and discussions. I have learnt so much from you all and gained experiences I never thought I would have so thank you, thank you, thank you. In particular, thank you to my primary supervisor Azra Ghani who inspired me on so many levels, thank you for your guidance, support, encouragement and ambition. Thank you for the opportunity way above my paygrade to be involved in the MPAG submission and for all your time also spent working with me over the COVID-19 response. Thank you for trusting me when often I didn't think myself capable. To Dr Alexandra Hogan thank you for all of your help getting me up to speed with and useful discussions on the malaria transmission and vaccine models. Thank you for your endless enthusiasm and positivity and sense of calm that I aspire to. And thanks to Dr Patrick Walker for having far too many ideas and for helping me through so many a mathematical modelling problem. Your enthusiasm and knowledge are truly inspiring.

Alongside my supervisors I need to thank everyone in the Malaria Group, the work you do is inspiring and has been a big motivator for me during my PhD. Special mention to Pete Winskill for fielding all my model code questions and to Lucy Okell for her guidance in the very early stages of Chapter 3 when my vaccine focussed mind did not know where to look for SMC related studies. And to everyone else (there's too many of you to name individually) for many an enjoyable meeting, Christmas party, corridor conversation and ASTHM dinner.

I would also like to acknowledge the other external collaborators for sharing data that contributed to the work in this thesis and for their valuable insights and comments on both published and unpublished manuscripts. I am particularly grateful to Matt Cairns, Brian Greenwood, Aubrey Cunningham, Christian Ockenhouse, Issaka Zongo, Issaka Sagara, Halidou Tinto, Jean-Bosco Ouedraogo, Alassane Dicko and Daniel Chandramohan. And to the team at PATH that helped to facilitate the inclusion of Chapter 5 in the MPAG evidence submission.

In addition, I would like to thank the UK Medical Research Council for funding my project.

To my VA5 office mates – Andria, Kelly, Nora, Sarah and Charlie, thank you for making this PhD a little less lonely and for providing endless laughs, basin walks, tea-breaks and colour palette discussions. I feel grateful to have been surrounded by such wonderful people during my PhD. I can't wait to see what the future has in store for you all. And to others in the department including Natsuko Imai who's

support during my time at the WHO helped to forge a truly wonderful friendship (perhaps the only thing I can thank COVID for?). And Gina Cuomo-Dannenburg for many many tea-breaks.

A massive thanks to my friends both old and new many of whom started this crazy journey with me 10 (?!) years ago as fresh-faced undergraduates and have been with me all the way. To my amazing housemates over this PhD: Georgie, Jack, Millie and Issy thank you especially for never letting me bring the PhD home (well until COVID) and for the endless laughs.

And most importantly thanks to my parents for having more belief in me throughout this process than I did and for providing endless support and love not just over my PhD but my entire life. To my brother for providing a sunny apartment in Australia when I most needed it and for always supporting me. And thanks to my dog Hector for knowing exactly when to prop his head onto my knee and demand a walk for both our sanity. This brief acknowledgement does not do justice to your contributions, and I will endeavour to thank you as often as I can for your support and love.

And finally, to my Grandpa Bob Noble, without your encouragement and wisdom I doubt I would have ever started this journey at Imperial 10 years ago. You never got to see this final adventure, but I hope I've made you proud. In loving memory always.

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AB-RTS,S	Age-based RTS,S dosing
ACCESS-SMC	Achieving Catalytic Expansion of SMC
ACT	Artemisinin-combination therapies
AL	Artemether-lumefantrine
AMA1	Apical membrane antigen 1
AQ	Amodiaquie
ASC	Antibody-secreting cells
CAP	Chemically attenuated parasites
CHIRPS	Climate Hazards Group InfraRed Precipitation Station
CI	Confidence Interval
CrI	Credible Interval
CSA	Chondroitin sulfate A
CSP	Circumsporozoite protein
D1	Domain 1
DDT	Dichlorodiphenyltrichloroethane
DF-RTS,S	Delayed-fractional RTS,S dosing
DIC	Deviance Information Criterion
EIR	Entomological inoculation rate
ELISA	Enzyme linked immunosorbent assays
elpd	Explected log predictive density
EPA	ExoProtein
EPI	Expanded Programme on Immunization
EU	Elisa Units
FOI	Force of infection
GMEC	Global Malaria Eradication Campaign
GMT	Geometric Mean Titre
GTS	Global Technical Stratergy
HBsAG	Hepatitis B surface antigen
HMC	Hamiltonian Monte Carlo
HRP2	Histidine-rich protein 2
IFNγ	Interferon gamma
IgG	Immunoglobulin G
IL-10	Interleukin-10
IPTi	Intermittent preventitive treatment of infants
IPTp	Intermittent preventitive treatment of pregnant women
IRR	Incidence rate ratios
IRS	Indoor residual spraying
ITN	Insecticide treated bed-net
LLIN	Long last insectide treated bed-net
loo-cv	Leave-one-out cross-validation
MAP	Malaria Atlas Project
MCMC	Markov Chain Monte Carlo
MDA	Mass drug administration
MVIP	Malaria Vaccine Implementation Programme
MVTR	Malaria Vaccine Technology Roadmap
NANP	central repeat region
NUTS	No-U-Turn-Sampler
PCR	Polymerase chain reaction
$PfPR_{2–10}$	*P. falciparum* prevalence in 2- to 10-year-old individuals

PvPR	*P. vivax* prevalence rate
PKPD	Pharmacokinetic pharmacodynamic
RBM	Roll Back Malaria
RDT	Rapid diagnostic test
RTS,S	RTS,S/AS01$_E$
SMC	Seasonal Malaria Chemoprevention
SP	Sulfadoxine-Pyrimethamine
SP+AQ	Sulfadoxine-Pyrimethamine plus Amodiaquie
SSA	sub-Saharan Africa
SV	Seasonal vaccination
SV-RTS,S	Seasonally targeted vaccination RTS,S dosing
Swiss TPH	Swiss Tropical and Public Health Insitute
TBV	Transmission blocking vaccine
TGF-β	Transforming growth factor-β
WHO	World Health Organization

Chapter 1

1 Introduction

Vaccines are historically one of the most cost-effective interventions that have greatly reduced the global burden of infectious diseases, particularly for childhood infections (Greenwood, 2014; Li et al., 2021). However, the childhood burden of malaria remains significant even with widespread use of vector control interventions and effective treatment regimes. As vaccines are being introduced for more complex diseases, the development of an effective vaccine against malaria has remained a global priority. In this Chapter I first review the epidemiology and burden of malaria, following this I review the historical and current policy for malaria control and detail existing malaria interventions. Next, I provide an overview of the current understanding of naturally acquired immunity against malaria and how this provides evidence for potential vaccine development. Following this, I review the history of malaria vaccine development and detail recent progress and challenges, before introducing key mathematical models of malaria transmission dynamics and discussing how they have been used as tools to further malaria vaccine research and policy. Finally, the aims of this thesis are introduced.

1.1 Malaria lifecycle

Malaria is caused by the protozoan parasite *Plasmodium* of which there are five species that infect humans: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae,* and *P. knowlesi.* Of these species, *P. falciparum* and *P. vivax* pose the greatest threat to human health. There is growing concern around *P. knowlelsi,* as zoonotic transmission has been associated with severe malaria outcomes in certain areas of South East Asia (Singh & Daneshvar, 2013).

The lifecycle of *P. falciparum* is complex and involves many antigenically distinct stages and two hosts: the female *Anopheles* mosquito and humans (Figure 1.1) Mosquitos can inoculate between 15–200 sporozoites into a human host, and these infective parasites then undergo development inside hepatocytes to form merozoites (Vanderberg, 1977). After a period of around 7–10 days sporozoites

mature into schizonts which then rupture, and merozoites are released into the bloodstream where they invade red blood cells (Murphy et al., 1989). Within the red blood cells, merozoites replicate, producing around 16–32 daughter merozoites which are then released into the blood stream following red blood cell rupture, where they reinvade new red blood cells (Warrell & Gilles, 2017). This process of invasion, replication and release occurs with a periodicity of 24–26 hours, and it is these periodic cycles that are associated with clinical disease manifestations (Warrell & Gilles, 2017). After approximately 10 days, a subset of red blood cell invading merozoites will differentiate into gametocytes, and these gametocytes continue to circulate in hosts until they are ingested by a feeding mosquito. Sexual reproduction then occurs in the mosquito midgut where gametes fuse to produce a zygote that elongates to become a motile ookinete, invades the midgut wall, and forms an oocyst. Following a sporogonic period of approximately 8–10 days, the oocysts burst to release sporozoites that travel to the mosquito's salivary glands, where they are ready for the cycle to repeat when the mosquito host takes a new blood meal.

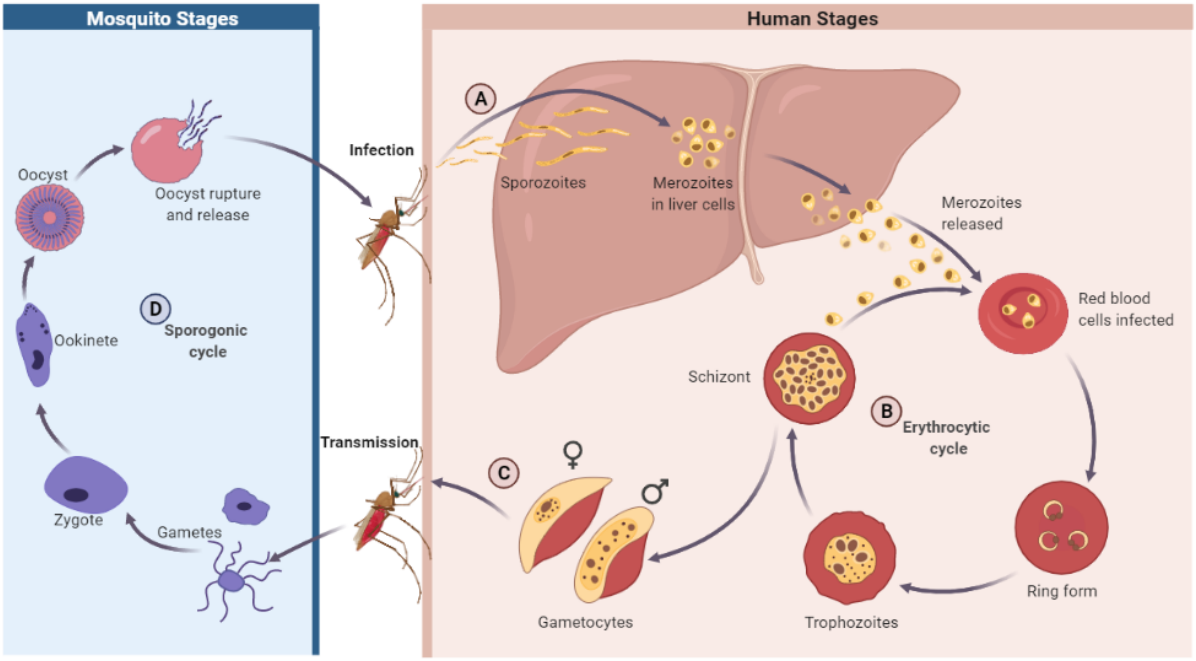

Figure 1.1 The lifecycle of *P. falciparum* in humans and mosquitos. A) Inoculation of sporozoites and the pre-erythrocytic infection stages in the liver; **B)** Asexual reproduction and blood stage infection; **C)** Gametocyte production and ingestion during a bloodmeal; and **D)** Sexual reproduction and developmental stages within the mosquito. Drawn using BioRender (www.biorender.com).

P. vivax differs from the *P. falciparum* lifecycle in several ways, however, one of the most epidemiologically significant is the ability of *P. vivax* to lie dormant and undetectable in the liver of infected human hosts. This hypnozoite stage can reactivate weeks, months, or even years after the initial infection and re-enters the bloodstream causing relapses in clinical malaria and further onwards transmission (Mueller et al., 2009). The variation in relapse times results from regional and seasonal

variations in mosquito vector populations, with tropical regions tending to experience shorter relapse periods, and longer periods in more temperate areas (White et al., 2016).

1.2 Anopheles mosquito vector

Malaria is a mosquito-borne parasitic disease that infects humans through the bite of an *Anopheline* mosquito vector. Of the approximately 70 *Anopheles* species that are able to transmit malaria to humans, an estimated 30–40 are dominant vector species, and are therefore of relevance to public health (Hay et al., 2010; Sinka et al., 2010a, 2010b; Warrell & Gilles, 2017). *Anopheles* species are found in varying geographic regions, and within regions distinct environments support different species, which affects malaria epidemiology and transmission.

Malaria parasites are transmitted by the female mosquito of the Anopheles genus. Anopheles species have four distinct life stages: egg, larva and pupa make up the juvenile aquatic stages before the final adult stage. Juvenile stages last for around 5–14 days depending on the species and the ambient temperature (World Health Organization, 2013a). Once at the adult stage mosquitos tend to mate within a few days of emergence and fed on sugar sources for energy. Female mosquitos will also require a blood meal for the development of her eggs (Harrison, Brown & Strand, 2021). It is this stage that links the female mosquito and human hosts in the malaria transmission cycle. Following a blood meal, the female must rest while the eggs are developed, again this process depends on the ambient temperature taking around two to three days in tropical conditions (World Health Organization, 2013a). Females will then lay their eggs in standing water and continue to seek further blood meals to sustain further egg production. This cycle continues until the female dies, around one to two weeks later (Matthews, Bethel & Osei, 2020). Chances of survival are dependent on temperature and humidity and the ability of the female mosquito to find a blood meal (World Health Organization, 2013a). In order to transmit parasites mosquitos must survive for longer than the extrinsic incubation period of plasmodium which is around 9–18 days depending on species and temperature (higher temperatures accelerate parasite growth) (Stopard, Churcher & Lambert, 2021; Ohm et al., 2018). Many *Anopheles* species are opportunistic in their feeding behaviour and will take a blood meal from whatever host is available either human or animal. The degree to which a species favours humans, known as anthrophily, determines their efficiency as a vector of malaria. *Anopheles gambiae* and *Anopheles funestus* are two highly anthropophilic species that makes them the primary vector in much of sub-Saharan Africa (SSA) (Sinka et al., 2010b).

1.3 Malaria disease

Infection with the malaria parasite can lead to parasitaemia resulting in uncomplicated clinical malaria, that may then progress to severe malaria or death. Uncomplicated malaria is associated with the

erythrocytic stage of the parasite lifecycle. The first symptoms of disease are usually non-specific and similar to many febrile illnesses. These initial symptoms of malaria occur around 7–14 days following an infectious bite, and patients present with a fever or flu-like illness including shaking, chills, headache, muscle ache and tiredness. Unlike other febrile illness however, malaria fevers are often characterised by their periodic presentation, approximately every two days coinciding with the erythrocyte rupture (Crutcher & Hoffman, 1996). At this stage of disease, with prompt treatment with an effective antimalarial, malaria is curable. However, if left untreated or if treatment seeking is delayed, severe malaria complications can occur which often lead to death especially in the case of *P. falciparum*. Severe malaria often causes vital organ dysfunction, or abnormalities in the patient's blood or metabolism, and manifestations can occur singularly but often present in combination. Manifestations of severe malaria include cerebral malaria, respiratory failure, acute renal failure, severe malarial anaemia, hypoglycemia and metabolic acidosis. Severe malaria can be treated with intravenous or intramuscular artesunate and symptoms of the severe manifestation are managed and treated in addition (World Health Organization, 2012a).

1.4 Malaria burden and epidemiology

According to the World Health Organization (WHO) World Malaria Report 2021 an estimated 241 million cases of malaria (95% Confidence Interval (CI) 218–269 million) and 627,000 deaths (95% CI 583,000–765,000) due to malaria occurred in 2020 (World Health Organization, 2021g). Globally the WHO Africa Region carries a disproportionately high burden of malaria and in 2020 95% of global malaria cases and deaths occurred in this region. In 2020, six countries in SSA accounted for just over half of all malaria deaths worldwide: Nigeria (27%), the Democratic Republic of the Congo (12%), Uganda (5%), Mozambique (4%), Angola (3%), and Burkina Faso (3%). Children under five years of age are particularly vulnerable to malaria and 77% of global malaria deaths occurred in this age group in 2020, predominantly in SSA.

The species responsible for the most severe and life-threatening form of malaria, *P. falciparum,* accounts for over 98% of global malaria cases and 99.7% of malaria cases in the WHO Africa Region. *P. vivax* is rare in SSA as a result of the high prevalence of the Duffy-negative phenotype which confers *P. vivax* resistance (Howes et al., 2011). *P. vivax* is, however, the dominant malaria parasite in most malaria endemic countries outside of SSA, having a much wider geographic range than *P. falciparum* in part due to its ability to lie dormant, allowing transmission to be sustained following seasons which are unsuitable for vectors (Figure 1.2)

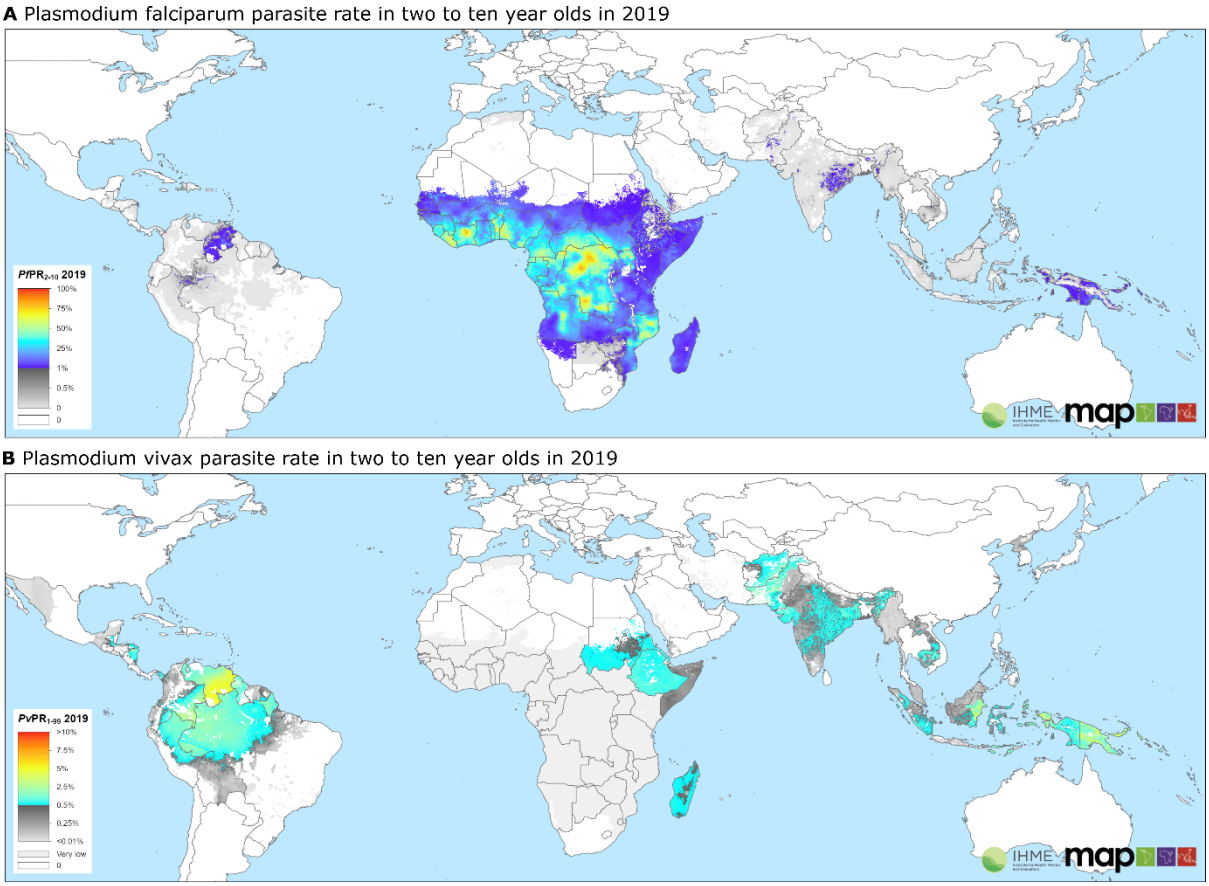

Figure 1.2 Global malaria epidemiology 2019. Predicted age-standardised parasite prevalence rates for A) Plasmodium falciparum and B) Plasmodium vivax for children two to ten years of age in 2019. The colour scaling is split to better differentiate within low endemic areas, with one linear scale between zero and 0·01 (grey shades) and a second linear scale between 0·01 and 1 (colours from blue to red). Malaria Atlas Project. Creative Commons 3.0 Unported Licence (CC BY 3.0s)

1.5 Malaria transmission intensity

Understanding the relationship between the prevalence of malaria infection, clinical incidence and transmission intensity is key to understanding the epidemiology and the impact of control interventions on malaria. Malaria transmission intensity varies greatly between populations, age-groups and over space and time and can be quantified using measurements from epidemiological studies. A wide variety of methods and metrics have been developed to quantify malaria transmission intensity.

The entomological inoculation rate (EIR) measures the average number of infectious bites per person per year (ibppy). This is a measure of the level of exposure of humans to infectious mosquitos. It is calculated as the product of the human biting rate (the number of bites per person per year) and the sporozoite rate (the proportion of mosquitos with sporozoites in their salivary glands) (Kelly-Hope & McKenzie, 2009). Human biting rates are estimated by catching and counting the number of

mosquitoes that attempt to feed on a human, and the sporozoite rate is found by examining those mosquitoes for the presence of sporozoites. While considered one of the mainstays in quantifying malaria transmission, measuring the EIR is time consuming and costly, requiring intensive and repeated measures throughout the year and large sample sizes (Kelly-Hope & McKenzie, 2009).

While the EIR measures the average number of infectious bites per year, not every infectious bite results in clinical malaria (Smith et al., 2010; Churcher et al., 2017; Rickman et al., 1990; Tall et al., 2009). The force of infection (FOI) is defined as the number of infections per person per unit time and counts all new human malaria infections in some time interval with or without clinical symptoms, and whether or not a person is already infected (Smith et al., 2010). The number of infectious bites that actually progress to malaria per unit of time describes the efficiency of transmission and can be estimated as FOI/EIR (Smith et al., 2010). The FOI is often quantified using transmission models of malaria but can also be estimated from cohort studies (Molineaux, Gramiccia & Organization, 1980; Smith et al., 2010; Mugenyi, Abrams & Hens, 2017; Mueller et al., 2012) or repeat cross-sectional surveys (Felger et al., 2012). Another method of measuring the FOI is using serological markers of malaria infection (Drakeley et al., 2005; Stewart et al., 2009; Pull & Grab, 1974). Antibody measurements in exposed populations can be used to estimate the seroconversion rate which is defined as the rate at which individuals become seropositive (Drakeley et al., 2005; Stewart et al., 2009; Pull & Grab, 1974; Cook et al., 2010; Pothin et al., 2016).

A further mainstay of malaria transmission metrics is the parasite prevalence rate, *PfPR/PvPR,* defined as the proportion of a population infected with malaria. This is measured through cross-sectional surveys and is widely collected. It is important to note that the method of parasite detection (microscopy or polymerase chain reaction (PCR) testing) will influence the estimate the parasite prevalence (Okell et al., 2009). Parasite prevalence rates measure the burden of both asymptomatic and symptomatic malaria infections at a specific period in time. Parasite prevalence rates have been crucial for mapping global malaria burden reductions and tracking declines in malaria over time (Bhatt et al., 2015; Weiss et al., 2019).

Measurements of clinical malaria incidence, defined as the number of clinical malaria episodes (usually defined as fever plus parasite density above a given threshold) per population over a given time period instead capture a direct measure of disease burden. Clinical malaria incidence can be measured by active or passive case detection or indirectly estimated using other routine health information data (Olotu et al., 2010; Snow et al., 2005; Hellewell et al., 2018; Epstein et al., 2020). In addition to clinical malaria incidence, measurements of severe malaria at the population level can be determined as the number of severe cases per person year at risk, but is often measured via the

number of cases presenting to the hospital (Njuguna et al., 2019). This can be biased by differential levels of access to care and differences in diagnosis of severe malaria (Camponovo et al., 2017).

A number of metrics derived from entomological data on vector behaviour are also of importance for measuring malaria transmission. Vectoral capacity describes the potential intensity of transmission by malaria vectors and is defined as the expected number of infectious bites that could eventually arise assuming perfect efficiency of transmission from all mosquito bites on a single human on a single day (Garrett-jones, 1964). The stability index provides a measure of the capacity of the environment to sustain malaria transmission and is defined as the number of human bites taken over the course of a vector's lifetime (Kiszewski et al., 2004).

Finally, the basic reproduction number of malaria, R_0, defined as the expected number of secondary infections arising from a single index case in a fully susceptible population, is another key metric (Smith et al., 2007). If R_0 is greater than one, transmission is sustained and if it is below one then transmission cannot be sustained of its own accord (Smith et al., 2007). Given the endemicity of malaria, we are often interested in understanding transmission in the context of pre-existing immunity and ongoing interventions meaning that populations are not fully susceptible. In this case, the effective reproduction number, R, is calculated.

When thinking about malaria transmission intensity, it is important to consider the variation in transmission. Heterogeneity in malaria transmission exists across all spatial scales, from differences within households to continental geographic variation. Large-scale geographic variation in transmission is primarily driven by climatic and environmental factors including temperature, altitude, land-use and urbanicity and the impact that these have on vector and parasite survival and breeding site availability, for example (Doumbe-Belisse et al., 2021; Gething et al., 2011; Garske, Ferguson & Ghani, 2013; Shah et al., 2022; Bødker et al., 2003). On a smaller scale, heterogeneity within communities can be driven by proximity to breeding sites, housing quality and host availability through the ownership and use of bed-nets, and attractiveness to mosquitos (Smith, Dushoff & McKenzie, 2004; Clark et al., 2008; Tusting et al., 2017; Atieli et al., 2011; Burkot, 1988). In addition, we also observe substantial temporal variation in malaria transmission which results from seasonal climate patterns, particularly rainfall, with transmission peaking during the rainy season and lowest during the dry season (Craig, Le Sueur & Snow, 1999; Reiner et al., 2015). Additionally, as mentioned above not all infectious mosquito bites result in blood-stage infection, and factors that impact the efficiency of a mosquito bite including immunity and heterogeneity in mosquito biting are also important determinants in the heterogeneity of malaria transmission.

1.6 Malaria control and elimination

Given the global burden of malaria, its control and elimination are a public health priority. The promising results of early Dichlorodiphenyltrichloroethane (DDT) insecticide spraying campaigns during World War II led the WHO to commence a Global Malaria Eradication Campaign (GMEC) in 1955 with DDT at the forefront (World Health Organization, 1955; Mendis et al., 2009). Despite some success of the GMEC in the Americas, Europe and some island nations, there was growing concern that eradication was not technically or economically feasible in many areas (World Health Organization, 1967). As financial and political will for this cause diminished, coupled with developing resistance to DDT, the campaign was unsuccessful and in many areas malaria resurged to levels equivalent to and higher than before the campaign (Mendis et al., 2009; World Health Organization, 1969). The aim of eradication was subsequently replaced with longer-term control strategies and improvements in disease management (World Health Organization, 1969).

The Roll Back Malaria (RBM) initiative was then established in 1998 bringing together multilateral, bilateral, nongovernmental, and private organisations with the aim of significantly reducing malaria incidence (World Health Organization, 1999). In 2000 African leaders gathered at an RBM summit to sign a declaration which committed to reducing malaria mortality by half by 2010 (Global Partnership to Roll Back Malaria, 2000). This renewed political and financial commitment to the control, elimination and eradication of malaria was reflected in the Millennium Development Goals agenda in 2000 and the subsequent Sustainable Development Goals agenda in 2015 (United Nations, 2000, 2015). In this Development Goal era, vector and parasite-based control initiatives have been successfully implemented in SSA. These include the widespread deployment of long-lasting insecticide-treated bed-nets (LLIN), the use of indoor residual spraying (IRS) and the use of highly effective artemisinin-combination therapies (ACT), both in routine care and in seasonal malaria chemoprevention (SMC) campaigns (World Health Organization, 2015a). The widespread distribution of these highly cost-effective control measures coupled with a period of considerable economic growth, and improvements in housing and health systems is considered to have significantly reduced incidence rates of new malaria cases by approximately 40% in SSA between 2000–2015 (World Health Organization, 2015a; Bhatt et al., 2015). Between 2000-2015 it was estimated that global efforts averted 6.2 million malaria deaths and 1.2 billion cases (World Health Organization, 2015a). In addition, during this time increasing numbers of countries moved towards malaria elimination (certified when a country achieves at least three consecutive years of zero indigenous malaria cases). Between 1987 and 2007 no country achieved WHO certification of malaria elimination, but since 2007 thirteen countries have been certified (World Health Organization, 2021a).

However, global progress has stalled in recent years and clinical malaria case numbers have increased every year since 2015 (World Health Organization, 2020a). The increases from 2019 and 2020 were the highest between any two years in this time, with an estimated additional 14 million cases and 68,000 deaths occurring in 2020 compared to the previous year (World Health Organization, 2021g). While these trends have been occurring since 2015 this was exacerbated by the COVID-19 pandemic and other humanitarian emergencies that led to disruptions in the delivery of essential malaria, and other healthcare, services. The WHO African Region contributed to over 95% of the increase in cases and deaths between 2019 and 2020 (World Health Organization, 2021g). Furthermore, of the 21 countries identified as having the potential to eliminate malaria by the WHO in 2016, only seven countries achieved elimination certification (World Health Organization, 2021h). On 27 May 2021, the World Health Assembly adopted an updated Global Technical Strategy (GTS) for malaria (World Health Organization, 2021c). The updated strategy reflects lessons learned and experiences from the 5 years from when the GTS was first set out (World Health Organization, 2015b), including the plateau in global progress and the impact of the COVID-19 pandemic. The ambitious global targets now include:

- Reducing malaria case incidence by at least 90% by 2030
- Reducing malaria mortality rates by at least 90% by 2030
- Eliminating malaria in at least 35 countries by 2030
- Preventing a resurgence of malaria in all countries that are malaria-free

The development of next-generation malaria tools that complement or replace existing tools will be essential for sustainable control and elimination programmes (World Health Organization, 2021c) and for achieving the ambitious target of malaria eradication within a generation (Feachem et al., 2019). While interventions against malaria are used in countries in each stage of malaria control or elimination there are key differences to these aims outlined below:

- **Malaria control:** is the reduction of disease incidence, prevalence, morbidity, or mortality to a locally acceptable level resulting from targeted interventions. Continued intervention is required to sustain control.
- **Malaria elimination:** is the interruption of local transmission achieving zero locally acquired cases within a year in a defined geographic area. Continued measures are required to prevent the reestablishment of transmission.
- **Malaria eradication:** is the permanent reduction to zero of the worldwide annual incidence of malaria infection caused by all species of human malaria parasites. Interventions are no longer required.

1.7 Principal methods of malaria control and elimination

The current suite of malaria interventions focusses on both the prevention of malaria through vector control and chemoprophylaxis and the prompt treatment of cases coupled with improved surveillance and reporting of malaria.

Vector control is a vital component of malaria control and elimination strategies. Vector control aims to reduce transmission of the malaria parasite through preventing human contact with the *Anopheles* mosquito vector and reducing the vector population. The two mainstay vector control interventions currently recommended by the WHO are insecticide treated bed-bets (ITNs) and indoor residual spraying (IRS). ITNs are hung over beds to protect individuals from bites during the night; they act as a physical barrier and are impregnated with insecticides that repel, disable, or kill mosquitos that come into contact with the net (Lengeler, 2004; Lim et al., 2011). This means that ITNs not only protect the user but can also have a 'community effect' when high levels of coverage are achieved (>50%), protecting non-users due to the effect of the insecticide on mosquito survival and longevity (Killeen et al., 2007; Govella, Okumu & Killeen, 2010; Russell et al., 2010; Hawley et al., 2003; Howard et al., 2000). Improvements in net design now means that insecticides are impregnated within the fibre of the net resulting in long-lasting protection (~3 years) without the need to regularly retreat nets (Kilian et al., 2011; World Health Organization, 2019a). The scale-up of distributions of LLINs has been attributed as a major component in reducing malaria burden (Bhatt et al., 2015) however, increasing mosquito resistance to commonly used insecticide classes threatens the effectiveness of LLINs. To date, 29 countries have reported mosquito resistance to all main insecticide classes and 78 have reported resistance to at least one class (World Health Organization, 2021g). IRS involves the spraying of residual insecticide to potential vector resting surfaces within dwellings, again killing or repelling vectors from the house (Choi, Pryce & Garner, 2019; Pluess et al., 2010). The choice of insecticide used for IRS depends on the local vector susceptibility to the active compound (World Health Organization, 2015c; Sherrard-Smith et al., 2018).

Effective anti-malarial drugs are vital for both treatment of malaria but also as preventative interventions. Antimalarials act by targeting and clearing the blood-stage parasites from individuals, preventing the progression to severe disease or death. Historically chloroquine and sulphadoxine-pyrimethamine (SP) were widely used as first line treatment, however resistance to these monotherapies developed and spread, rendering these drugs ineffective in many countries (Roux et al., 2021). These regimes were replaced with artemisinin-based combination therapies (ACTs) – an artesunate drug combined with a longer lasting partner drug (White, 1999a, 1999b; World Health Organization, 2001). Prompt and effective case-management of malaria is critical, as early diagnosis and treatment reduces morbidity and mortality (Sinclair et al., 2009). In addition, currently

recommended ACTs have also been shown to have gametocidal activity which can contribute to transmission reductions (Okell et al., 2008b). The WHO recommends that all suspected cases of malaria are confirmed using diagnostic methods, ensuring appropriate treatment for individuals, a rationed use of ACTs to help prevent resistance and improved surveillance, and reporting of malaria cases to help national programmes understand disease burden (World Health Organization, 2000). The development of rapid diagnostic tests (RDTs) for malaria have been vital in the expanded use of malaria diagnostics, enabling quick testing without the need for laboratory facilities.

The development of resistance to currently available drugs is a large threat to malaria control. Resistance to artemisinin-based combination therapies is widespread across southeast Asia but to date has not been widely reported in SSA (Hamilton et al., 2019). Recent studies however have shown that genes associated with ACT resistance are present in Rwanda (Uwimana et al., 2020), and that such mutations correlated with an observable reduction in parasite clearance times in Ugandan patients (Balikagala et al., 2021; Asua et al., 2021). Given the historic prevalence of resistance to older drugs across Africa, the existence of partial artemisinin resistance in SSA is of great global concern (Conrad & Rosenthal, 2019). Not only is resistance to therapies a problem but genetic deletions that render parasites undetectable by currently available RDTs that detect histidine-rich protein 2 (HRP2) are also of concern, putting patients at risk of misdiagnosis, significant morbidity and death. Recent surveys in the Horn of Africa region found that over 50% *P. falciparum* cases are missed by RDTs due to the high prevalence of HRP2 deleterious parasites (World Health Organization, 2021d).

Preventative chemotherapy using full courses of anti-malarial drugs delivered regardless of infection status has also been introduced as control methods in specific contexts and for particular risk groups. WHO recommended approaches to preventive chemoprophylaxis include:

- Intermittent preventive treatment of infants (IPTi), SP delivered to infants (<12 months) at routine childhood immunisation appointments (World Health Organization, 2011a);
- Intermittent preventive treatment of pregnant women (IPTp), SP delivered to all women in their first or second pregnancy from the second trimester onwards (World Health Organization, 2013b);
- Seasonal malaria chemoprevention (SMC), SP+AQ delivered to all children under 6 years old at monthly intervals during the peak malaria transmission season (World Health Organization, 2012b);
- Mass drug administration (MDA) treats the entire population of a defined geographic area, either with or without first screening for parasites, at the same time (World Health Organization, 2018).

Again, preventative chemotherapy is reliant on the anti-malarial remaining effective against parasite strains in specific locations. For example, resistance to SP is widespread in eastern Africa due to historic use of SP as a first line treatment (Okell, Griffin & Roper, 2017; Naidoo & Roper, 2011). This means that despite suitable conditions for SMC this intervention cannot be deployed (Cairns et al., 2012).

Despite the threat of resistance, current interventions remain effective and their scale-up is a priority. However, novel and complementary tools are required to ensure the sustainability and success of malaria control and elimination programmes. This includes the development of novel drugs, insecticides, and RDTs that could be deployed in the face of resistance but also totally novel interventions. Some examples include: genetically modified mosquitos (Hoermann et al., 2021; Nolan, 2021), sterile insect techniques (Munhenga et al., 2011; Nolan, 2021), attractive toxic sugar baits (Traore et al., 2020; Fraser et al., 2021), transgenic fungi (Lovett et al., 2019), long-lasting antimalarial injectables (Burgert et al., 2022; Bakshi et al., 2018), monoclonal antibodies (Gaudinski et al., 2021; Kisalu et al., 2018), spatial repellent emanators (Hellewell et al., 2021; Ogoma et al., 2017), eave tubes (Sternberg et al., 2021; Snetselaar et al., 2017) and housing improvements (Furnival-Adams et al., 2021; Tusting et al., 2017), ivermectin (Slater et al., 2020; Foy et al., 2019) and antimalarial treated bed nets (Paton et al., 2019). Notably a vaccine against malaria has been a long-term goal, and perhaps one of the most important classes of novel interventions against malaria and this now looks to be within reach.

1.8 Naturally acquired immunity to malaria – the case for a vaccine

Morbidity due to *P. falciparum* infections can vary from mild clinical symptoms of febrile illness to severe and life-threatening disease due to vital organ dysfunction. Individuals living in malaria endemic areas, however, do acquire substantial protection against clinical and severe forms of malaria, but rarely, if ever, is sterile immunity achieved (Doolan, Dobaño & Baird, 2009). Generally, immunity against severe malaria develops rapidly, followed by immunity against clinical disease and finally, more slowly, the build-up of immune tolerance to blood-stage parasites (Figure 1.3) (Gupta et al., 1999; Griffin et al., 2015; Snow et al., 1998). While immunity to patent parasitaemia can develop by adulthood, subpatent infections that are detectable with advancements in molecular diagnostic techniques still occur. It is this immune tolerance that results in asymptomatic carrier infections among adult populations in malaria endemic areas (Bousema et al., 2014; Okell et al., 2012; Mosha et al., 2013; Nguyen et al., 2018). The acquisition of immunity to malaria has been shown to be both age- and exposure-dependent leading to a high degree of variability in patterns of immunity across populations (Rodriguez-Barraquer et al., 2018; Greenwood, Marsh & Snow, 1991; Griffin et al., 2015; Baird, 1995; Reyburn et al., 2005; Idro et al., 2006). This progressive acquisition of immunity to malaria

is why younger children are particularly vulnerable to episodes of severe malaria, once a period of protection provided from maternally derived antibodies wanes and before the acquisition of this effective immunity (Riley et al., 2001).

The development of naturally acquired immunity therefore forms a strong rationale for the development of a malaria vaccine that could protect children from the deadly consequences of malaria infections. Epidemiological patterns of anti-disease and anti-parasite immunity have been described across a range of transmission settings, however there are still fundamental gaps in our understanding of the mechanisms of immunity. Understanding the immunological basis of naturally acquired immunity is of further importance for rational vaccine development.

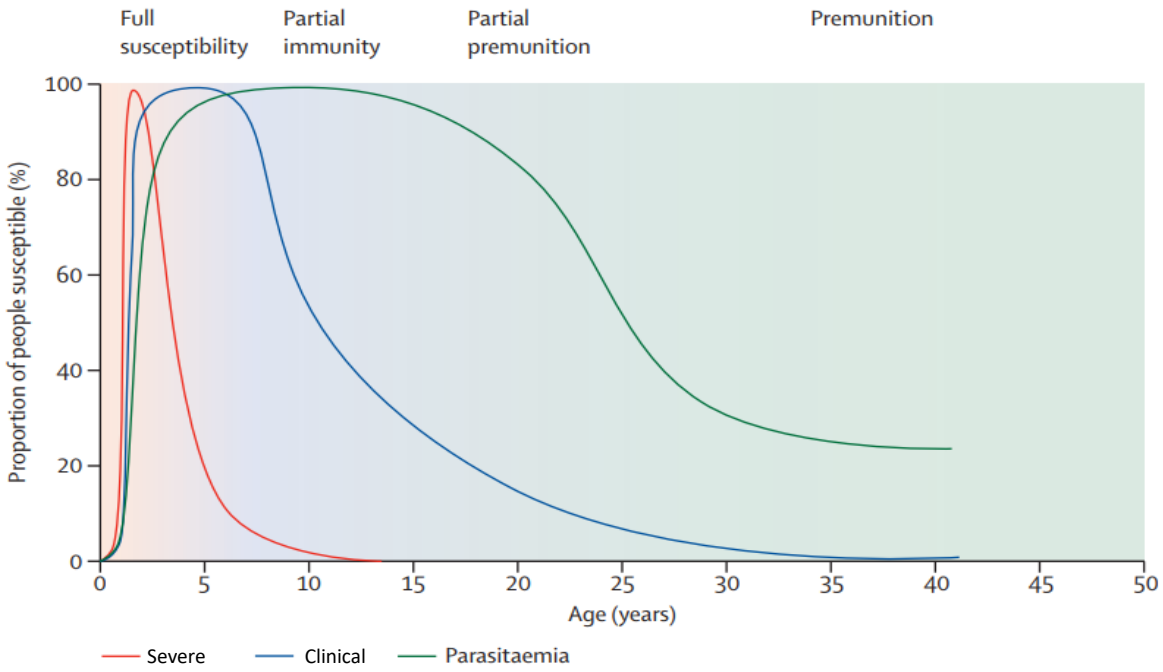

Figure 1.3 Progression of Naturally Acquired Immunity to Malaria. The relationship between age and malaria severity in an area of moderate transmission intensity shows how with repeated exposure by early childhood, protection is first acquired against severe disease, then more slowly protection builds up against clinical disease and finally much more slowly develops against parasitaemia. In areas with higher transmission intensity the rate of acquisition of naturally acquired immunity can increase. Reproduced from Griffin et al 2015 doi: 10.1098/rspb.2014.2657.

1.8.1 Immunological responses to *P. falciparum*

Due to the parasite multistage lifecycle, there are several points at which the immune system could respond to the invading threat. Upon first exposure to blood stage parasites, the innate immune system launches a non-specific immune response triggering a release of pro-inflammatory cytokines which help to limit parasite growth (Stevenson & Riley, 2004). These cytokine responses also allow for the effective priming of the humoral and cellular-mediated immune responses which then provide adaptive responses upon re-exposure (Stevenson & Riley, 2004). Key to the adaptive immune

responses is the phenomenon of pattern recognition of parasite antigens by B and T cells, which enables a rapid and more effective parasite specific protective response to each lifecycle stage (Tew et al., 1997). However, the exact immune effector mechanisms involved in parasite regulation, control and elimination at each lifecycle stage are not fully characterised. The ability of malaria parasites to evade and interfere with effective immune responses also presents several challenges in mounting and understanding successful immune responses (Langhorne et al., 2008; Ramasamy, 1998; Gomes et al., 2016). The key mechanisms understood to play a significant role at each parasite life-stage are shown in (Figure 1.4).

Antibodies directed against sporozoite antigens are a key mediator of pre-erythrocytic immunity (Beeson et al., 2019). The circumsporozoite protein (CSP) is the most abundant protein on the surface of sporozoites and is thought to be a major target for antibody responses (Nussenzweig & Nussenzweig, 1985); other antigen targets include liver-stage antigen 1 and 4 and Thrombospondin-related adhesive protein (John et al., 2008, 2005). Antibodies can opsonise and immobilise free sporozoites in the skin and blood preventing hepatocyte invasion (Schwenk et al., 2003), and a strong role of antibody mediated phagocytosis and activation of the classical complement pathway has recently been eluded to in this response (Feng et al., 2021). Once sporozoites have invaded the liver, CD8+ T cells and interferon gamma (IFNγ) cytokine production have been shown to be instrumental at recognising and killing infected hepatocytes (Lefebvre & Harty, 2020; Epstein et al., 2011). However, this response is under heavy regulation to prevent inflammatory damage to liver cells and is frequently insufficient (Bertolino & Bowen, 2015; Riley & Stewart, 2013). There is also some evidence of long-lasting protective immunity through memory B cells to pre-erythrocytic lifecycle stages from individuals in malaria endemic regions and international travellers (Weiss et al., 2010; Ndungu et al., 2013; Nogaro et al., 2011). These naturally occurring immune responses to sporozoite life-stages are poor and frequently inefficient at eliminating parasites and are generally considered inadequate to confer protection against clinical malaria.

The majority of naturally acquired immunity is directed against blood stage parasites – both freely circulating merozoites and infected red blood cells. Merozoites express a much larger number of proteins on their surface compared to the sporozoite life stage, and these proteins exhibit many more genetic polymorphisms and functional redundancies making identification of immune targets difficult. Key antigens are thought to include merozoite surface protein 1 and 2, apical membrane antigen 1 and erythrocyte binding antigen 175 (Reiling et al., 2019; Richards et al., 2013; Mu et al., 2006; Volkman et al., 2006). Antibodies have been shown to be important for blocking merozoite invasion of red blood cells, opsonising free merozoites in the blood stream and activating complement pathways leading to parasite removal (Irani et al., 2015; Bouharoun-Tayoun et al., 1995; Boyle et al.,

2015; Healer, Chiu & Hansen, 2018). Antibodies have also been shown to correlate with naturally acquired protection in longitudinal studies in malaria endemic regions (Reiling et al., 2019; Hill et al., 2016; Osier et al., 2014; Boyle et al., 2015). In addition, a broad range of cellular mediated immune responses are induced at this stage. T cells support antibody production and secrete IFNγ, a pro-inflammatory cytokine, which activates macrophages leading to phagocytosis of opsonised parasites and infected red blood cells (Stanisic & Good, 2016). If these inflammatory responses are not carefully regulated however, they can contribute to significant immunopathology (Coban, Lee & Ishii, 2018). The regulation of this inflammatory response requires production of anti-inflammatory cytokines such as interleukin-10 (IL-10) and transforming growth factor-β (TGF-β), the balance and timing of which are critical for determining disease outcomes (Stanisic & Good, 2016). Long-term memory B cell production for blood stage antigens has been demonstrated following a limited number of clinical episodes, and there is further evidence to suggest that repeat infections in endemic areas of moderate to high transmission could actually have a detrimental impact on the generation of memory B cells which could hamper immunological memory in certain settings (Pérez-Mazliah et al., 2020; Ly & Hansen, 2019).

Additional anti-gametocyte immune responses that reduce onwards transmission in malaria exposed individuals have been shown to occur (Ouédraogo et al., 2011). Key antigen targets for naturally acquired sexual stage immunity include pre-fertilization antigens Pfs230 and Pfs48/45 (Ouédraogo et al., 2011; Stone et al., 2016). Naturally acquired antibodies against gametocytes can act by blocking development in human hosts, facilitating complement-mediated gamete killing or by interrupting fertilization and development in the mosquito following ingestion (Tonwong et al., 2012; Bousema et al., 2011; Stone et al., 2018). However, gametocyte specific antibodies are often poorly induced and not widely circulating in populations (Bousema et al., 2011).

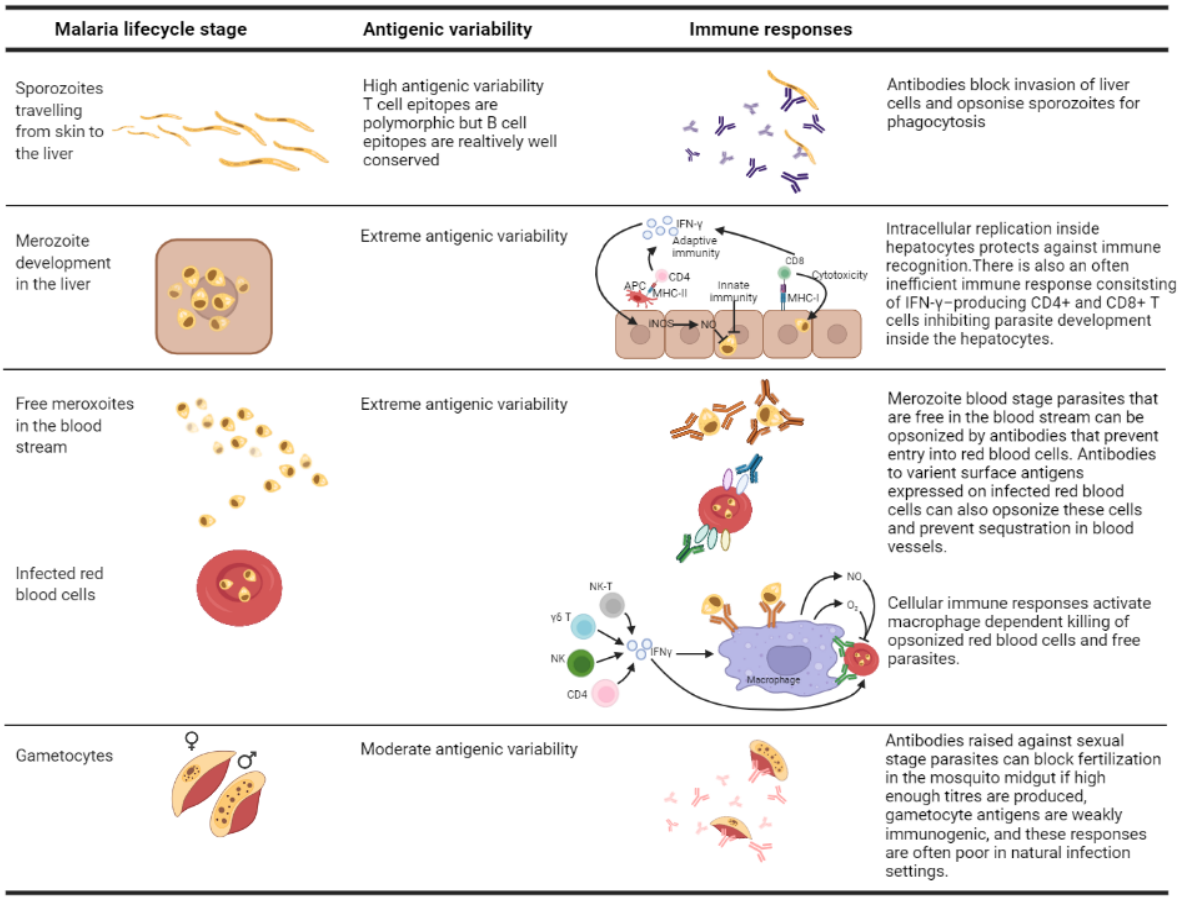

Figure 1.4 Overview of the immune responses directed against each malaria parasite life stage. Drawn using BioRender (www.biorender.com).

1.9 Malaria vaccine candidates

While a deeper understanding of the mechanisms that mediate immunity to malaria is needed, the evidence of protective responses at each stage of the parasite lifecycle provides a strong rational for vaccine approaches to malaria. Modern vaccine development can therefore be split into three categories depending on the stage of the parasite lifecycle being targeted:

1. **Pre-erythrocytic vaccines**: target inoculated free sporozoites and the intra-cellular liver-stage parasites. A pre-erythrocytic vaccine that completely blocks the establishment of parasites in the liver or prevents maturation of the liver stage parasite would prevent both disease and onwards transmission.
2. **Blood-stage vaccines**: target free merozoites or merozoite infected red blood cells. Sterile immunity will not be achieved with a blood-stage vaccine but instead it would induce protection against severe and clinical disease manifestations by reducing parasite densities.
3. **Transmission blocking vaccine**: target the sexual stages of the parasite lifecycle (gametocytes, gametes, ookinetes) to prevent parasite development within the mosquito. A transmission blocking vaccine would have no impact on individual disease outcomes but would reduce onwards transmission with the aim of inducing community level protection against infection and thus disease.

In the absence of a single life-stage vaccine that induces sterilising immunity, combination vaccines targeting multiple stages of the parasite's lifecycle are an attractive developmental target. Through a vaccine platform that combines antigenic targets for each stage of the parasite lifecycle, an effective immunological safety net could be induced such that broad biological effects prevent infection, disease and onwards transmission (Moore & Hutchings, 2014).

1.9.1 Malaria vaccine development

In 2006 the WHO launched the Malaria Vaccine Technology Roadmap (MVTR) which formed a strategic framework to underpin the activities of the global malaria vaccine research and development process (Malaria Vaccine Funders Group, 2006). The framework was revised in 2013 in response to the changing epidemiological landscape of malaria and the renewed goals of eventual malaria eradication (Malaria Vaccine Funders Group, 2013). The roadmap sets ambitious targets to develop a malaria vaccine of at least 75% efficacy against clinical malaria after one year and the development of a vaccine to significantly reduce transmission of the parasite (Malaria Vaccine Funders Group, 2013). Malaria vaccine development is a challenging and difficult process, hampered by our still relatively

limited understanding of functional immunity that confers protection against malaria, and a lack of understanding of specific immune correlates of vaccine induced protection.

Modern malaria vaccine development dates back to early immunisation studies in the 1960s using radiation-attenuated whole sporozoites, conducted first on mice (Nussenzweig et al., 1967) and then on humans in the 1970s (Clyde et al., 1973). While demonstrating that a high level of protection against malaria was possible, these approaches were not practical on a large scale, requiring numerous bites from irradiated mosquitos. However, this was the first proof of principle helping to stimulate many avenues of malaria vaccine research. In the 1980s the identification of the circumsporozoite protein (CSP) on the surface of sporozoites and the subsequent cloning and sequencing of this gene (Nardin et al., 1982; Dame et al., 1984) led to the development of vaccines that isolate and deliver specific antigens instead of whole parasites. By 1997 the first subunit vaccine based on the CSP antigen demonstrated protection against experimental challenge with malaria (Osé et al., 1997).

Candidate malaria vaccines must be tested for safety, immunogenicity and efficacy in clinical trials. Clinical trials are carried out in phases with each phase focussing on some overlapping and some new research questions to inform researchers on the next steps of development. In addition, clinical trials provide data allowing policy makers to assess how vaccines could potentially be integrated into country health systems and contribute to knowledge on vaccine technology as well as infection and disease specific questions on immunity. The following are a summary of the typical phases associated with malaria vaccine research (MVI PATH, 2015):

- **Research and preclinical development** includes identifying antigen targets, creating the vaccine platform, conducting preclinical studies in animal models or cell lines, and developing vaccine manufacturing processes.
- **Phase 1 clinical trials** assess the safety and immunogenicity of a vaccine candidate in humans. Early trials typically involve small numbers of trial participants (<100 volunteers) and take around a year from recruitment to initial data analysis. Phase 1 trials for malaria vaccines are often conducted in two stages, the first in malaria naïve volunteers known as Phase 1a trials which then progress into Phase 1b trials in malaria exposed populations in endemic countries. A favourable safety and immunogenicity profile means the vaccine candidate can progress to Phase 2 trials.
- **Phase 2 clinical trials** monitor the safety, potential side effects, immune response and preliminary vaccine efficacy against infection and/or clinical disease. These trials can also be used to determine the optimum dosage and schedules for vaccine candidates. Phase 2 trials

are larger in size, recruiting up to several thousand volunteers and last around 2 years. Phase 2 trials are again conducted in two stages, Phase 2a trials in malaria naïve volunteers in non-endemic countries are vaccinated and then exposed to the bites of infectious mosquitos in human challenge studies with close monitoring to see how long it takes for them to become infected, if infection is detected volunteers are treated with antimalarials. These challenge trials allow for the assessment of a vaccine efficacy before moving into Phase 2b trials in endemic-populations under natural exposure to malaria. If the vaccine maintains suitable safety and demonstrates efficacy in Phase 2 trials, it can proceed to Phase 3 testing.

- **Phase 3 clinical trials** are large-scale long-term trials, recruiting thousands of participants and lasting for three to five years. Phase 3 trials monitor the safety and potential side effects and can evaluate vaccine efficacy on a large scale. Trials need to be large to ensure that vaccine efficacy can be measured under varied transmission settings. Once a vaccine has demonstrated safety and sufficient efficacy, manufacturers submit for licensure to regulatory authorities along with a plan for post-licensure safety monitoring.
- **Phase 4 clinical trials** is an additional monitoring step that ensures any rare, serious adverse events including delayed side-effects are detected early as they might not become evident until sufficiently large numbers of people have been vaccinated. Phase 4 studies continue to monitor the effectiveness of the vaccine and can monitor the duration of protection over longer-time frames and impacts on secondary health outcomes for example reducing severe malaria outcomes or mortality.

Over the past 20 years malaria vaccine development has progressed rapidly, and since 2000 around ten new malaria vaccine clinical trials were registered each year on ClinicalTrials.gov and vaccines targeting each stage of the *P. falciparum* lifecycle are now in pre-clinical or clinical studies. A full set of clinical trials for a successful candidate can take up to 15 years and is highly costly (MVI PATH, 2015). Table 1.1 highlights a selection of vaccine candidates, in clinical and pre-clinical trials in each of these categories. The most advanced of these candidates is the RTS,S/AS01$_E$ pre-erythrocytic vaccine (referred to as RTS,S) which has been developed in partnership with GlaxoSmithKline and the Malaria Vaccine Initiative at PATH since 2001. The vaccine recently received a positive recommendation from the WHO for widespread use in sub-Saharan Africa (World Health Organization, 2021f).

Table 1.1 Select malaria vaccine candidates in clinical and preclinical studies.

Vaccine candidate	Immunogen type	Antigen	Clinical trial status	Summary	Key references
Pre-erythrocytic stage vaccines (infection blocking)					
RTS,S	Subunit	CSP	Phase 4 implementation studies	RTS,S is a subunit vaccine created by fusing a portion of the CSP and the hepatitis B surface antigen (HBsAg) and is delivered with a potent adjuvant system $AS01_E$ developed by GSK. RTS,S is the first malaria vaccine to be recommended for widescale use by the WHO. Phase 3 clinical trials completed in 2014 in 11 sites across sub-Saharan Africa. Efficacy of 68% (95% CI 64%-72%) was reported after 6 months, with efficacy waning significantly to 36% (95% CI 32%-41%) by the end of follow up. 7 year follow up studies shown vaccine derived protection to reach zero by four years with potential negative efficacy in the fifth year.	(RTSS Clinical Trials Partnership, 2015; Olotu et al., 2016; World Health Organization, 2021b)
R21	Subunit	CSP	Phase 3	The first next generation RTS,S-like vaccine. The R21 vaccine is a virus-like particle based on the C-terminal portion of the circumsporozoite fused to the N-terminus of HBsAg. R21 is manufactured using the Matrix-M adjuvant produced by Novavax. Recently completed Phase 2 field trials showed vaccine efficacy of 77% (95% CI 67%-84%) after 6 months in a cohort of children aged 5-17 months resident in a highly seasonal transmission setting in Burkina Faso with a background of substantial use of bednets, moderate implementation of seasonal malaria chemoprevention and minimal indoor residual spraying. Due to differences in case detection methods between the R21 and RTS,S trials and differences in the timing of vaccinations to the beginning of the malaria transmission season, the question of superiority or non-inferiority between these two vaccines remains unanswered.	(Datoo et al., 2021; Collins et al., 2017)
Full length CSP	Subunit	CSP	Phase 1	rCSP/GLA-LSQ malaria vaccine features a full length recombinant CSP protein which includes a region critical to sporozoite cell attachment and hepatocyte invasion that are not present in RTS,S. Human antibodies against the junction between the N-terminal and central repeat region have been isolated and protected against controlled human malaria infection suggesting potential advantage of including this junctional region in a CSP-based vaccine. A Phase 1 dose escalation study was recently completed with this rCSP/GLA-LSQ formulation and showed a favourable safety and tolerability and immunostimulatory results and is moving forward with next clinical trials.	ClinicalTrials.gov Identifier: NCT03589794 (Friedman-Klabanoff et al., 2021)

PfSPZ PfSPZ-CVac PfSPZ-GA1	Whole sporozoite, radiation attenuation, chemical attenuation, genetic attenuation	Whole sporozoite	Phase 2	In 2010 the company Sanaria introduced the PfSPZ vaccine, utilising a new technology that entailed harvesting sporozoites from the salivary glands of mosquitos infected with laboratory cultured parasites. Harvested sporozoites can then be attenuated by different mechanisms: through radiation, through chemoattenuation (concomitant administration of antimalarial drugs in vivo) or through genetic attenuation (deletion of genes required for liver-stage development). Efficacy of these PfSPZ approaches have been demonstrated in controlled human infectious challenge studies in malaria naïve-adults and Phase 1 clinical trials. PfSPZ demonstrated 52% (95% CI 31%-86%) efficacy against malaria infection after 24 weeks in healthy adults living in malaria endemic areas in Mali. Larger-scale Phase 2 field trials of PfSPZ have since been completed but the routine under examination showed no significant protection against infection with malaria by 6 months follow up but showed efficacy against clinical malaria of up to 46% (95% CI 7%-69%) after 3 months. PfSPZ-CVac administered under the prophylactic cover of pyrimethamine or chloroquine demonstrated after 3 months high levels of protection against homologous and heterologous challenge up to 100% in human challenge studies. PfSPZ-GA1, a PfSPZ vaccine attenuated by deletion of b9 and slarp genes has been tested for safety, immunogenicity, and preliminary efficacy in malaria-naïve Dutch volunteers. While no conclusions on protection from PfSPZ-GA1 could be drawn from this study due to the small sample size, the favourable safety profile and immunogenicity warrant further clinical evaluation which is ongoing. PfSPZ vaccines require liquid nitrogen cold chains, are delivered intravenously and these do present a number of challenges for scale-up and associated costs.	(Sissoko et al., 2017; Oneko et al., 2021; Steinhardt et al., 2020; Oneko et al., 2020) (Mwakingwe-Omari et al., 2021; Sulyok et al., 2021; Murphy et al., 2021) ClinicalTrials.gov Identifier: NCT03952650 (Roestenberg et al., 2020)
Blood stage stage vaccines (disease blocking)					
PfRH5	Subunit	RH5	Phase 2	*P. falciparum* reticulocyte-binding protein homolog 5 (PfRH5) is a novel antigen that binds to an essential red blood cell receptor and shows limited levels of polymorphisms. PfRH5 vaccine candidates induced broadly neutralising antibodies in preclinical studies and has demonstrated protection in non-human primate models of malaria. Most recently an RH5 recombinant protein vaccine formulated in $AS01_B$ adjuvant was found to be safe and well tolerated and while the current formulation was not able to prevent parasitaemia the authors observed a significant reduction in parasite growth rate in human challenge studies. A panel of human monoclonal antibodies against RH5 from this clinical trial were isolated and a subset identified with neutralizing, non-neutralizing and potentiating activity to help guide the next generation of immunogen design.	(Payne et al., 2017; Douglas et al., 2011; Minassian et al., 2021; Alanine et al., 2019) ClinicalTrials.gov Identifier: NCT02927145
AMA1-RON2	Subunit	AMA1	Preclinical	Previously apical membrane antigen 1 (AMA1) showed poor efficacy in early blood stage vaccines trials but is known to be an important protein involved in parasite growth. Following the identification of a complex between AMA1 and rhoptry neck protein 2 (RON2) that is essential for parasites to enter red-blood cells. Recently this AMA1-RON2 complex was tested in non-human primate models of malaria infection and was shown to protect Aotus monkeys.	(Srinivasan et al., 2013, 2017)

Chemically attenuated parasite vaccines (CAP)	Whole blood-stage parasite (chemical attenuation)		Phase 1	Whole parasite asexual blood-stage vaccines are also being investigated. Chemoattenuation in this life-stage approach currently involves using DNA-binding drugs known as Cyclopropylpyrolloindole analogues administrated in vitro. Early clinical studies in malaria naïve volunteers in Australia showed chemically attenuated parasites to be immunogenic, safe and well tolerated and they induced *P. falciparum* specific T cell responses but not antibody responses. This approach was also shown to induce protection against challenge in rodent models of malaria and has begun the process of a human trial of a 3-dose regime to assess efficacy in a controlled human malaria challenge study.	(Good et al., 2013; Raja, Stanisic & Good, 2017; Raja et al., 2016; De et al., 2016; Stanisic et al., 2018) ACTRN12618001314213
Malaria in Pregnancy blood stage vaccines					
VAR2CSA	Subunit	chondroitin sulfate A (CSA)	Phase 1	A finding central to vaccine development against malaria in pregnancy is that the associated adverse health outcomes of malaria in pregnancy are gravidity-dependent with first time mothers in areas of sustained transmission at substantially higher risk of morbidity. In subsequent pregnancies women acquire adaptive immunity that restricts the extent and consequences of placental infection. This protective immunity is believed to be driven by protective antibodies that block the parasite binding to chondroitin sulfate A (CSA) molecules in the placenta and opsonise infected red blood cells. VAR2CSA is a protein expressed by parasites and is the leading candidate antigen for placental malaria vaccines. Two VAR2CSA-derived placental malaria vaccines have begun Phase 1 clinical trials: PRIMVAC and PAMVAC. Both trials were designed as randomised, double-blind placebo-controlled dose escalation trials to evaluate the safety and immunogenicity of vaccine candidates. PRMVAC was tested in both malaria-naïve French volunteers and *P. falciparum* exposed non-pregnant women in Burkina Faso and PAMVAC tested in malaria-naïve German volunteers. Both formulations were safe and well-tolerated and immunogenic.	(Fried et al., 1998; Fried & Duffy, 2015; Mordmüller et al., 2019; Sirima et al., 2020)
Transmission blocking vaccines					
Pfs25	Subunit	Zygote surface proteins	Phase 1	Transmission blocking vaccines (TBVs) aim to incorporate surface antigens of the mosquito and sexual stages of the parasite (gametes and zygotes) to induce antibodies that kill parasites preventing parasites from successfully infecting a mosquito. Zygote surface proteins are expressed post-fertilization in the mosquito gut, two leading candidates are Pfs25 and Pfs28, with Pfs25 the first TBV candidate to be prepared as a recombinant protein and the leading candidate in clinical trials to date. Early iterations of recombinant Pfs25 vaccine candidates expressed antigens as monomers however, these showed poor immunogenicity in early clinical trials and several new approaches are now in development. The leading candidate is a Pichia pastoris produced Pfs25 (PpPfs25H-A) chemically conjugated to the mutant, non-toxic ExoProtein A (EPA) of Pseudomonas aeruginosa to generate nanoparticles. This was shown in 2016 to be well-tolerated, immunogenic and induced functional antibodies that blocked transmission in membrane feeding assays which correlated with antibody titres, but responses and activity waned	(Barr et al., 1991; Kapulu et al., 2015; Menon et al., 2018; Radtke et al., 2017; Talaat et al., 2016; MacDonald et al., 2016; Chichester et al., 2018; Jones et al., 2015)(de Graaf et al., 2021; Sagara et al., 2018)

				rapidly. Further viral vectored Pfs25 candidates have also been tested and while demonstrating good safety profiles and being immunogenic, significant transmission reducing activity has not yet been demonstrated and alternative vaccine formulations need to be studied. Functional immunogenicity and durability must be improved before advancing transmission-blocking vaccines further in clinical development.	
Pfs230	Subunit	Gamete surface proteins	Phase 1	Gamete surface proteins are expressed in the human host and classified as pre-fertilisation antigens. Pfs230 antibodies have been shown to lyse gametes in the presence of compliment. Pre-clinical studies found Pfs230 to induce high levels of functional antibodies in animal testing. Recombinant Pfs230 vaccine candidate (Pfs230 domain 1 (D1)-EPA conjugate vaccine) is currently being tested in Phase 1 clinical trials in the US and Mali. Early prior results from Pf230D1-EPA formulated in Alhydrogel showed Pfs230D1 to be significantly more potent than Pfs25-EPA as a vaccine, and the addition of Pfs25 to Pfs230D1 did not appear to improve on this activity.	(Read et al., 1994; Healy et al., 2021) ClinicalTrials.gov Identifier: NCT02942277; NCT03917654; NCT05135273; NCT02334462;
Pfs48/45	Subunit	Gamete surface proteins	Preclinical	Pfs48/45 is a binding partner for Pfs230 and is considerably smaller but has been challenging to prepare as a properly folded recombinant protein. Recently Pfs48/45 domain 3 has been expressed in Lactococcus lactis as a fusion with the R0 region of asexual stage Glutamate Rich Protein, resulting in a construct called R0.6 C. R0.6 C induced transmission blocking antibodies in animal studies and a subsequent fusion protein that incorporated the pro-domain of Pfs230 induced significantly higher serum functional activity than in preclinical studies suggesting a benefit of combining Pfs230 and Pfs48/45.	(Singh et al., 2017, 2019, 2021)

1.9.2 RTS,S/AS01 pre-erythrocytic malaria vaccine

RTS,S is a pre-erythrocytic subunit vaccine created by fusing a portion of the *P. falciparum* circumsporozoite protein (CSP) and the hepatitis B surface antigen and is delivered with a potent adjuvant system $AS01_E$ which was found to be more immunogenic than the previous AS02 adjuvant (Gordon et al., 1995; Leroux-Roels et al., 2014). The CSP is the dominant feature of the sporozoite surface; effectively forming a dense coat around the parasite making it a prime vaccine target (Nardin et al., 1982). CSP consists of an immunodominant central repeat region which is flanked by conserved motifs at the N-terminus and C-terminal (Nardin et al., 1982). As a pre-erythrocytic vaccine, the aim of RTS,S is to induce a CSP-specific antibody driven immune response to prevent inoculated sporozoites traversing the peripheral blood circulation and causing liver-stage infection. A single successful hepatocyte invasion is sufficient to initiate and maintain blood stage infection and so it was also hoped that vaccine induced CSP specific CD4+ T cells that could recognise and kill infected liver cells would act as an additional protective immune barrier.

Phase 3 RTS,S efficacy trials were completed in 11 locations in seven countries in SSA, representative of different transmission settings following over 30 years of development (RTSS Clinical Trials Partnership, 2015). Vaccine efficacy against clinical malaria for a 0-, 1-, and 2-month dosing schedule was found to be 35.2% (95% CI 30.5%–39.5%) in children aged 5–17 months during 32 months of follow up. In those given a fourth booster dose of vaccine 18 months after the third dose efficacy reached 43.9% (95% CI 39.7%–47.8%)(RTSS Clinical Trials Partnership, 2015). Efficacy estimates were lower in the younger age group, aged 6–12 weeks at first immunisation and waned across both age groups through the course of follow up. Many sites experienced too few cases to generate reliable estimates for efficacy against severe malaria as a result of the high levels of access to care in the trial (RTSS Clinical Trials Partnership, 2015). Extended follow-up studies of RTS,S have since been completed and demonstrated that efficacy in these cohorts wanes significantly over time and that over seven years follow up efficacy was around 4.4% (95% CI −17.0%–21.9%) (Olotu et al., 2016). In this setting significant negative efficacy was measured in the fifth year among children with higher-than-average exposure to malaria parasites (Olotu et al., 2016). Following Phase 3 trials, RTS,S was given a positive scientific opinion from the European Medicines Agency under Article 58 (European Medicines Agency, 2015). Starting in 2018, Ghana, Kenya and Malawi in partnership with the WHO's Malaria Vaccine Implementation Programme (MVIP) began implementation of a large-scale Phase 4 pilot implementation of RTS,S/AS01 (Adepoju, 2019).

Within these three countries, RTS,S has been delivered under an age-based schedule with doses delivered to children as part of routine childhood immunization services integrated with Expanded

Programme on Immunization (EPI) schedules (World Health Organization, 2021b). Ghana and Kenya provide the four doses at 6-, 7-, 9-, and 24-months of age and in Malawi the four doses were given at 5-, 6-, 7-, and 22-months of age, in an effort to administer the primary vaccination series as early as possible (World Health Organization, 2021b). The MVIP has reached over 900,000 children and administered over 2 million vaccine doses (World Health Organization, 2021b). Coverage data showed that 62% of age-eligible children had received at least three vaccine doses which resulted in reductions in hospitalisations with severe malaria of 29% (95% CI 8%–46%) and hospitalisations with malaria parasitaemia or antigenaemia of 21% (95% CI 7%–32%) (World Health Organization, 2021b). Demand and uptake of the malaria vaccine has been strong across all three countries despite the challenges brought about by the COVID-19 pandemic and vaccine uptake was shown to be equitable and had no negative effects on the uptake of other vaccines, malaria interventions (LLIN use) or health seeking behaviour (World Health Organization, 2021b). In addition, the MVIP found that introduction of RTS,S increases equity in access to malaria control interventions with more than two-thirds of children who did not sleep under an LLIN receiving a malaria vaccine, meaning vaccine introduction resulted in over 90% of children in the three countries having access to one or more preventative intervention (World Health Organization, 2021b).

The evidence generated from the ongoing MVIP combined with results from mathematical modelling studies, cost-effectiveness studies and expert opinion led the WHO to recommended widespread use of RTS,S in sub-Saharan Africa and in other regions with moderate to high *P. falciparum* malaria transmission (World Health Organization, 2021b). Subsequently international financing of vaccine doses for country implementation was secured when the Board of Gavi, the Vaccine Alliance, approved funding for the malaria vaccine programme (Gavi The Vaccine Alliance, 2021). The initial investment of US$ 155.7 million will support malaria vaccine introduction, procurement, and delivery for Gavi-eligible countries in sub-Saharan African initially between 2022-2025.

1.9.3 Next generation vaccines – progress and challenges

One of the challenges surrounding RTS,S has been the relatively modest efficacy and its decay to low levels within a few years necessitating a fourth dose of vaccine. The results of the MVIP highlight the importance of delivering RTS,S in combination with other control interventions and ensuring good levels of access to care due to this partial protection. The MVTR goal of a vaccine with 75% protective efficacy against clinical malaria and a vaccine that reduces transmission is a target that RTS,S currently falls short of, and as such the development of second-generation vaccines are needed.

As described in Table 1.1 studies are already underway to assess the next generation of vaccine platforms. Two pre-erythrocytic vaccine candidates R21/MatrixM and PfSPZ are approaching late-

stage clinical evaluation. Additional vaccine candidates targeting other malaria life-stages, the blood stage vaccine candidate Rh5 and potential transmission blocking vaccines utilising Pfs25 and Pfs230 for example are also progressing through clinical testing. In addition, new vaccine technologies along with the ongoing development of adjuvants and delivery platforms are also being explored for use in next-generation malaria vaccines.

For example, new technologies including mRNA vaccine platforms that have been so successful over the COVID-19 pandemic are being applied to malaria. BioNTech recently announced plans to initiate a mRNA malaria vaccine clinical trial by the end of 2022 (BioNTech, 2021). Further to this, researchers at the Walter Reed Army Institute recently evaluated a potential *P. falciparum* CSP mRNA vaccine candidate and found it to be well expressed in mammalian cells when delivered in an encapsulated lipid nanoparticle, immunogenic in mice and protective in homologous and heterologous transgenic rodent models (Mallory et al., 2021). These results make for a compelling candidate for further investigation. In addition to novel mRNA-based platforms other new technologies are also being directed towards malaria vaccine research. These include nanoparticle display platforms, whose technology allows for self-assembling proteins that are conformationally stable, that can incorporate multiple epitopes that can then stimulate both B and Tcell responses [234–238, ClinicalTrials.gov Identifier: NCT04296279]. Continued development of novel virus like particle delivery platforms are also being directed towards malaria vaccine research, for example the SpyCatcher and novel bacteriophage platforms have recently shown promising results in pre-clinical studies (Janitzek et al., 2016; Jelínková et al., 2021).

In addition to vaccine platform development, the continued search for new vaccine candidate antigens from across the parasite's lifecycle, that are ideally conserved, essential and susceptible to antibodies, are important for enhancing the potential breadth of current vaccine targets. Furthermore, vaccines that target multiple antigens either from the same or different life-cycle stages that could provide additive or synergistic benefits to efficacy are attractive next-generation vaccine targets. Multi-component/multi-stage/multi-antigen formulations of malaria vaccines are in early stages of development with several promising approaches combining both pre-erythrocytic and transmission blocking antigens and pre-erythrocytic and blood-stage antigens in early pre-clinical studies (Yusuf et al., 2019; Collins et al., 2021; Hill et al., 2014).

While these technologies and second-generation vaccine candidates remain in the early stages of clinical development, novel approaches to RTS,S vaccination are also being evaluated that aim to improve upon the current efficacy of first-generation RTS,S. These changes have included alterations to the dosing schedule and dose amount (Regules et al., 2016) and an alteration to the timing of

vaccination (Chandramohan et al., 2021). These two novel approaches to RTS,S delivery are the focus of this Thesis and are discussed further in Chapter 2, 4 and 5.

The licensure of RTS,S as a first-generation vaccine also raises several challenges for the testing and evaluation of second-generation vaccines. Various options will remain for trial design dependent on factors including whether RTS,S is adopted as national policy and in use in counties where trials are planned which raises ethical issues for performing placebo-controlled trials (Fowkes, Simpson & Beeson, 2013; Vannice et al., 2012; World Health Organization, 2015d). Without regulatory accepted correlates of vaccine-induced protection second-generation vaccines will need to demonstrate safety and efficacy through randomised controlled trials with clinical endpoints. Whether superiority, non-inferiority or equivalence trials are selected for evaluation will depend on the type of vaccine being evaluated. A superiority trial would be used to test whether the second-generation vaccine was more efficacious than first-generation vaccine as defined by a pre-specified margin (World Health Organization, 2015d). A non-inferiority trial has the objective of understanding if a second-generation vaccine is not "unacceptably worse" than the currently licenced vaccine within a pre-specified margin (World Health Organization, 2015d) and an equivalence trial aims to understand if the second-generation approach is clinically equivalent in efficacy (Fowkes, Simpson & Beeson, 2013). Properly designed non-inferiority trials are considered justified in the context of development of new products which may bring advantages such as reduced cost, fewer doses, a simpler schedule, ease of administration, delivery and storage or an improved safety and tolerability profile (Fowkes, Simpson & Beeson, 2013). The determination of the non-inferiority margin will have to be carefully justified, taking into account scientific, clinical and public health opinion and needs and choice of the margin will have implications for the sample size required with narrower margins requiring larger sample sizes (Fowkes, Simpson & Beeson, 2013). If second-generation vaccines target different life-stages to the currently licenced vaccine this will also require careful planning of trial design. In this situation the vaccines may be delivered concurrently and could be compared with either first-generation or first-generation and placebo. Table 1.2 highlights some of the important implications for field trial designs considering second-generation vaccines and their comparisons.

Table 1.2 Considerations of different trial designs and comparisons for second-generation malaria vaccines. Modified from (World Health Organization, 2015d)

Field efficacy options	**2nd generation vs placebo**	**2nd generation vs 1st generation**	**1st & 2nd generation vs 1st generation**	**1st & 2nd generation vs 1st generation vs placebo**
Efficacy estimate	Absolute efficacy estimated	Relative efficacy estimated	Relative efficacy estimated	Absolute and relative efficacy estimated
Trial type	Superiority to no vaccine	Non-inferiority to 1st generation or superiority to 1st generation	Superiority to 1st generation	Superiority to 1st generation and no vaccine
Limitations and considerations	May be considered unethical to randomize to placebo, if 1st generation vaccine is available and recommended in country.	Large sample sizes may be needed. Non-inferiority design would not clearly show progress towards 75% efficacy goal, but could make alternative vaccines available.	Large sample sizes may be needed. 1st and 2nd generation vaccines could be given together or as prime-boost.	Large sample sizes may be needed (may not be feasible). May be considered unethical to randomise to placebo is 1st generation is available and recommended in country.
	Efficacy relative to 1st generation would not be estimated with confidence.	Efficacy relative to no vaccine would not be estimated with confidence.	This design would not demonstrate efficacy of the 2nd generation vaccine independent of the 1st generation vaccine. Efficacy relative to no vaccine would not be estimated with confidence.	This design would not demonstrate efficacy of the 2nd generation vaccine independent of the 1st generation vaccine.

Malaria vaccine candidates are progressing in clinical trials and with RTS,S advanced to implementation, the question remains of how best we can deploy malaria vaccines to the advantage of those populations at risk. Continued antigen discovery, improved vaccine platforms and an expanding portfolio of vaccine candidates highlights the potential success on the horizon. As more candidates transition to licensure optimal deployment strategies that maximise vaccine impact in malaria endemic countries will be essential. One aspect that can help guide our understanding of optimal strategies is the use of mathematical modelling which I discuss in the following sections.

1.10 Mathematical modelling of malaria transmission

Mathematical models that describe the dynamics of infectious diseases are tools which can help provide insights into the epidemiology of infection and disease as well as insights into host immunity and the parasite lifecycle and can assist in the design and evaluation of different intervention programmes. Transmission models have formed a vital component of malaria research.

The first model of malaria transmission was developed by Ronald Ross in the early 1900s following his discovery that mosquitos transmit malaria parasites and while working on malaria control in Mauritius (Ross, 1908, 1910, 1911). This simple model which explained the relationship between the number of mosquitos in a location and the incidence of malaria in humans was used to show that the reduction of mosquitos below a certain threshold would be sufficient for the malaria parasite to die out. The model formulation described a value known as the critical threshold (m') the mosquito density in an area below which transmission could not be sustained. Ross' insights have been standardised into the following formula (Smith et al., 2012a):

$$m' > \frac{gr}{a^2 bce^{-gv}} \tag{1.1}$$

where m is the ratio of mosquitos to humans, g the instantaneous death rate of a mosquito, r the daily rate each human recovers from infection, a the rate at which a mosquito takes a blood meal, b the probability that a bite by an infectious mosquito infects a human, c the probability a mosquito becomes infected after biting an infected human, and v the number of days from infection to infectiousness in the mosquito (Smith et al., 2012a). Ross extended his original model, which was built in discrete time, to take a continuous time form using a pair of differential equations to describe how the number of infected humans X and mosquitoes Z change over time:

$$\frac{dX}{dt} = ma\frac{Z}{M}(H - X) - rX \tag{1.2}$$

$$\frac{dZ}{dt} = ac\frac{X}{H}(M - Z) - gZ \tag{1.3}$$

where parameters are described as above and where H and M are the total number of humans and mosquitoes in the population. Ross's model drove the first few decades of malaria control when interventions focussed on destroying larval breeding sites.

Following the initiation of the GMEP, George Macdonald built on Ross's model, testing his critical threshold theory with data from epidemiological and entomological field trials (Macdonald, 1957).

The initial Ross model did not consider the delay from infection to infectiousness in the mosquito. MacDonald incorporated this latency period and introduced the concept of an exposed class to the mosquito component of the model. MacDonald also incorporated other biological processes such as the concept of superinfection (the potential for individuals who are already infected to be reinfected) (Macdonald, 1950, 1952b, 1952a)

A schematic of the Ross-MacDonald model is shown in Figure 1.5, where each compartment represents the proportion of humans and mosquitos at equilibrium that are either infected (I) or susceptible (S) or for mosquitoes only exposed (E) i.e infected but not yet infectious. The parameters describing the rate of flow between compartments are as described above. This model captures key aspects of the transmission cycle and can be used to estimate important metrics such as the EIR, force of infection, parasite prevalence rate and the basic reproduction number. MacDonald's work helped to explain how insecticides reduce malaria transmission through reducing the number of mosquitos that live long enough to allow the parasite to complete its lifecycle within the mosquito host (Macdonald, 1956). These insights helped to demonstrate the efficacy of using the insecticide DDT as a malaria control strategy for the GMEP.

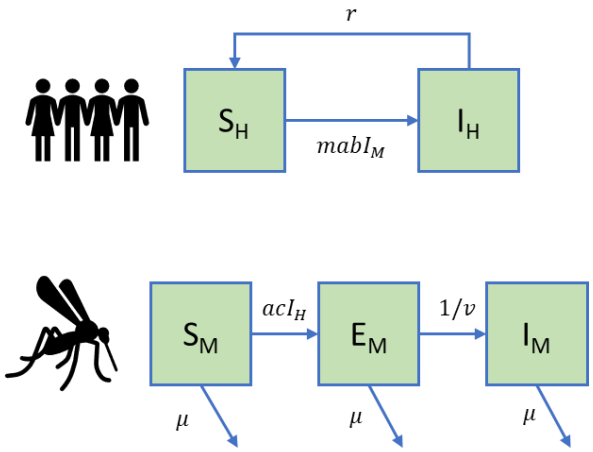

Figure 1.5 Schematic representation of the Ross-MacDonald malaria transmission model, parameters, and rates between compartments.

Following the GMEP the WHO launched the Garki Project in the 1970s to evaluate whether malaria could be controlled in a high transmission intensity setting in sub-Saharan Africa (Molineaux, Gramiccia & Organization, 1980). In planning and analysing this project, extensions to the Ross-Macdonald model of malaria were developed that incorporated processes of human immunity and specific malaria control interventions (Dietz, Molineaux & Thomas, 1974). The model was validated

against the data from the project and was able to replicate the observed trends in age-specific patterns of malaria and provided important insights into the project outcomes. As well as providing important insights into the successes and failures of the project, the model developed helped to identify key indices of malaria transmission, including the vectorial capacity and the human blood index.

Since the 1900s advances in our understanding of mosquito behaviour, human immunity and parasite transmission coupled with advances in mathematical and statistical methods and computational power has driven the evolution of the early Ross-Macdonald model. Extensions to the early models have incorporated latent periods of infection in mosquitos and humans (Anderson & May, 1992), age-related differences in susceptibility to and the development of immunity to malaria (Anderson & May, 1992; Aron & May, 1982; Dietz, Wernsdorfer & McGregor, 1988; Aron, 1988; Filipe et al., 2007), spatial heterogeneity of hosts and parasites (Hasibeder & Dye, 1988; Gupta & Hill, 1995; Gupta, Swinton & Anderson, 1994; RodrÍguez & Torres-Sorando, 2001; Torres-Sorando & Rodríguez, 1997), behavioural differences in vector feeding (Dye & Hasibeder, 1986) and individual based model structures (Gu et al., 2003). As a result of progressive improvements to models alongside the advancements in modern computing. Current models are now capable of assessing the impact of a variety of current and potential future malaria control interventions.

For example, mathematical modelling was a vital component of the creation of the WHO Global Technical Strategy for Malaria 2016–2030 helping to assess the long-term impacts of interventions on malaria (World Health Organization, 2015b, 2021c). Modelling found that if the coverage of malaria interventions remains at current levels, incidence could increase moderately. However, this rise, and its consequences could be averted through a concerted effort to optimize the use of currently available interventions at levels above 80% coverage of at-risk populations and by improving the quality of services. Furthermore, a number of WHO guidelines and policy recommendations for malaria interventions have incorporated findings from mathematical modelling studies or been supported by modelling studies. For example, increasing treatment coverage with ACTs (Okell et al., 2008a; Johnston et al., 2014; Penny et al., 2015b), the introduction of SMC and other IPT programmes (Cairns et al., 2012; Walker et al., 2017), ITNs and IRS (Le Menach et al., 2007; Smith et al., 2009; Chitnis et al., 2010; Okumu & Moore, 2011; White et al., 2011a), larval control methods (Killeen, Fillinger & Knols, 2002; Eckhoff, 2011), MDA for malaria elimination (Brady et al., 2017) and now RTS,S vaccination (Penny et al., 2016; World Health Organization, 2021b). Additionally, throughout the COVID-19 pandemic mathematical models have been important to understanding the potential impact of the pandemic on malaria intervention coverage, morbidity, and mortality (Hogan et al., 2020; Sherrard-Smith et al., 2020; Weiss et al., 2021).

The WHO relies on several contemporary models of malaria transmission and interventions to aid in our understanding of different aspects of malaria control programmes. Models of malaria transmission developed by teams at Imperial College London (Griffin et al., 2010), the Swiss Tropical and Public Health Institute (Swiss TPH) (Smith et al., 2006a) and the Institute for Disease Modelling (Eckhoff, 2013), have been instrumental to understanding the potential impacts of malaria vaccine candidates in particular. Critically these three dynamic individual based models were employed in a consensus modelling exercise aligned with WHO processes to support WHO guidance on RTS,S deployment prior to the MVIP programme (Penny et al., 2016). These models have continued to be used to address questions about future roles of RTS,S during the current phase of pilot deployment. I briefly describe the OpenMalaria model used by researchers at Swiss TPH and the EMOD DTK model used by researchers at the Institute for Disease Modelling and the model used by researchers at Imperial College London. As I will be using the Imperial College London model in three of my Thesis Chapters I outline this model in detail in Appendix 8.1. I then briefly discuss the contributions of these models to malaria vaccine research and their impact on policy.

1.10.1 Swiss TPH OpenMalaria model

OpenMalaria is a stochastic individual-based, single location simulation model of malaria in humans linked to a deterministic model of malaria in mosquitos (Smith et al., 2006a, 2012b; Chitnis, Hardy & Smith, 2012). The model includes sub-models of infection in humans (Smith et al., 2006b), blood-stage parasite densities (Maire et al., 2006a), infectiousness to mosquitos as a lagged function of asexual parasite density (Ross, Killeen & Smith, 2006), and incidence of morbidity and mortality with the model including all severe malaria and hospitalisations with malaria (Ross et al., 2006). Immunity is comprised of separate sub-models of pre-erythrocytic and blood-stage immunity, with blood-stage immunity predominating as infection-blocking immunity results from very high cumulative malaria exposure within the model structure (Maire et al., 2006a). OpenMalaria is formulated as an ensemble of 14 model variants with varying assumptions in the decay of natural immunity, within host variability between infection and entomological exposure, heterogeneity in transmission and heterogeneity in susceptibility to co-morbidities (Smith et al., 2012b). The model has been parameterised using age-incidence clinical malaria data from sites in Senegal and Tanzania. The severe disease and mortality model has been fitted to all-cause and cause-specific age-specific mortality from the pre-LLIN and pre-ACT era, to hospitalisation rates by prevalence for multiple sites, and to age incidence of hospitalized severe malaria (with age-specific case fatality rate based on Tanzanian data). The model incorporates the current suite of malaria interventions including pre-erythrocytic, blood-stage and transmission blocking vaccines, LLINs, IRS, larviciding, repellents and screening, zooprophylaxis, odour-baited traps, sugar-baited traps, treatment and case-management of clinical and severe disease by specified drug,

facility level and diagnostic, mass screen and treat, mass drug administration, IPTi/IPTc and SMC and the model allows for drug resistance.

1.10.2 Institute for Disease Modelling EMOD DTK model

EMOD DTK is a discrete, stochastic, individual-based model of malaria in either local or spatially distributed settings. It includes a comprehensive model of the vector life cycle coupled to a detailed mechanistic representation of intra-host parasite and immune dynamics (Eckhoff, 2011, 2012a). The individual infection and immunity modules track the distribution of parasites by surface-antigen type with both innate and antigen-specific adapted immune responses, while human infectiousness is calculated directly from the mechanistic dynamics of parasite densities (Eckhoff, 2012a). Blood-stage immunity is acquired through cumulative exposure to different parasite variants in terms of their expressed variant surface antigens (Eckhoff, 2012b). Heterogeneity in individual biting rates is included in the model structure. The model relationships between transmission intensity, parasite prevalence, clinical episodes, and severe disease were calibrated to historical study-site data from several sites in Nigeria, Tanzania, Senegal and The Gambia (McCarthy et al., 2015). The model accounts for the combined effect of an extensive set of both vector- and human-directed interventions including vaccines, LLIN, IRS, larviciding and other novel vector control including: ivermectin, genetically modified mosquitoes, individual and spatial repellents, oviposition traps and sugar-baited traps.

1.10.3 Imperial College London malaria transmission model

An individual-based transmission model has been developed and validated by researchers at Imperial College London. The model has previously been fitted to data on the relationship between vector density, EIR, parasite prevalence, uncomplicated malaria, severe disease and death from data across SSA (Griffin et al., 2010, 2015, 2016; White et al., 2011a). The model links a single population of humans to a stochastic compartmental mosquito model. The model tracks individuals through the stages of infection with pre-erythrocytic immunity and blood-stage immunity incorporated that capture the changing patterns of clinical disease, severe disease and asymptomatic infection with age and exposure (Griffin et al., 2015). The vector model incorporates larval and adult stages to capture the feedback of vector control on population dynamics (White et al., 2011a). Multiple vector species and heterogeneity in exposure are incorporated. The model captures the combined effects of multiple interventions including vaccines, LLIN, IRS, mass screen and treat, IPTi, SMC, IPTp, larval control, and novel interventions including ivermectin and sugar-baited traps. Treatment of clinical and severe disease is specified by drug type and diagnostic.

1.10.4 Contribution of mathematical models to malaria vaccine research and policy

Mathematical models of malaria transmission have been used to evaluate the potential impacts of stage-specific malaria vaccines on transmission dynamics since the late 1980s following the incorporation of immunity dynamics into model structure following the Garki project (Struchiner, Halloran & Spielman, 1989; Halloran, Struchiner & Spielman, 1989; May & Gupta, 1989; Koella, 1991; Halloran & Struchiner, 1992).

Following the demonstration of potential efficacy from RTS,S and prior to large-scale field trials, the three contemporary mathematical models described above have all been used to evaluate the potential impacts of pre-erythrocytic vaccination with hypothetical efficacy profiles (Maire et al., 2006b; Penny et al., 2008; Griffin et al., 2010; Smith et al., 2012b; Brooks et al., 2012; Wenger & Eckhoff, 2013). These studies helped to describe the relative importance of vaccine properties namely the initial efficacy and its decay over time on potential burden reductions and how these two characteristics can help to achieve different operational targets namely reductions in morbidity and mortality or the interruption of transmission. Furthermore, these studies demonstrated the impact of transmission intensity on the potential burden reductions with pre-erythrocytic vaccination and highlighted the need for additional preventative interventions to counteract the potential for vaccines to reduce population-level immunity leading to increases or rebounding in clinical disease and death at older ages.

In addition to modelling the population level impacts of malaria vaccines, within host models of malaria infection have been developed and used to characterise and predict vaccine impact at an individual level as a function of the vaccine induced immune response. *White et al.* used established pharmacological dose-response methods and fitted dose-response curves to immunological data from an early RTS,S human challenge study (White et al., 2013). Analysis of the challenge data enabled the characterisation of the relationship between antibodies and CD4+ T cell responses and vaccine induced protection from infection, showing that anti-CSP antibodies and CSP-specific CD4+ T cells were immunological surrogates of protection, with antibodies playing a dominant role in protection. Characterisation of this relationship enabled models to be fitted to predict vaccine efficacy for different levels of response titres, an important step in understanding the potential efficacy achievable if immunogenicity of RTS,S could be improved.

Following the completion of Phase 3 RTS,S clinical trials modelling groups used data from the trial, in combination with their transmission models to estimate an efficacy profile for RTS,S. *White et al.* (2015) used the Imperial College London malaria transmission model to estimate the efficacy profile of RTS,S against malaria infection and clinical malaria using individual-level data on the incidence of

clinical malaria from the 11 trial sites of the Phase 3 trial along with antibody titre data from these individuals over the course of the follow up (according-to-protocol population) (White et al., 2015). Firstly, the kinetics of RTS,S-induced anti-CSP antibodies were described using a bi-phasic model of exponential decay. The decay in antibody titres was then related to efficacy against infection using a Hill function that captures the dose-response relationship between antibody titre and vaccine efficacy against infection over time. Vaccine efficacy against infection was then related to efficacy against clinical malaria using their model of age- and exposure-dependent acquisition of clinical immunity (Griffin et al., 2015). For the childhood cohort (5–17 months) the initial efficacy against infection estimated from this model following the third dose was 74%, waning to 28% by 12 months (White et al., 2015). This analysis was important for demonstrating the role of anti-CSP antibody titres to act as surrogate markers for protection against infection from malaria following RTS,S vaccination corroborating the earlier dose-response within host modelling work by *White et al* (White et al., 2013).

Penny et al. used the OpenMalaria model to estimate the efficacy profiles of RTS,S against malaria infection and clinical malaria using pooled three-monthly incidence data from the intention-to-treat population of the Phase 3 trial (Penny, Pemberton-Ross & Smith, 2015). Each arm of the trial in each study site was explicitly simulated using an ensemble of six models assuming many different hypothetical profiles for vaccine efficacy against infection. The efficacy profile of RTS,S was described by a Weibull decay function and was determined through Bayesian model fitting comparing the resulting model predictions of clinical incidence over time to those reported in the trial. For the childhood cohort (5–17 months) the initial efficacy against infection estimated from this model following the third dose was 91% with an estimated half-life of seven months (Penny, Pemberton-Ross & Smith, 2015). The EMOD DTK model also used the pooled three-monthly incidence data from the intention-to-treat population of the Phase 3 trial to estimate RTS,S efficacy over time (Penny et al., 2016). Again, each arm of the trial in each study site was explicitly simulated and vaccine efficacy described by exponential curve parameterized by an initial efficacy and half-life of protection. Simulations were run over a range of parameter values and Poisson regression was performed to compute the likelihood for the relationship between simulated case counts and trial data for each vaccine parameter set and the best fitting set chosen by selecting the vaccine properties that yielded the highest likelihood. For the childhood cohort (5–17 months) the initial efficacy against infection estimated from this model following the third dose was 80% with an estimated half-life of 13.5 months (Penny et al., 2016).

Despite the differences in fitting approaches and transmission model structures, all three modelling groups estimated a high initial efficacy post dose three in the 5–17-month-old cohort, with similar rates of waning protection in the first 12 months, which then diverged with EMOD DTK and

OpenMalaria suggesting a more rapid decay than the model from Imperial (Penny et al., 2016). Following the fourth dose both Imperial and EMOD DTK estimated a higher initial efficacy and slower decline than the OpenMalaria model (Penny et al., 2016). Discrepancies in model estimates of RTS,S protection reflect differences in the model structures and differences in the parametric assumptions of efficacy.

Once RTS,S efficacy profiles had been fitted, the three modelling groups were part of a consensus modelling exercise co-ordinated by the WHO to make more precise predictions on the potential long-term public health impact of RTS,S (Penny et al., 2016). Results confirmed the results from earlier modelling studies that RTS,S could have considerable public health impact when delivered at scale. The results of this consensus modelling were used to support guidance on the deployment of RTS,S and the need for the large-scale pilot implementation (MVIP) (World Health Organization, 2016). The consensus modelling highlighted two important considerations that were not apparent from the results of the Phase 3 trial. The first is that modelling predicted an overall positive impact of RTS,S on severe disease and mortality due to the incorporation of realistic levels of access to care in the modelling exercise. This differed from the Phase 3 trial results in that very high levels of access to treatment meant there was significantly low levels of mortality in all trial participants. The second is that there was a lack of consensus between the modelling groups on the impact of the fourth dose of RTS,S with the OpenMalaria model predicting only marginal benefit of a fourth dose in terms of reducing severe malaria outcomes as a result of parameterisation of the protection following this fourth dose. The ongoing MVIP was therefore powered to understand the impact of RTS,S on both severe malaria outcomes and mortality, and the feasibility of delivering and the impact of the fourth RTS,S dose on these outcomes.

Further to the consensus modelling exercise, modelling groups have expanded predictions of the public health impacts of RTS,S into country specific predictions of impact (Penny et al., 2015a) and estimates of the prioritisation of vaccine allocation among countries or administrative units to maximise cases or deaths averted (Hogan, Winskill & Ghani, 2020). Modelling has also been used to assess the potential cost-effectiveness of RTS,S vaccination in both generic transmission settings and for specific countries (Penny et al., 2016; Winskill et al., 2017; Sauboin et al., 2019a; Galactionova et al., 2017). Furthermore, modelling has also considered the impact of RTS,S not only as a morbidity reduction intervention but as a potential tool in low-prevalence settings to aid MDA in transmission interruption (Camponovo et al., 2019). Models of the duration of RTS,S protection over time have also been applied as potential transmission blocking vaccine efficacy profiles to understand the impact a vaccine with RTS,S like peak protection and duration could have on malaria (Challenger et al., 2021).

The use of modelling in the assessment of RTS,S has provided many insights for vaccine developers in terms of the relationship of immune responses and vaccine protection over time, and policy makers and countries in understanding the potential for RTS,S to form a part of intervention packages moving forwards. These modelling platforms are now well placed to tackle the next generation of RTS,S and other vaccine candidates moving through development.

1.11 Thesis aims and structure

The aim of this thesis is to use mathematical models to provide insights into key problems in the field of second-generation malaria vaccine development and deployment, ranging from immunological correlates of protection to exploration of changes in efficacy profiles under novel schedules and optimal immunisation strategies for maximal public health impact in setting of seasonal malaria transmission. Throughout this thesis I have maintained a focus on questions relevant to malaria policy. Specific objectives for each proposed thesis chapter are as follows:

Chapter 2: I use a Bayesian framework to investigate the relationship between RTS,S induced antibody responses and protection from malaria infection in malaria-naïve volunteers in a human challenge study.

Chapter 3: I use a Bayesian survival analysis framework to fit an updated efficacy profile of Sulfadoxine-Pyrimethamine plus Amodiaquine (SP+AQ) the drug combination currently used for Seasonal Malaria Chemoprevention (SMC) using clinical trial data.

Chapter 4: I use the efficacy model of SP+AQ fitted in Chapter 3 and an existing model of first-generation RTS,S protection to replicate the first large-scale field trial of an alternative seasonal RTS,S vaccination approach. In this I aim to validate the efficacy model parameterisation of RTS,S under this new delivery approach in this trial.

Chapter 5: Taking these updated efficacy models forwards from the fitting and validation exercises in Chapter 3 and 4 I use a transmission model to investigate the long-term public health impact of this alternative seasonal RTS,S vaccination schedule compared to first-generation RTS,S in seasonal settings in the presence and absence of SMC. I examine impact by degree of seasonality, transmission intensity and by wider health system and operational factors.

Chapter 6: I summarise and discuss the implications of my findings in light of other literature, as well as outline the main limitations and provide suggestions for future work.

Chapter 2

2 Characterising second-generation immunological markers of vaccine-induced protection

In this chapter I investigate the potential for antibody avidity measurements to be used as markers of RTS,S/AS01$_E$ (RTS,S) vaccine-induced protection. I extend a previously published individual-based mathematical model of sporozoite inoculation to characterise the relationship between the quantity, measured as the antibody titre and the quality, measured as an avidity index of anti-circumsporozoite protein (CSP) antibodies and their relationship with protection from malaria infection in malaria-naïve adults in human challenge studies.

Published as: Thompson HA, Hogan AB, Walker PG, White MT, Cunnington AJ, Ockenhouse CF, Ghani AC. *Modelling the roles of antibody titre and avidity in protection from Plasmodium falciparum malaria infection following RTS, S/AS01 vaccination*. Vaccine. 2020 Nov 3;38(47):7498-507.

2.1 Background

The ongoing RTS,S Malaria Vaccine Implementation Programme (MVIP) in Ghana, Keyna, and Malawi has so far provided valuable information on the impact and safety of RTS,S, as well as the feasibility of delivering four doses of RTS,S through national programmes. The pilot programme also highlights the scale required for RTS,S studies to be able to fully understand the public health impact of the vaccine. Testing second-generation vaccines against RTS,S in comparative field trials will also require large sample sizes, extensive resources, and time commitments, and may also raise ethical and licensing dilemmas (Fowkes, Simpson & Beeson, 2013).

Some second-generation vaccines for other childhood infections have bypassed these requirements as their licensure was based on an acceptable immunological output that conferred clinical efficacy

(Andrews, Borrow & Miller, 2003; World Health Organization Expert Committee on Biological Standardization, 2009; Van Els et al., 2014). However, this approach is currently not possible for comparing malaria vaccine candidates as we still do not fully understand the definitive mode of action by which the current RTS,S vaccine confers protection and no threshold for a protective immune marker has been established (Greenwood, 2011). Establishing immunological makers of protection that can predict individually or within the population whether a vaccine candidate will protect against natural infection will significantly accelerate the evaluation and downstream selection of new vaccines. Antibody titres to the repeat region of the circumsporozoite protein (NANP) have been established as the leading major correlate of protection, with higher titres shown to be associated with protection against infection and the rate of waning of antibody responses following vaccination associated with the magnitude and duration of efficacy over time (White et al., 2013, 2014, 2015).

In addition to the concentration of antibodies, many other properties govern antibody function and may be important determinants of protective capacity against malaria sporozoites. One of these aspects is the quality of the antibody response. High quality antibodies are determined by the measurements of affinity and avidity, and the production of high affinity antibodies is a sign of successful priming by antigens or vaccines that have led to the development of antigen-specific B cell clones (Good-Jacobson & Shlomchik, 2010). These B cells have undergone affinity maturation through a process of somatic hypermutation of their VDJ genes to produce high affinity antibodies specific to the parasite antigen (De Silva & Klein, 2015). Affinity maturation results in mutated antibody variants with improved antigen-binding properties to better protect from invading pathogens (Imkeller et al., 2018). Antibody avidity, measured using inhibition enzyme linked immunosorbent assays (ELISAs), is a representation of the overall strength of interaction between antibodies and antigens in a complex, and it takes into account the intrinsic affinity of antibodies to their specific epitopes and also valences of antibodies and any structural features of antibody binding confirmations (Goldblatt, Vaz & Miller, 1998; Klasse, 2016; De Souza et al., 2004). High avidity antibodies have been shown to be important in the protection conferred by several viral and bacterial vaccines (Vermont et al., 2002; Alam et al., 2013; Siegrist et al., 2004; Schlesinger & Granoff, 1992; Bachmann et al., 1997) and *Haemophilus influenzae* Type b vaccine failure has been shown to occur when individuals do not reach a threshold level of antibody avidity (Yeh et al., 2008). However, studies of antibody avidity responses following RTS,S vaccination during field trials have provided conflicting evidence on the relationship between avidity and protection from infection (Olotu et al., 2014; Reed et al., 1996; Ajua et al., 2015; Dobaño et al., 2019). Given the variation in protection that remains unexplained by anti-CSP antibody titre alone, the quality of these antibodies has continued to be theorised to contribute to vaccine efficacy

and may act as a marker for successful vaccine efficacy in combination with antibody titre (Kazmin et al., 2017; Chaudhury et al., 2016).

Controlled human malaria infection challenge studies offer a unique opportunity to study immune mechanisms and correlates of protection during early Phase 1 and Phase 2 clinical trials (Figure 2.1) (World Health Organization Expert Comittee on Biological Standardization, 2016; Sauerwein, Roestenberg & Moorthy, 2011; Spring, Polhemus & Ockenhouse, 2014). In addition to providing efficacy estimates, challenge trials are carried out in a controlled environment in malaria naïve volunteers, allowing detailed immunological markers to be taken. This enables us to identify potential immune correlates and mechanisms of protection and establish if there are any observable dose-response relationships between these and protection from infection. As more second-generation malaria vaccines are developed, challenge studies will be vital in helping to compare candidates and provide essential data for predicting their potential field impact.

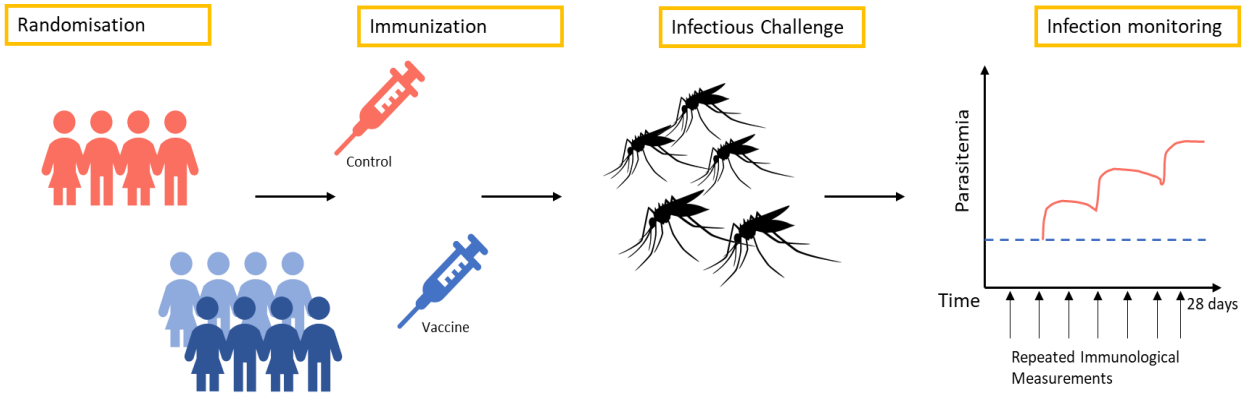

Figure 2.1 Malaria Human Challenge Study Protocol. Standardized methodology for pre-erythrocytic vaccine testing involves the randomisation of volunteers into vaccine recipient and control groups, following immunizations volunteers are exposed to the bites of 5 infectious mosquitos and microscopy and PCR monitoring of disease progression for 28 days post exposure enables rapid diagnosis and disease progression characterisation. During this time immunological measurements can also be taken at specified timepoints

A Phase 2a challenge study of a second-generation RTS,S vaccine has demonstrated an increase in initial vaccine efficacy following a delayed-fractional immunisation regime, whereby the third dose of the primary RTS,S schedule was given with a five-month delay and at one-fifth of the standard dose compared to the standard full dose monthly schedule (Regules et al., 2016). Efficacy against infection was 86.7% (95% CI 66.8–94.6%) for those volunteers in the delayed-fractional arm at first challenge compared to 62.5% (95% CI 29.4–80.1%) for volunteers on the standard 0-, 1-, 2-month full dose regime (Regules et al., 2016). The immunological reasons for the difference in efficacy are not fully understood, and the increase in efficacy did not correlate with increases in antibody titre. Instead, it

was postulated that the improvement in efficacy resulted from an increase in affinity maturation resulting in a higher quality of antibody response following the delayed-fractional schedule (Regules et al., 2016). This trial provides us with key data to further understand the relationship that antibody quantity and quality might have with protective efficacy following RTS,S vaccination.

2.2 Aims

In this chapter I reanalyse the immunological data from this challenge trial using a biologically motivated mathematical model of *P. falciparum* infection. Using this approach, I quantify the potential amount of protection that is derived from each aspect of the antibody response through fitting dose-response curves that relate the magnitude of each immune measurement to sporozoite killing. I aim to relate these probabilities to vaccine efficacy providing insights into the likely contribution of antibody characteristics in driving RTS,S efficacy. Using this approach I am able to combine data on the relative risk of infection and delays in the time to onset of detectable parasitaemia in those infected volunteers into measures of vaccine efficacy.

2.3 Methods

2.3.1 Challenge study data

Data was obtained from the Phase 2a RTS,S/AS01B fractional third and fourth dose challenge study, full trial methodology can be found in the corresponding publication (Regules et al., 2016). Briefly, 46 malaria naïve adults received full vaccination schedules, 30 received the updated delayed-fractional regime whereby the third dose was delayed by five months and given at one-fifth the standard dose and 16 volunteers received the standard 0,1,2-month regime of full dose vaccine. Three weeks following the third dose, volunteers underwent mosquito challenge through the bite of five *P. falciparum* (3D7, a clone of the NF54 strain) infected *Anopheles stephensi* mosquitos. Daily blood slide reading and PCR was performed to monitor parasitaemia levels from days five through 20 and then on every other day through day 28 post challenge. At the onset of detectable parasitaemia volunteers were treated with antimalarials. From the 46 volunteers who underwent vaccination and challenge infection status and time to onset of parasitaemia were recorded.

Immunogenicity assessments are outlined in detail in the Supplementary Information of Regules et al (Regules et al., 2016). Briefly, IgG antibody levels against the NANP repeat region, C-terminal pf16 and full length recombinant CSP were measured using standard ELISAs (Clement et al., 2012). Plate absorbed R32LR antigen was used to measure antibodies against the repeat region with total IgG titre reported in ELISA Units. ELISA-based avidity assays were conducted to assess antibody binding to the repeat region NANP. These findings were reported as an avidity index calculated by dividing the serum titre in the presence of 4M urea (the chaotropic reagent) to the serum titre obtained without exposure

to the chaotropic agent. For the following analysis I used measurements of end-point total IgG titre and avidity indices against the immunodominant NANP repeat region taken from a single time point from each volunteer on the day of mosquito challenge.

2.3.2 Statistical methods

Immunological data was reanalysed to assess for any statistical differences in immune measurements between the two arms of the trial. I used non-parametric Mann-Whitney U tests to compare antibody titres and avidities from all volunteers from each arm of the trial, due to the non-normality in data distributions. I calculated Spearman's rho to test for any correlation between the immune measurements from each individual and a log rank test was performed to test for significant difference in time to onset of parasitaemia between vaccination schedules.

2.3.3 *P.* falciparum infection model

The *P. falciparum* infection model was developed and previously described in *White et al.,* (White et al., 2013). The model itself is an individual based mathematical model that captures the following parasite dynamics following challenge: sporozoites inoculated into the skin then migrate to the liver where they undergo intrahepatic development and merozoites are released into the blood stream. Merozoite progeny replicate in the blood until they become detectable by microscopy and treatment begins (Figure 2.2). The above biological processes are described by the models presented below.

2.3.3.1 Sporozoite inoculation

Following challenge with five mosquito bites there is a high level of variation in the number of sporozoites each mosquito deposits (Beier et al., 1991; Churcher et al., 2017; Vanderberg, 1977), which can be described by a Negative Binomial distribution. Therefore, the number of sporozoites that go on to complete intrahepatic development can be characterised by a Negative Binomial distribution with mean (n) and standard deviation (σ_n). The probability that after challenge a given number of sporozoites k will successfully initiate blood stage infection is given by:

$$S_k = \binom{k+r-1}{k} \frac{r^r n^k}{(n+r)^{r+k}} \tag{2.1}$$

Where r is a shape parameter of the Negative Binomial distribution $r = \frac{n^2}{\sigma_{n-n}^2}$ and $k = [1, \infty]$.

2.3.3.2 Merozoite progeny

To initiate blood stage infection the sporozoites that survive intrahepatic development will release merozoite progeny into the blood stream, which occurs around $T_L = 6.5$ days after challenge (Murphy et al., 1989). The number of merozoites released is assumed to be Gamma distributed with a mean $(\mu = 30{,}000)$ (Coffeng et al., 2017) and standard deviation (σ_μ). Once in the blood merozoites will

replicate at a given rate ($m = 3.8/day$) (Bejon et al., 2005) until they reach the threshold number for detection of parasitaemia in the blood by microscopy ($P_T = 50{,}000{,}000\ parasites/mL$) and treatment is initiated (Bejon et al., 2006). If blood-stage infection is initiated with Q merozoites, then the number of parasites (P) at time t is

$$P(t) = \begin{cases} 0, & t < T_L \\ Qm^{t-T_L}, & t \geq T_L \end{cases} \tag{2.2}$$

Given the observed day of the onset of detectable parasitaemia (T), then the initial infectious dose of merozoites emerging from the liver (Q) can be estimated as:

$$Q = p_T m^{-(T-T_L)} \tag{2.3}$$

This equation allows us to relate the delays observed in the time it takes for parasites to reach the detectable threshold in the blood to an initial reduction in the number of parasites that established blood stage infection.

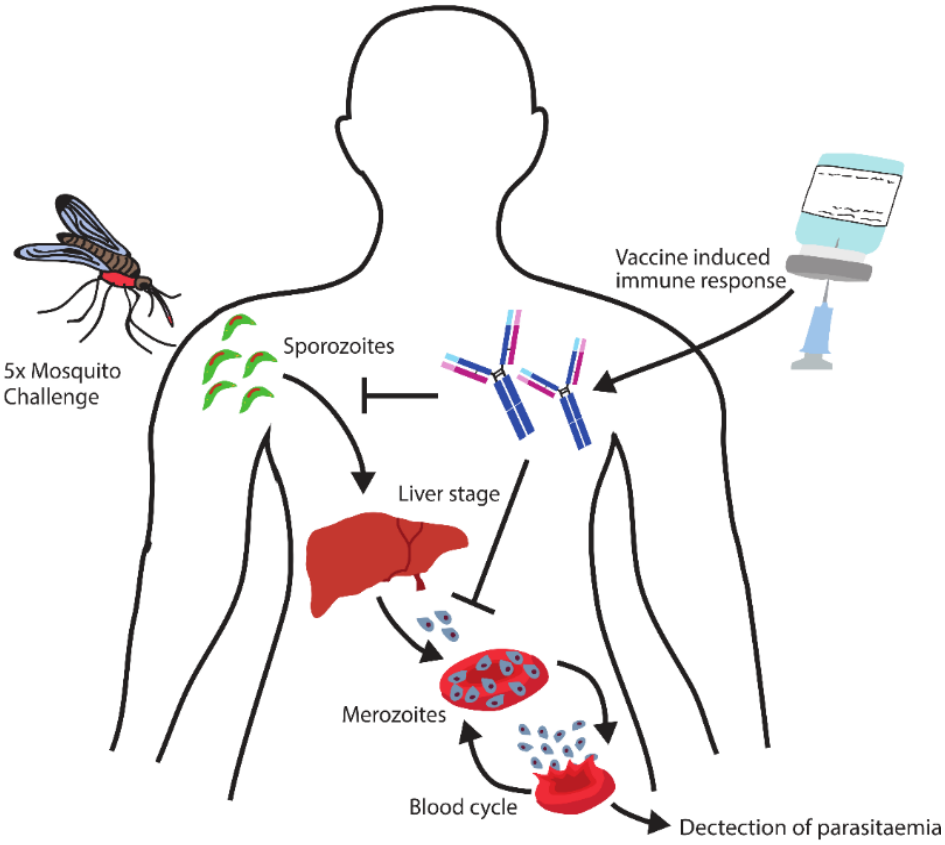

Figure 2.2 Depiction of the sporozoite infection model. The sporozoite infection model mathematically captures the parasite and immune dynamics following vaccination and challenge. This schematic conceptualises the model: following mosquito challenge with 5 infectious mosquitos, inoculated sporozoites migrate to the liver, undergo intrahepatic development, and release an initial infectious dose of merozoites. These merozoites cycle through blood stage development until they reach a threshold level for detection. Following vaccination with RTS,S the number of antibodies and the avidity of antibodies will influence the survival probability of the inoculated sporozoites. And subsequently, the antibody immune response will also influence the initial infectious dose of merozoites that emerge from the liver, which can be related back to longer delays in detection of parasitaemia. If an individual is protected, then *I* assume all infectious sporozoites will have been prevented from completing liver stage development.

2.3.3.3 Vaccine component

Following challenge, an individual's innate or adaptive immune response will act to detect and eliminate invading parasites. Given vaccination with RTS,S the prevention of infection and the killing of sporozoites is assumed to be driven by the vaccine-induced antibody response and can depend on both the number of antibodies and the relative avidity of these antibodies (Figure 2.2). If a volunteer is protected following challenge, then all sporozoites must have been cleared preventing blood stage infection. However, if an individual becomes infected then a proportion of sporozoites will have escaped the antibody response to initiate breakthrough infection. To quantify the contribution of the antibody response to protection dose–response curves were incorporated into the sporozoite infection model that relate IgG titre and avidity indices ('dose') to probability of sporozoite success ('response'). Two parametric dose–response curves were considered: exponential and Hill functions.

$$\text{Exponential: } f(x) = e^{-\log(2)\frac{x}{\beta}} \quad (2.4)$$

$$\text{Hill function: } f(x) = \frac{1}{1+\left(\frac{x}{\beta}\right)^{\alpha}} \quad (2.5)$$

In these equations, $f(x)$ is the probability of sporozoite survival for a given immune measurement (x) and α and β are the shape and scale parameters of the respective distributions. Immune markers were considered alone and in combination. Where these immune characteristics provide protection independently of each other, dose-response curves were multiplied for each branch of the antibody response: IgG titre (x_t) and IgG avidity index (x_{ai}):

$$f(x_t, x_{ai}) = f(x_t) f(x_{ai}) \quad (2.6)$$

Interaction dose-response curves were also considered whereby synergistic or less than multiplicative effects of each immune component were modelled according to:

$$f(x_t, x_{ai}) = e^{-\frac{\log(2)}{2}\left(\left(\frac{x_t}{\beta_t}+\frac{x_{ai}}{\beta_{ai}}\right)+\sqrt{\left(\frac{x_t}{\beta_t}+\frac{x_{ai}}{\beta_{ai}}\right)^2+4\gamma\frac{x_t x_{ai}}{\beta_t \beta_{ai}}}\right)} \quad (2.7)$$

where $\beta_{t/ai}$ are scale parameters and γ is a shape parameter. Here γ >0 suggests a synergistic interaction between immune markers, γ=0 suggests immune markers act independently, and -1 < γ <0 suggests a less than multiplicative effect of immune markers (White et al., 2011b; Greco, Bravo & Parsons, 1995).

2.3.3.4 Vaccine modified sporozoite inoculation

Given vaccination status the number of successful sporozoites following challenge will be reduced as a function of the vaccine-induced immune response: from n to $nf(x)$. This relationship results in a sporozoite infection model with the probability that k sporozoites initiate infection as:

$$S_k(x) = \binom{k+r-1}{k} \frac{r^r \left(nf(x)\right)^k}{(nf(x)+r)^{r+k}} \tag{2.8}$$

2.3.3.5 Model Likelihood

Under the sporozoite infection model an individual's infection status and time to onset of parasitaemia will depend on several parameters:

- The mean and standard deviation of the number of successful sporozoites (n, σ_n)
- The mean and standard deviation of the number of merozoites released per successful sporozoite (μ, σ_μ)
- The shape and scale parameters (α, β) describing the dose-response relationship between anti-NANP IgG antibody measurements and sporozoite survival probability.

Let θ denote the vector of these parameters to be estimated I an indicator for protection status (0 protected, 1 infected), T the observed time to onset of parasitaemia (days) and the resulting estimate of the liver-to-blood inocula (Q) and x the volunteer specific vector of IgG antibody titre and avidity on the day of challenge. The above parameters can be estimated using the following likelihood equation:

$$L(\theta|I,T,x) = \prod_{j=1}^{j} \left(\left(\sum_{k=1}^{\infty} S_k(x_j) \left(\frac{\mu}{\sigma_\mu^2}\right)^{\frac{\mu^2}{\sigma_\mu^2}} \frac{Q_j^{\frac{\mu^2}{\sigma_\mu^2}-1} e^{-\frac{Q_j \mu}{\sigma_\mu^2}}}{\Gamma\left(\frac{\mu^2}{\sigma_\mu^2}\right)} \right)^{I_j} S_0(x_j)^{1-I_j} \right) \tag{2.9}$$

The likelihood can be explained when taken in its constitute parts. The index j ($j = 1, \ldots, 46$) represents the number of volunteers in the challenge study and the index k represents the number of sporozoites inoculated in a 5-mosquito challenge. For a volunteer who was protected ($I_j = 0$) then $k = 0$ sporozoites will be successful with the probability $S_0(x_j)$. If an individual becomes infected ($I_j = 1$), then infection will have been initiated by *k* number of sporozoites theoretically from 1 to ∞ with the probability $S_k(x_j)$. Each of these successful sporozoites will then release a given number of merozoites into the blood stream following a Gamma distribution $\Gamma\left(\frac{\mu^2}{\sigma_\mu^2}, \frac{\sigma_\mu^2}{\mu^2}\right)$.

2.3.4 Model fitting

Model parameters were jointly estimated in a Bayesian framework using Markov chain Monte Carlo (MCMC) sampling and fitting to data from both vaccine schedule arms. Ten separate models were fitted to the individual data from the challenge study, those where antibody measurements were considered alone, in combination and a model where antibody measurements were not considered (Table 2.1).

Parameters were jointly updated at each MCMC iteration using a random walk Metropolis-Hastings algorithm with update stages illustrated below, where ' denotes an attempted update:

- Update parameters $\theta' = (n', \sigma_n', \sigma_\mu', \alpha_t', \beta_t', \alpha_{ai}', \beta_{ai}')$
- Calculate updated model likelihood $L(\theta'|I,T,x)$ and updated prior probability density $P(\theta')$
- Accept the parameter update with probability: $\min\left(1, \frac{L(\theta'|I,T,x)\ P(\theta')}{L(\theta|I,T,x)\ P(\theta)}\right)$

The MCMC algorithm was implemented in R and the code can be viewed at: https://github.com/ht1212/thesis_chapter_2. All updates were attempted with a multivariate-Normal proposal distribution. The covariance of the multivariate-Normal proposal distributions for Metropolis-Hastings updates were adaptively tuned using the estimated posterior distributions during iterations 500 – 5000. This ensured the MCMC process adapted to the target distribution to keep the search effective at all times due to correlation between the parameters to be estimated. Further the magnitude of the proposed step size was calibrated using a Robbins-Munro algorithm to ensure an acceptance rate of approximately 23% (Gelman, Gilks & Roberts, 1997; Robbins & Monro, 1951; Atchadé & Rosenthal, 2005). The Robbins-Munro sampler will increase the value of the step-size if the acceptance rate is too high and will decrease the value of the step-size if the acceptance rate it too low. The total number of MCMC iterations was 200,000 with a burn-in period of 40,000 iterations. All Markov chains were visually examined for appropriate mixing and convergence. The MCMC fitting process was repeated multiple times to ensure consistent results and to test for lack of convergence.

Prior distributions for parameters describing the parasite distributions were derived from the studies of *White et al*., (White et al., 2013) and *Coffeng et al.,* (Coffeng et al., 2017). Note that informative gamma priors were selected due to the small number of data-points available to fit the model leading to problems in model convergence. Where prior information was unavailable for dose-response parameters, a diffuse gamma prior was required for β_t to ensure convergence, a normal prior was selected for γ and uniform priors over reasonable ranges were selected for the remaining parameters (Table 2.1). Fixed parameters during model fitting are listed in Table 2.2.

2.3.5 Model comparisons

Model parameter estimates were summarised in terms of the median and 95% Bayesian Credible Intervals (95% CrI) of the posterior distributions for each parameter. I compared the fit of the ten proposed models using Deviance Information Criterion (DIC) methods, with the best fitting model selected as that with the lowest DIC.

2.3.6 Predicted vaccine efficacy

Following model fitting, parameters from the best fitting model were used to obtain estimates of vaccine efficacy given possible combinations of IgG antibody measurements (x). Vaccine efficacy against infection (VE_i) was defined as the reduction in the probability of infection following infectious challenge in vaccinated volunteers compared to control volunteers. Given the assumption that the number of successful sporozoites follows a Negative Binomial distribution, efficacy against infection can be estimated as:

$$VE_i(x) = 1 - \frac{1 - \left(\frac{r}{nf(x) + r}\right)^r}{1 - \left(\frac{r}{n + r}\right)^r} \tag{2.10}$$

where n is the mean number of successful sporozoites multiplied by the dose-response relationship $f(x)$ and r the shape parameter of the negative binomial distribution $r = \frac{n^2}{\sigma^2_{n-n}}$.

Vaccine efficacy per sporozoite (VE_s) was defined as the proportional reduction in liver-stage parasite load (the liver-blood inoculum) and is calculated as: $VE_s(x) = 1 - f(x)$.

2.3.7 Predictive time to onset of parasitaemia

From our model calculations of the liver-to-blood parasite inocula from each volunteer $\big(Q(x)\big)$, expected time to onset of parasitaemia can be estimated via the following equation:

$$T(x) = t_L + \frac{\log\left(\frac{P}{Q(x)}\right)}{\log(m)} \tag{2.11}$$

Here I assume that all individuals have the same merozoite blood stage replication rate (m).

2.3.8 Binary infection model

An alternative way to parameterise the sporozoite infection model is to take into account only the binary outcome of protection status from trial volunteers (I) and ignore the data on the time taken for blood-stage infection to be detectable in infected volunteers. This way the model reduces to a binomial likelihood where the dose-response curves relate directly to vaccine efficacy and not

sporozoite survival. After challenge it is thus assumed that volunteer $j(\ j = 1, \ldots, 46)$ will either become infected ($I = 1$) or will be protected ($I = 0$) depending on the combination of their anti-NANP IgG titre and avidity index x_j. The likelihood equation for the parameters of the dose-response curves (θ) can be written as:

$$L(\theta|I, x) = \prod_{j=1}^{J} f(x_j)^{I_j} \left(1 - f(x_j)\right)^{1-I_j} \tag{2.12}$$

Results from the binary infection model are shown in Appendix 8.2 for reference.

Table 2.1 Estimates of the sporozoite infection model parameters. Priors and Posteriors are presented as parameter median and 95% Credible intervals in brackets. U denotes a uniform distribution, N normal and G gamma. Posterior estimates are shown for models with different combinations of dose-response curves and are ordered left to right based on model fitting comparisons with Deviance Information Criterion. Hill denotes a Hill-function dose response curve and Exp and exponential dose-response curve, read as "titre dose-response curve - avidity index dose-response curve".

Parameter	Description	Prior	Posterior								
			Hill-Exp	Interaction	Exp-Exp	Hill-Hill	Exp-Hill	-Hill	-Exp	Hill-	Base-	Exp-
n	Mean number of successful sporozoites per challenge	G 150 (75, 237)	152 (68-291)	142 (61-267)	143 (63-278)	157 (73-299)	157 (71-290)	156 (73-295)	145 (62-280)	141 (63-282)	102 (38-212)	127 (53-224)
σ_n	Standard deviation of the number of sporozoites per challenge	G 194 (93, 324)	197 (32-629)	214 (38-635)	215 (39-655)	210 (31-665)	191 (29-608)	204 (32-589)	283 (76-751)	331 (104-829)	324 (58-874)	523 (180-948)
σ_μ	Standard deviation in the number of merozoites per sporozoite	G 62,700 (47,700-10,680)	71,692 (56,580-91,061)	72,518 (56,772-92,295)	72,107 (56,652-91,989)	71,421 (56,248-91,770)	70,939 (56,141-90,658)	70,607 (55,703-89,773)	72,633 (56,960-92,541)	73,901 (57,641-94,076)	75,690 (60,066-96,780)	76,818 (59,750-97,827)
β_t	Anti-NANP antibody titre needed for 50% reduction in sporozoite survival probability	G 16,666 (10,000-70,000)	3,612 (553-38,658)	29,986 (7,490-98,883)	16,051 (6,458-63,718)	7,873 (573-78,358)	20,708 (7,770-77,207)	-	-	944 (510-4,997)	-	5,472 (3,586-13,235)
α_t	Shape parameter for antibody dose-response	U(0,30)	1.3 (0.3-3.6)	-	-	1.4 (0.2-5.7)	-	-	-	1.9 (1.4-3.2)	-	-
β_{ai}	Anti-NANP antibody titre needed for 50% reduction in sporozoite survival probability	U(0,100)	8.7 (5.8-49.8)	8.5 (5.8-69.2)	7.1 (5.4-22.7)	13.9 (1.3-55.9)	10.3 (1.3-28.1)	7.6 (1.5-23.8)	5.9 (4.8-7.7)	-	-	-
α_{ai}	Shape parameter for antibody dose-response	U(0,30)	-	-	-	3.7 (1.3-12.2)	3.4 (1.5-7.8)	3.4 (1.8-7.8)	-	-	-	-
γ	Shape parameter for interaction dose-response curve	N(0,10)	-	4.6 (-0.5-19.7)	-	-	-	-	-	-	-	-
VE	Sporozoite killing probability for vaccination status only	U(0,1)	-	-	-	-	-	-	-	-	0.99 (0.82-0.99)	-
ΔDIC	Difference in deviance informative criterion		0.00	7.35	10.98	11.20	15.34	16.52	18.07	22.15	22.34	34.61

Table 2.2 Fixed model parameters.

Parameter	Description	Value	Reference
t_L	Duration of liver stage development	6.5 days	(Murphy et al., 1989; Coffeng et al., 2017)
m	Daily blood stage parasite multiplication rate	3.8 day^{-1}	(Bejon et al., 2005)
P_T	Threshold number of parasites for detection of infection (occurrence of parasitaemia, defined by positive blood slide)	50,000,000 Parasites/mL	(Bejon et al., 2006)
μ	Mean number of merozoites released per sporozoite	30,000	(Coffeng et al., 2017)

2.4 Results

2.4.1 Challenge study data

On the day of challenge volunteers vaccinated with the delayed-fractional regime had lower anti-NANP IgG titres than in those receiving the standard regime on the day of challenge (Mann-Whitney:

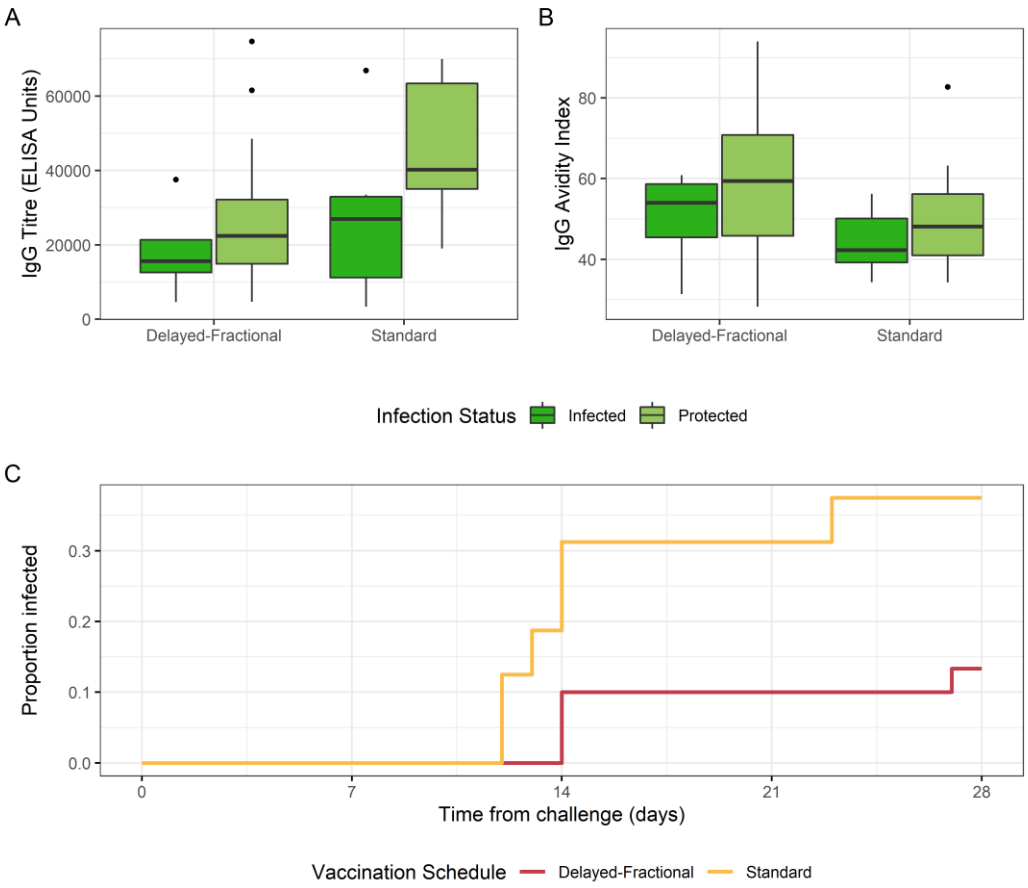

Figure 2.3 Challenge study data summary. Box and whisker plots of the observed differences in anti-NANP IgG measurements by **A)** IgG titre (ELISA Units) (n=16) and **B)** IgG Avidity Index (n=30). Measurements are stratified by vaccine schedule and by infection status with outlying values shown by grey circles. **C)** Cumulative malaria incidence stratified by vaccine schedule arm. Follow up period for the challenge study reached 28 days after which point volunteers were deemed protected from infection following challenge. Infection was determined by a positive blood slide.

p-value=0.02). Whereas anti-NANP IgG avidity was significantly higher in volunteers from the delayed fractional arm (Mann-Whitney: p-value=0.03). In addition, there was a trend for protected volunteers to achieve higher avidity and titre measurements within arms (Figure 2.3). There was no significant correlation between these two immune measurements (Spearman's rho: -0.05, p-value=0.72). The time to onset of parasitaemia in those volunteers who became infected was significantly different between the two arms (log rank test: p-value=0.04) with the delayed-fractional arm showing longer delays (Figure 2.3).

2.4.2 Model fitting

The model that provided the best fit to the data included both anti-NANP IgG titre and IgG avidity, with the DIC indicating this to provide a better fit than models with either antibody titre or avidity alone (Table 2.1). Furthermore, based on the DIC, avidity alone provided a better fit to the trial data than antibody titre alone (Table 2.1). I found that the best fitting dose-response curves modelled antibody titre according to the Hill function and avidity according to the exponential, parameter posterior distributions and MCMC trace plots for the best fitting model are shown in Figure 2.4.

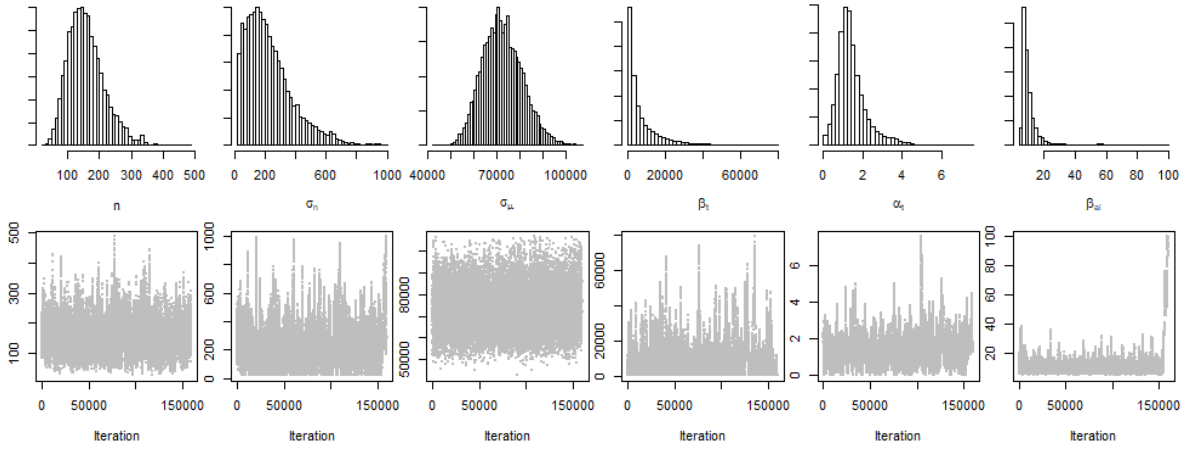

Figure 2.4 MCMC posterior distributions and trace plots.

Despite the low number of data points available, the titre-avidity model replicated well the observed vaccine efficacy (Figure 2.5A) and recorded time to onset of parasitaemia (Figure 2.5C) in the trial arms. When only titre was accounted for this model failed to capture the increase in vaccine efficacy of the delayed-fractional arm over the standard arm and instead overestimated standard arm efficacy and underestimated delayed-fractional arm efficacy (Figure 2.5B). Again, the titre only model predicted earlier onsets of infection for individuals across both arms of the trial, whereas the inclusion of avidity dose-response terms better replicated the observed relationship and distinguished the later times to onset from the delayed-fractional arm (Figure 2.5D). The model does not take into account

inter-individual variation in innate immune responses and variations in the blood-stage parasite replicate rate which might result in underestimates of the time to onset for several volunteers.

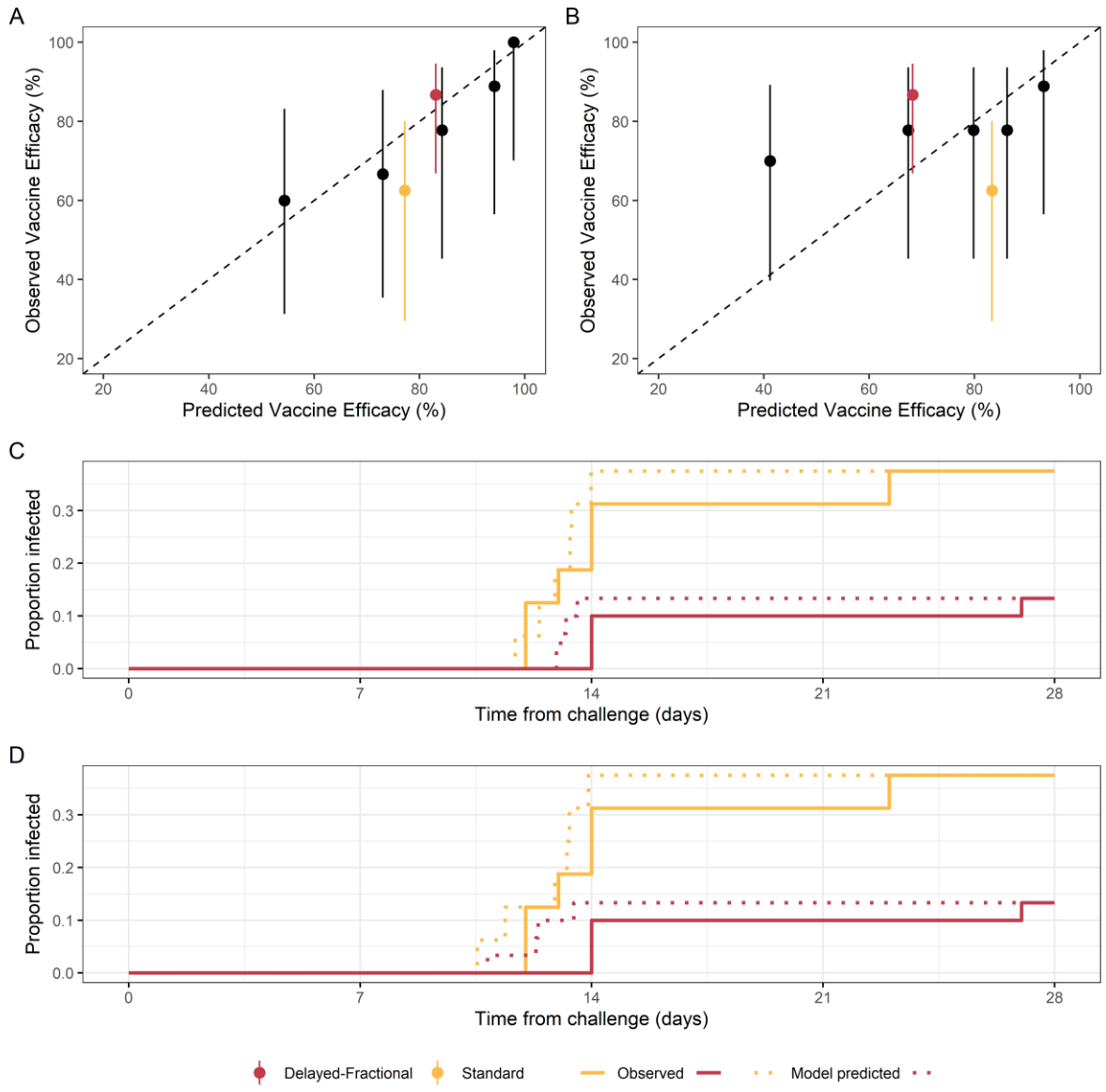

Figure 2.5 Comparisons of model predicted outcomes to challenge trial data. A) Model predicted vaccine efficacy resulting from the best fitting model including dose-response curves for IgG titre and avidity and **(B)** a comparison with the model when only IgG titre was included as a predictor of vaccine efficacy. Dashed black lines on both plots represents equivalent predicted and observed efficacy. Pink points represent the overall efficacy of the delayed-fractional arm and yellow the standard arm. Black points represent individuals grouped into quintiles of their predicted vaccine efficacy, with vertical lines representing binomial 95% Confidence Intervals for observed efficacy measurements. **C)** Model predicted time to onset of parasitaemia (dotted lines) for the best fitting model including dose-response curves for IgG titre and avidity and **D)** model predicted time to onset of parasitaemia (dotted lines) for the IgG titre only model. All model results at median predicted values.

Table 2.3 Comparison of model predicted (green) and observed (black) vaccine efficacy against infection at first challenge. Comparisons are stratified into terciles based on observed immune marker measurements. Number in brackets for observed rows represents the number of individuals infected in each tercile/total number in each tercile

IgG Avidity Index	Anti-NANP IgG Titre (ELISA Units)			
	Low (3,399-18,512)	**Med** (18,513-34,139)	**High** (34,140-74,710)	**All** (3,399-74,710)
Low (28-44)	44.6%	63.5%	76.8%	63.9%
	66.7% (1/3)	57.1% (3/7)	80.0% (1/5)	66.7% (5/15)
Med (45-59)	65.7%	84.9%	91.7%	77.1%
	62.5% (3/8)	100% (0/3)	80% (1/5)	75% (4/16)
High (60-94)	84.9%	95.2%	97.6%	93.2%
	75.0% (1/4)	100% (0/6)	100% (0/5)	93.3% (1/15)
All (28-94)	66.1%	78.4%	89.0%	78.0% (95% CrI 60.3% - 88.8%)
	66.7% (5/15)	81.3% (3/16)	86.7% (2/15)	78.2% (10/46)

2.4.3 Vaccine efficacy against infection

Protection against infection was estimated to increase with both increasing antibody titre and avidity. bb shows the observed and model-predicted vaccine efficacy against infection for volunteers stratified into terciles (low, medium, high) based on their titre and avidity measurements. Of the five individuals with the highest recorded immune response measurements, all were protected following challenge, with modelled efficacy extremely close to sterile protection upon first challenge at 97.5% (95% CrI 87.9%–99.6%). Efficacy was predicted to be higher if high avidity levels (avidity index≥60) were achieved but titres remained low, efficacy: 84.9% (95% CrI 48.0%–97.0%), compared to when high titre measurements were achieved but avidity remained low (≤44), efficacy: 76.8% (95% CrI 52.7%–92.9%). For the lowest observed titre and avidity measurements efficacy was predicted to be almost half that for the highest terciles (Table 2.3). The estimated distribution of individual efficacy against infection is shown in Figure 2.6 and shows high between individual-variation due to the underlying variation in immune responses between individuals. Efficacy against infection predicted by the model for continuous combinations of both IgG antibody titre and avidity is shown in Figure 2.7A, noting the presence of a highly protected subgroup of volunteers all of whom had avidity measurements greater than 65 but whose titre measurements varied significantly across the observed range. Increasing avidity was estimated to substantially increase vaccine efficacy for all but the highest antibody titres.

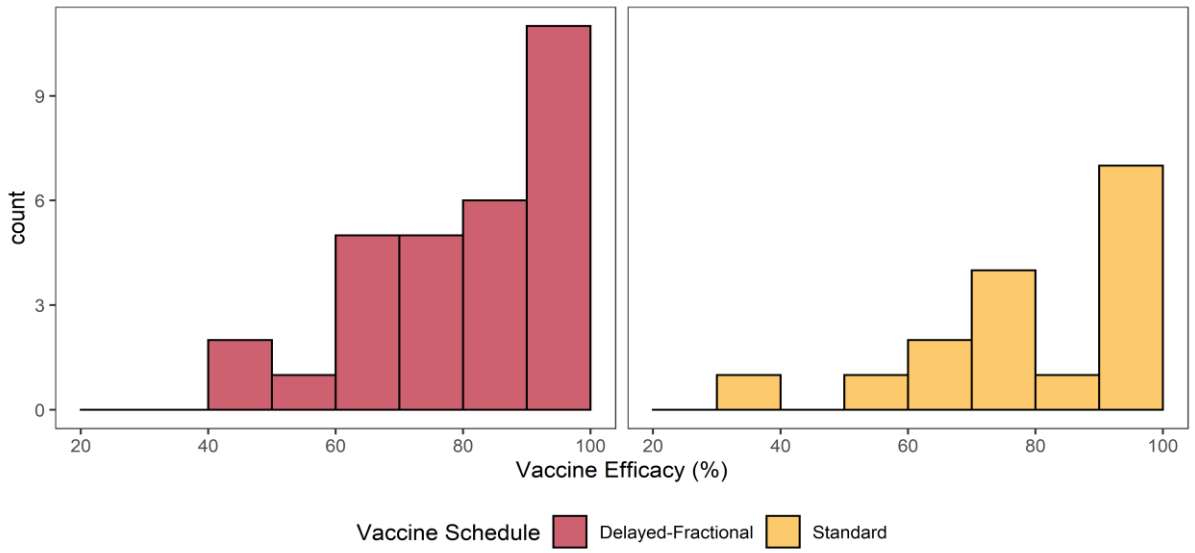

Figure 2.6 Distribution of model predicted vaccine efficacy against infection. Estimated for each volunteer (n46) in the challenge trial stratified by vaccination schedule.

2.4.4 Vaccine efficacy per sporozoite

In addition to estimating vaccine efficacy against infection, the model estimates the percentage reduction in the number of sporozoites initiating blood stage infection for a given level of immune response. I estimated the efficacy per sporozoite across the vaccination arms to be extremely high at 99.7% (95% CrI 98.7 %–99.8%). This suggests that even if 1% of the infecting sporozoites escape the immune response they can go on to initiate breakthrough infection, all-be-it at a slower rate than in unvaccinated populations. Both efficacy against infection and efficacy per sporozoite increase with increasing titres and avidity (Figure 2.7B-E).

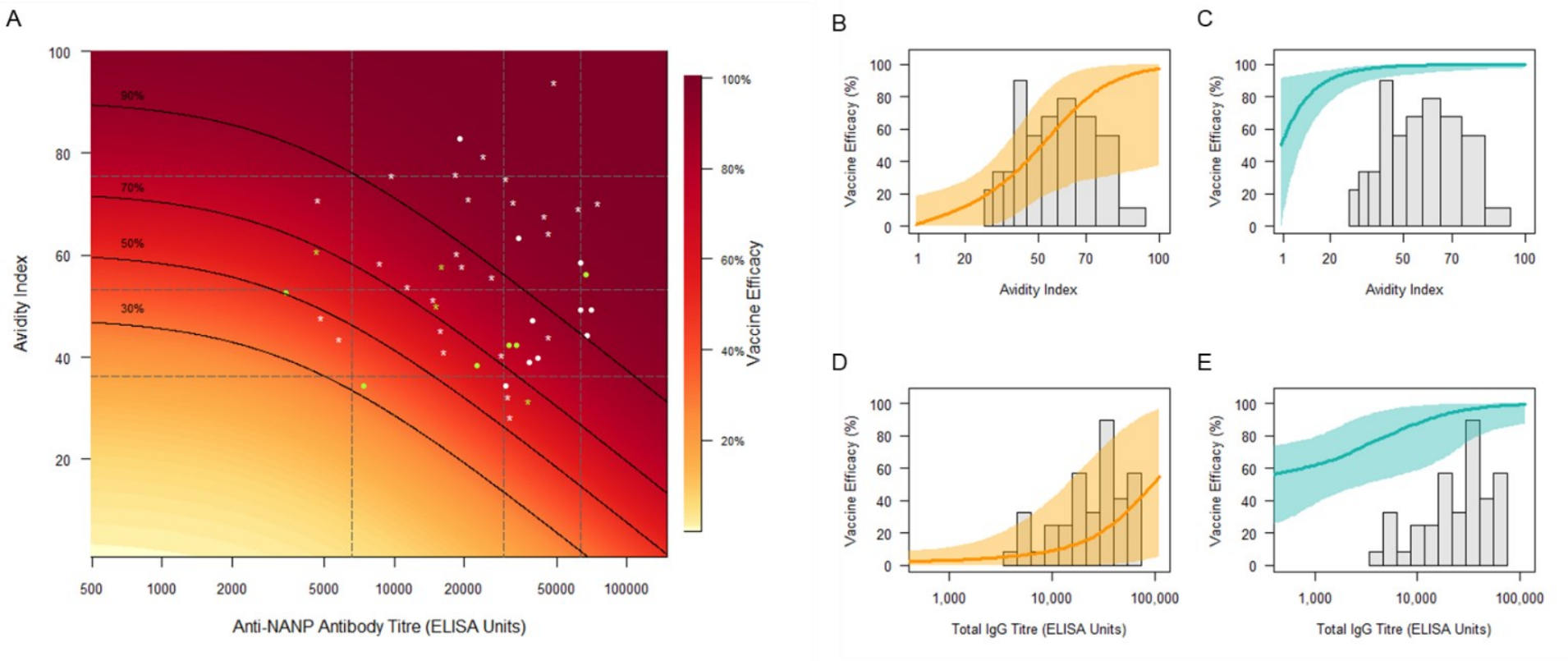

Figure 2.7 Predicted efficacies as a function of the antibody immune response. A) Efficacy against infection as a function of anti-NANP IgG antibody titre (ELISA Units) and avidity index. Vertical dashed lines represent the median and 90% ranges of the observed antibody titres. Horizontal dashed lines represent the median and 90% ranges of the observed avidity index measurements. Isoclines represent the 30%, 50%, 70% and 90% estimated vaccine efficacies for combinations of avidity and titre. Green represents infected volunteers and white protected volunteers, * the Delayed-Fractional arm and • the standard arm. **B,D)** Efficacy against infection as a function of each single immune measurement with the other held constant at the value of the estimated β parameter from model fitting (avidity 8.7 and titre 3,612). **C,E)** Efficacy per sporozoite as a function of each single immune measurement with the other held constant at the value of the estimated β parameter from model fitting (avidity 8.7 and titre 3,612). Shaded areas represent 95% credible intervals. A histogram of the distribution of observed immune measurements is shown in grey.

2.5 Discussion

Understanding the nature of the protective immune response elicited by second-generation RTS,S vaccines and the identification of immunological markers that reliably predict protection against malaria infection is critical for vaccine development and evaluation. Here I have shown that the quality of vaccine-induced responses is also an important consideration when evaluating associations between immune markers and protection from infection. This work highlights that high levels of protection can be achieved without further increases in antibody titre. Further, model fitting demonstrated how the combination of both IgG titre and avidity help to explain the observed efficacy improvements of the delayed-fractional regime better than if titre alone was used to predict efficacy.

Across the volunteers in this trial the model predicts that RTS,S induced partial protection in everyone with subgroups of individuals highly protected against first challenge. It is important to note that this variation in protection stems from both the observed variation in immune responses and also the underlying variation in the potential infectious dose of sporozoites received per-challenge-per-volunteer. Despite the presence of a highly protected sub-group of individuals who all achieved avidity indices greater than 60, no thresholds of protection for either immune marker were identified in this study. The model does predict efficacy against infection in excess of 70% if avidity indices greater than 70 are achieved, regardless of the number of antibodies induced, which suggests that close to sterile protection could be achieved with further increases to avidity.

At the lower levels of avidity achieved in this trial a higher antibody titre was required to achieve a given level of vaccine efficacy, suggesting that low functional avidity could be compensated for by high IgG titres and vice versa (Bachmann et al., 1997; Reverberi & Reverberi, 2007). High avidity antibodies that recognise and strongly bind sporozoites might be especially important in the short time frame following inoculation to successfully clear invading parasites. However it is not yet clear how avidity measurements relate to functional responses and if high avidity antibodies exert better biological functions (Mota & Rodriguez, 2004; Yang et al., 2017; Sidjanski & Vanderberg, 1997). One aspect of the antibody response not captured in this work is the subtype composition of IgG antibodies. A recent study suggested that the observed avidity improvements might result from an underlying change in IgG subclass composition, with a dominant IgG4 response in those volunteers from the delayed-fractional regime (Chaudhury et al., 2017). Given that IgG subclass has implications for antibody function and it has been shown that differing subclass compositions associate with protection or risk of malaria infection, it will be important to further characterise whether total IgG avidity can potentially act as a successful surrogate marker of both serum antibody composition and protection

(Kurtovic et al., 2018, 2019, 2020; Chaudhury et al., 2017; Ubillos et al., 2018; Dobaño et al., 2019; Vidarsson, Dekkers & Rispens, 2014).

While this challenge study was performed in malaria naïve adults in the United States, the measurements of avidity to repeat region antigens recorded from the standard arm mean 45.5 reflect those recorded from RTS,S/AS01 vaccinated children in malaria-endemic countries (median 41.2, mean 45.5 and geometric mean 39 (Ajua et al., 2015; Olotu et al., 2014; Dobaño et al., 2019)). Whilst the approaches and methodologies of the studies vary making comparisons difficult, if the improvements in avidity and efficacy observed in the delayed-fractional arm translate to target populations in malaria endemic countries modelling work suggests this could potentially result in an additional 21–25% more clinical cases averted compared to the standard RTS,S/AS01 dosing regimen over 10-years of routine delivery (Hogan et al., 2018). To further optimise the protective efficacy and delivery of RTS,S it will be important to disentangle the influence of the fractional dose versus the delay on both the immune response pathways and vaccine efficacy and such evaluations are currently ongoing in naturally exposed populations (Clinicaltrials.gov identifiers: NCT03276962). Preliminary results of these ongoing field trials as assessed in an interim analysis and presented to the 2021 WHO Malaria Policy Advisory group showed that fractional only regime was not superior to the standard regimen over either 6.5 or 12 months for the same outcomes and that there were no significant differences in antibody avidity among vaccinated groups (World Health Organization, 2022). While full trial results are not yet available results suggests the potential for the concurrent delay and fractioning might be critical for improvements in efficacy.

Critically children in malaria endemic countries will have pre-exposure to *P. falciparum*, experience repeat exposure, between dose exposure and a diverse parasite strain environment all of which are not captured in challenge trials, which could influence the adaptive immune response and thus achievable efficacy of delayed-fractional RTS,S. Our results show that it will be important to measure both antibody markers in these studies to further assess their utility as predictors of protection in naturally exposed populations.

Finally, vaccine dose reductions and dose spacing have been associated with improving affinity maturation responses in vaccinees, resulting in higher affinity protective antibodies (Lambert, Liu & Siegrist, 2005). Evidence suggests these changes can preferentially give rise to the formation of memory B cells and prioritise germinal centre formation over short term effector responses (Lambert, Liu & Siegrist, 2005). The increased levels of somatic hypermutation along with the improvements in avidity and recent work characterising the formation of CSP-specific memory B cells suggests that the delayed-fractional regime alters affinity maturation to some degree (Chaudhury et al., 2017; Regules

et al., 2016; Pallikkuth et al., 2020). Due to the limited number of data points in this study I was unable to fit separate *P. falciparum* infection models to each vaccination arm and test this hypothesis that the schedule changes result in different relationships between efficacy against infection and immune markers. Further largescale trials will be key to exploring this hypothesis further. As noted previously delineating the role of dose spacing and dose reductions will be important for further optimising efficacy of RTS,S. Monitoring avidity in all future RTS,S clinical trials should be considered and further work into understanding its utility as a correlate of vaccine efficacy but also as a potential marker of underlying B cell changes should be a priority.

2.6 Limitations

There are several limitations to the results presented in Chapter 2. Firstly, the findings presented here only apply on the day of challenge and do not give information on the duration of protection. The dynamics of these antibody responses over time will be important for understanding the duration of protection afforded by delayed-fractional RTS,S and its overall public health impact. In addition to only looking at a single time point following challenge the dataset from this trial was small with only 46 volunteers, 10 of whom developed infection. This limits the precision of the estimated biological parameters and leads to wide uncertainty intervals on all estimates. Given this small sample size informative priors were needed to ensure convergence during model fitting. The model itself being a simplification of a complex biological process also carries intrinsic limitations and cannot account for any periodic fluctuations in parasite density, short term influences of innate immunity or inter-individual differences in parasite growth rates. The focus here is on the antibody response to a single region of the CSP protein, however differences in cellular immune responses and responses to other regions of the CSP protein should be considered in future field trials and subsequent analysis. Recently a study by *Das et al.,* highlighted that the delayed-fractional regime breaches the immunodominance of the humoral immune response, inducing a balanced response across the C-terminal and the NANP region of CSP which were both found to be linked to protection following vaccination (Das et al., 2021). Conversely, the less efficacious standard regimen induced a more focused response to the NANP repeat region. The authors suggested that the fractional dosing regimen appeared to retain responses to the NANP repeat region that were induced by the standard regimen, while broadening the response to the C-terminal region that might also contribute to vaccine protection. Furthermore, the authors highlighted that IgG avidity to full-length CSP and C-terminus antigens did correlate to antibody effector functions suggesting that avidity could act as a surrogate marker for function as well as for improved vaccine outcomes but more work again is needed to explore this further. A further limitation and generalisability problem with this current work is that I rely on avidity measurements from inhibition ELISA with only 4M urea used as the chaotropic regent. The use of this assay has its own

limitations and these results therefore might not correlate to avidity indices evaluated from different assays (including thiocyanate or guanidine hydrochloride inhibition ELISA, plasma magnetic resonance, bilayer interferometry or multiplex assays) (Abu-Raya et al., 2020; Dimitrov, Lacroix-Desmazes & Kaveri, 2011). Further work is needed to standardise avidity measurements in malaria vaccine research to improve the validity and precision in avidity as a potential correlate of vaccine efficacy.

2.7 Conclusions

In malaria vaccine development, considerable focus has been on the quantity of the vaccine-induced immune response. Here I have shown that the quality of these induced responses is also an important consideration when evaluating associations between immune markers and protection from infection. Given the need for continued development of a highly efficacious malaria vaccine and the challenges of testing new vaccine formulations in large field trials, the establishment of immune correlates will be invaluable. These results provide an early insight into the use of avidity as a surrogate marker of the quality of the vaccine-induced antibody response to form part of future malaria vaccine evaluation frameworks.

Chapter 3

3 Fitting and validating the protective efficacy profile of Sulfadoxine-Pyrimethamine plus Amodiaquine against clinical malaria when used in seasonal malaria chemoprevention

In this chapter, I use Bayesian methods to fit an updated efficacy profile of Sulfadoxine-Pyrimethamine plus Amodiaquine (SP+AQ) the drug combination currently used for Seasonal Malaria Chemoprevention (SMC) using clinical trial data. I then validate this profile by comparing model estimated impact outcomes with those of published trial data. This is the first of two chapters where I aim to validate seasonal malaria interventions against clinical trial data. Before using these models in further population level impact modelling in Chapter 5.

3.1 Background

The diverse climatic environment across sub-Saharan Africa (SSA) leads to significant variations in transmission patterns of malaria. In areas where the annual temperature is suitable for malaria transmission, seasonality in rainfall patterns is a key driver of seasonality in malaria transmission. In areas of the Sahel and sub-Sahel region that experience intense seasonality in rainfall, transmission of malaria is also highly seasonal with cases typically concentrated in three to six months of the year. Since 2012, the WHO has recommended seasonal malaria chemoprevention (SMC) in areas where around 60% of malaria cases fall within four months of the year, or 60% of the annual rainfall falls within three months of the year (World Health Organization, 2012b). Given these criteria two broad areas of SSA, the Sahel and sub-Sahel and a stretch of southern Africa from Namibia to Mozambique and Southern Tanzania in the east, were identified as suitable for SMC as an intervention (Cairns et al., 2012). However, due to high levels of resistance to the recommended drug combination used for SMC in south and east Africa following historic use of Sulfadoxine-Pyrimethamine as a first line

treatment, SMC has not been recommended in these areas despite suitable seasonality patterns (Cairns et al., 2012; World Health Organization, 2012b; Okell, Griffin & Roper, 2017; Naidoo & Roper, 2011).

Sulfadoxine-Pyrimethamine plus Amodiaquine (SP+AQ) is the current drug combination recommended for use in SMC campaigns, to be delivered at monthly intervals to children aged 3–59 months old during the transmission season (World Health Organization, 2012b). Three- or four-monthly cycles of SMC were initially recommended by the WHO in 2012 (World Health Organization, 2012b). SP is delivered as a combined dose on day one and three daily doses of AQ are required for a complete monthly cycle of SMC.

Through providing a full treatment course of antimalarials, SMC aims to prevent clinical disease by maintaining therapeutic levels of drug concentrations in the blood over the period of greatest malaria risk. A series of clinical trials of SMC with SP+AQ have shown SMC to be a highly promising tool for controlling malaria in areas of seasonal transmission, with a pooled protective efficacy against clinical malaria across the transmission season of 83% (95% CI: 72%–89%) (Wilson, 2011; World Health Organization, 2011b). Estimates of efficacy following a single monthly treatment course are reported to be between 78%–87% (Bojang et al., 2011; Cairns et al., 2020). In addition, estimates of time-varying efficacy over follow-up suggests SP+AQ provides a high level of protection against uncomplicated malaria for around four weeks after administration of each treatment course whereafter protection appears to decay rapidly (Zongo et al., 2015).

Thirteen countries in the Sahel and sub-Sahel region have now adopted SMC programmes, delivering four-monthly cycles to populations at risk, reaching around 33.5 million children in 2020 (World Health Organization, 2021g). Similar to that observed in randomised trials, protective effectiveness of SMC against clinical malaria when delivered in routine settings was estimated at 88% within 28 days of administration and 61% between 29–42 days following administration in seven countries participating in the Achieving Catalytic Expansion of SMC (ACCESS-SMC) project (Baba et al., 2020). In addition, the routine use of SMC has been shown to reduce the number of malaria cases at outpatient clinics and the number of malaria deaths in hospitals (Baba et al., 2020).

Understanding and characterising the efficacy profile of malaria interventions over time is essential for ensuring realistic model projections of intervention impact that can contribute to policy discussions. As currently implemented in the Imperial College London malaria transmission model RTS,S vaccine efficacy profile was determined through model fitting to large scale Phase 2 and Phase 3 clinical trial data and has since been validated against the longer term follow up studies from these trials (White et al., 2014, 2015). Antimalarial treatment efficacy of first-line Artemisinin-based

combination therapies (ACT) was characterised using a previously published pharmacokinetic-pharmacodynamic (PKPD) model, fitted to clinical trial data from six different sites in sub-Saharan Africa and validated against data from a long-term trial in Uganda (Okell et al., 2014). The temporality in efficacy of insecticide-treated bed nets and indoor residual spraying with several classes of insecticides along with the impact of insecticide resistance on effectiveness has also been characterised through statistical fitting to experimental hut trial data and validation to randomised control trials (Churcher et al., 2016; Nash et al., 2021; Sherrard-Smith et al., 2018). We lack the same level of validation and quantification of the uncertainty in the effect for our current SP+AQ efficacy profile that is used in the Imperial College malaria transmission model. In order to compare seasonal malaria interventions in Chapters 4 and 5 and incorporate uncertainty in intervention effects a statistical refitting and validation of the efficacy profile of SP+AQ is required.

3.2 Aims

In this chapter I aim to use a Bayesian framework to estimate a functional form of SP+AQ efficacy over time using previously published clinical trial data. I then aim to validate this efficacy profile by using the individual based Imperial College London malaria transmission model to simulate SMC clinical trials and compare efficacy estimates to those observed in the trial. Further, I will investigate the impact of different SMC protocols (four-, five-, six- or seven-monthly cycles) on burden reductions in countries that experience seasonality in malaria transmission to assess how seasonality influences the potential impact of SMC at scale. This fitting procedure will allow propagation of uncertainty surrounding SP+AQ protection in modelling the potential public health impact of SMC in comparison to RTS,S vaccination.

3.3 Methods

3.3.1 Clinical trial data

Individual level data were made available through requests to investigators of the Zongo et al study (Zongo et al., 2015). These data are derived from the SP+AQ arm and the control arm in this trial from the final month of SMC delivery through to the following month (Oct – Nov 2009). In brief, the trial was conducted in three trial sites, served by three health centres (Satiri, Kadomba and Balla), in the district of Lena, Burkina Faso. The control arm was recruited from the same area served by the Balla health centre. All SP+AQ treatments were directly observed, and children were given SP in a dosage of 25 mg sulfadoxine and 1.25 mg pyrimethamine per kg of body weight and AQ in a dosage of 10 mg/kg. Parents were asked to bring their children to the study clinics whenever the child was unwell and, two weeks after drug administration, a study nurse would visit each household to check the child was well and refer any children to the clinic. Children who presented with a history of fever were administered a rapid diagnostic test for malaria, and if this was positive, they were treated with artemether-lumefantrine (AL), and a blood smear was taken. If a child was diagnosed with malaria on the day SMC was scheduled to be given (all children were tested before delivery), SMC was withheld that month and the child was treated with AL. In this analysis, clinical malaria was defined as: axillary temperature ≥ 37.5°C or history of fever in the last 24 hrs with any level of parasitaemia. Anonymized data from 940 individuals were provided along with their age at enrolment (in years), bed-net usage (yes/no), study site and their time until first malaria event (in days) or when they exited the study due to withdrawal, migration, death of another cause (last time point child was known to be malaria free).

Ethical approval from the Head of the School of Public Health at Imperial College London was sought and approved for the analysis of this secondary data.

3.3.2 SP+AQ profile

Several functional forms were explored to describe the decay in efficacy of SP-AQ against clinical malaria over time. I define $P_{SPAQ}(t)$ as the probability that an individual is protected from infection at time t following the third SP+AQ cycle in this trial. The functional forms used were the following: (a) the Weibull cumulative distribution function:

$$P_{SPAQ}(t) = \exp^{-(t/\lambda)^k} \tag{3.1}$$

where k and λ are the shape and scale parameters to be estimated, (b) the Gamma cumulative distribution function:

$$P_{SPAQ}(t) = 1 - \frac{1}{\Gamma(w)}\gamma\left(w, \frac{t}{\theta}\right) \quad (3.2)$$

where w and θ are the shape and scale parameters to be estimated, Γ the gamma function and γ the lower incomplete gamma function and finally, (c) a Hill-function:

$$P_{SPAQ}(t) = \frac{1}{1 + \left(\frac{t}{l}\right)^{r}} \quad (3.3)$$

where r and l are the shape and scale parameters to be estimated.

3.3.3 Infection model

The control group in this trial reflects the baseline rate of malaria infections in the absence of SMC treatment. I assume that the timing of observed malaria events can be described by an exponential distribution $\left(Exp(\alpha)\right)$ such that the time-dependent hazard $h(t)$ of an episode of clinical malaria over the trial is constant:

$$h(t) = \alpha \quad (3.4)$$

If an individual is sleeping under an insecticidal treated bed-net (LLIN) then the hazard of clinical malaria is assumed to be adjusted by a factor δ_{LLIN}:

$$h(t) = \alpha * \delta_{LLIN} \quad (3.5)$$

Further, given that the gradual acquisition of natural immunity to clinical malaria will influence patterns of clinical malaria in this trial a further age modification term for each age group ($\delta_{age\ 0-1}, \delta_{age\ 1-2}, \delta_{age\ 2-3}, \delta_{age\ 3-4}, \delta_{age\ 4-5}$) in the trial cohort is included:

$$h(t) = \alpha * \delta_{LLIN} * \delta_{age} \quad (3.6)$$

Seasonal malaria chemoprevention is assumed to reduce the hazard of clinical malaria by a factor $1 - P_{SPAQ}(t)$:

$$h(t) = \alpha * \delta_{LLIN} * \delta_{age} * \left(1 - P_{SPAQ}(t)\right) \quad (3.7)$$

This formulation of the model assumes that the hazard of clinical malaria over the final two months of the trial is constant which may be an unrealistic assumption given the seasonality of transmission in the trial site. Therefore, I also compare this constant hazard model to three formulations of a piecewise-hazard model. In these piecewise-hazard models the time axis is separated at defined cut points; I test $n = 2$ monthly intervals using the following cut points $c = \{(0,30], (30,60]\}$, $n = 5$ bi-weekly intervals using the following cut points $c = \{(0,14], (14,28], (28,42], (42,56], (56,60]\}$, and $n = 9$ weekly intervals defined using the following cut points: $c = \{(0,7], (7,14], (14 - 21], (21 -$

$28], (28-35], (35-42], (42-49], (49-56], (56-60]\}$. The corresponding baseline time-dependent hazard of an episode of clinical malaria is therefore:

$$h(t) = \begin{cases} \alpha_1, & if\ 0 \leq t\ \leq\ c_1 \\ & \cdots \\ \alpha_n\ if\ c_{n-1} < t\ \leq\ c_n \end{cases} \tag{3.8}$$

The corresponding time-dependent survival function describing the probability of remaining malaria-free at time t is described by the following equations for the control arm and SMC arm respectively:

$$S(t) = \exp\big(-\alpha_n *\ \delta_{LLIN} * \delta_{age} * t\big) \tag{3.9}$$

$$S(t) = \exp\left(-\alpha_n * \delta_{LLIN} * \delta_{age} * \sum_{\tau=1}^{t}\big(1 - (P_{SP\,AQ}(\tau))\big)\right) \tag{3.10}$$

3.3.4 Model likelihood for survival analysis

Given the total number of individuals J in the trial, during follow-up an individual $(j, j = 1,2, \dots J)$ will either be diagnosed with malaria $(I_j = 1)$, or be censored by remaining malaria-free $(I_j = 0)$, by a certain time point t_j. For each individual j the time spent in each interval is denoted by $t_{j1}, t_{j2}, \dots, t_{jn'}$ where n' indicates the time interval in which the jth subject is diagnosed with malaria, or the time interval in which they were last known to be malaria-free. In this way, we define I_{jn} to be 0 for each interval n where individual j remains malaria free and 1 if they are diagnosed with malaria in that interval. I estimate the parameters pertaining to the baseline hazard of malaria (α_n), the age-group and bed-net modifiers $(\delta_{LLIN}, \delta_{age})$ and the parameters of the SP-AQ efficacy profile (φ). The above parameters can be estimated using the following likelihood equation:

$$L\big(\alpha_n, \delta_{LLIN}, \delta_{age}, \varphi \big| I, t\big) = \prod_{j=1}^{J} \prod_{n=1}^{n'(j)} h_{jn}\big(t_{jn}\big)^{I_{jn}}\, S_{jn}\left(t_{jn}\right) \tag{3.11}$$

If an individual j remains malaria free $(I_{jn} = 0)$ in time interval n their contribution to the likelihood is simply the probability of remaining malaria-free up to time $t\ \Big(S_{jn}\big(t_{jn}\big)\Big)$. If an individual is diagnosed with malaria $(I_{jn} = 1)$ in the time interval n, their contribution to the likelihood is the instantaneous rate of malaria infection at time $t\ \Big(h_{jn}\big(t_{jn}\big)\Big)$ multiplied by the probability of remaining malaria-free up to time t $(S_{jn}\big(t_{jn}\big))$.

3.3.5 Model fitting

Model parameters were jointly estimated in a Bayesian framework using Markov Chain Monte Carlo (MCMC) sampling fitting to the data from the control and SP+AQ arms. The model was written in RStan

(Stan Development Team, 2020) and all code can be viewed at https://github.com/ht1212/thesis_chapter_3. Stan implements a Hamiltonian Monte Carlo (HMC) algorithm along with the No-U-Turn Sampler (NUTS), effectively generating an Adaptive Hamiltonian Monte Carlo algorithm. HMC supresses the random-walk behaviour of the Metropolis algorithm used in Chapter 2 which allows for a much more efficient searching of the posterior space (Neal, 2011). As with all Bayesian model fitting, the goal of sampling is to elucidate the posterior density of our model parameters (q) given our data: $p(q|data)$. HMC augments this posterior density with an independent momentum variable (ϕ) giving the joint density:

$$p(\phi, q) = p(\phi|q)p(q) \tag{3.12}$$

It is this joint density that defines a Hamiltonian as:

$$H(\phi, q) = T(\phi|q) + V(q) \tag{3.13}$$

which is composed of the sum of the potential energy: $(V(q))$ and the kinetic energy: $(T(\phi|q))$. The potential energy is given by the negative log of the unnormalized posterior density of the model $(V(q) = -\log(p(data|q) * p(q)))$ (Stan Development Team, 2020; Betancourt, 2017; Neal, 2011).

The algorithm updates following the stages below:

1. Starting from the current value of parameters q
2. Generate a random initial momentum ϕ from a multivariate Normal proposal distribution: $\phi \sim MVN(0, M)$, where M is the Euclidean metric.
3. The parameter values are updated using the Leapfrog integrator step size ϵ and number of steps L according to Hamiltonian dynamics
4. By applying L leapfrog steps a total of $L\epsilon$ time is simulated the resulting state at the end of each simulation is recorded (ϕ', q')
5. A metropolis acceptance step is then applied where the probability of keeping the proposal (ϕ', q') generated by transitioning from (ϕ, q) is: $\min\left(1, \exp\left(H(\phi, q) - H(\phi', q')\right)\right)$

To ensure efficient sampling, Stan is able to automatically optimise ϵ to match an acceptance-rate target, is able to estimate M, based on warmup sample iterations, and can dynamically adapt L using the NUTS algorithm (Stan Development Team, 2020; Hoffman & Gelman, 2014). This algorithm automatically selects an appropriate number of leapfrog steps in each iteration to allow the proposals to traverse the posterior without doing unnecessary work (Stan Development Team, 2020; Hoffman & Gelman, 2014). The total number of MCMC iterations during model fitting was 5,000 with half of these discarded as warmup samples and 4 chains were run in parallel. All Markov chains were visually

examined for appropriate mixing and convergence along with R-hat statistics for each parameter (Vehtari et al., 2019).

Posterior median parameter estimates with 95% Credible Intervals (95% CrI) are presented in the results. Non-informative Gamma priors were placed on all α_n parameters, log-normal priors were placed on each age-group parameter δ_{age}, and a uniform prior on δ_{LLIN}, parameters and weakly informative Gamma priors were placed on the P_{SPAQ} parameters based on the previous trial results that suggested the mean duration of protection of SP-AQ is around 28 days (Zongo et al., 2015; Cairns et al., 2020) (Table 3.1).

The four models with different numbers of parameters describing the baseline hazard of malaria over time were compared visually and the model that provided the most representative fit to the observed trial data was selected. I then compared the fit of each model describing the shape of P_{SPAQ} using leave-one-out cross-validation (loo-cv) implemented in the *loo* package in R (Vehtari, Gelman & Gabry, 2015). loo-cv compares the difference in the expected log predictive density (elpd) of models, relative to the model with the largest elpd to select the best fitting model (Vehtari, Gelman & Gabry, 2015). The model with the highest elpd is the model with the predictions that are the closest to the ones of the true data generating process.

3.3.6 Efficacy profile validation

In order to validate the fitted efficacy profile of SP+AQ I used the Imperial College London malaria transmission model described in Appendix 8.1 to recreate previously published SMC clinical trials inside the model framework and compare model predicted trial endpoints of efficacy to those reported in the trials.

3.3.6.1 Literature search

I performed a literature search using Embase and MEDLINE with no language restrictions up to 20-04-2021 to identify SMC clinical trial papers. The following search terms were used in all fields: (seasonal malaria chemoprevention OR intermittent preventative treatment) AND (sulfadoxine pyrimethamine AND amodiaquine). Studies were included in the validation process if they met the following criteria: 1) randomised controlled trial 2) seasonal administration of SP+AQ as one of the therapeutic regimens and 3) evaluated the effect of SP+AQ administration on clinical malaria (with parasitological confirmation). Summary data were extracted on study design, trial location, study year, number of monthly cycles of SMC delivered and the months of delivery, coverage at each round, age ranges of delivery, bed-net usage, age distributions of the cohort in each trial and reported efficacy. An

assessment of the risk of bias in each identified study was performed using the Cochrane RoB2 tool (Sterne et al., 2019).

3.3.6.2 Parameterising the model for trial locations

For each trial the first administrative unit (admin-1) of the trial location was used for simulations with admin-1 boundaries sourced from GADM (GADM, 2021). The transmission model described in Appendix 8.1 was calibrated to Malaria Atlas Project (MAP) parasite prevalence in children aged 2-10 years and clinical cases aligned with the World Malaria Report median case numbers for the year preceding the trial start (Pfeffer et al., 2018). Seasonality in malaria incidence for each admin-1 location was derived from Fourier transformations of historical rainfall data from the Climate Hazards Group InfraRed Precipitation with Station data and offset by 35 days to reflect mosquito abundance (Funk et al., 2015; CHIRPS, 1999; Garske, Ferguson & Ghani, 2013). Local vector species composition is captured for each admin-1 unit derived from MAP datasets (Pfeffer et al., 2018; Sinka et al., 2016; Wiebe et al., 2017; Malaria Atlas Project, 2022). Country specific population data were sourced from the United Nations World Population Prospects (United Nations, 2019). Historic treatment coverage levels for each location from the year preceding the trial were derived from the Demographic and Health Surveys and historic insecticide-treated bed-net usage from MAP (Pfeffer et al., 2018; Demographic and Health Surveys, 2019).

SMC with SP+AQ within the model structure acts to directly treat any existing infections with a 95% clearance probability which moves infected individuals in the model to a state of prophylaxis before moving back to the susceptible class. It also provides a period of drug-dependent prophylaxis to individuals who weren't infected. This period of protection is characterised in this chapter and parameters describing SP+AQ protection over time can be found in the results section.

SMC delivery was simulated according to the data presented in the trial publications, replicating the number of monthly cycles, age-ranges, delivery timings, and SMC coverage levels. Efficacy against clinical malaria was calculated as $1 - Incidence\ Rate\ Ratio$, comparing the modelled SP+AQ group to the modelled control group over the time-period as it was measured in the trials. I simulate across 50 posterior draws of the SP+AQ efficacy profile estimated in this chapter to explore the minimal and maximal model-predicted impact.

3.3.7 SMC protocol extension

Motivated by ongoing policy discussions surrounding the optimal number of SMC monthly cycles (Word Health Organization, 2019), using the validated SP+AQ efficacy profile, I modelled the impact of increasing the number of monthly cycles of SMC in countries of the Sahel and West African region. I compare the percentage and absolute reductions in clinical malaria for SMC delivery schedules of 4-

, 5-, 6- or 7-monthly cycles over a 12-month period in children aged 0-5 years old. Simulations were carried out at the resolution of the first administrative unit across countries of the Sahel and West African region that experience some degree of seasonality in malaria transmission (Cairns et al., 2012). For each administrative unit, the Imperial College London malaria transmission model was calibrated to *P. falciparum* prevalence in 2- to 10-year-old individuals ($PfPR_{2-10}$) based on Malaria Atlas Project (MAP) prevalence estimates and World Malaria Report cases up to 2018 (Pfeffer et al., 2018; World Health Organization, 2019b). Again, seasonality in malaria transmission was derived from Fourier transformations of historical rainfall data from the Climate Hazards Group InfraRed Precipitation with Station data and offset by 35 days to reflect mosquito abundance (Funk et al., 2015; CHIRPS, 1999; Garske, Ferguson & Ghani, 2013). Local vector species composition is captured for each admin-1 unit derived from MAP datasets (Sinka et al., 2016; Wiebe et al., 2017; Malaria Atlas Project, 2022). Country specific population data were sourced from the United Nations World Population Prospects (United Nations, 2019). Historic treatment coverage levels for each location from the year preceding the trial were derived from the Demographic and Health Surveys and historic insecticide-treated bed-net usage from MAP (The DHS Program, 2019; Pfeffer et al., 2018). Vector control depends on the level of insecticide resistance in the local mosquito populations which diminishes the effectiveness of LLINs, levels of resistance for each administrative unit were incorporated into the model based on data from WHO bioassays over time and experimental hut trials (Churcher et al., 2016; Nash et al., 2021; Sherrard-Smith et al., 2018). Coverage and resistance levels were kept constant for future simulations.

SMC coverage was calculated as the mean of those observed in the trials identified in the literature review (92%), which is at the upper limit of the reported levels following wide-scale routine implementation (Baba et al., 2020).

SP+AQ delivery was timed in the model to coincide with the peak in the transmission season and delivered to children aged 3-59 months old. Clinical cases averted were calculated for a population of 10,000 children aged under 5-years and percentage reductions in clinical malaria were calculated as $1 - Incidence\ Rate\ Ratio$, comparing the modelled SMC scenario to a counterfactual baseline scenario with the removal of SMC delivery unless otherwise stated.

Table 3.1 Estimates of the infection model parameters. Posterior estimates are shown for models with different functional forms of SP+AQ efficacy and are ordered left to right based on model fitting comparisons with loo-cv.

Parameter	Description	Prior Distribution	Posterior (95% Credible Intervals)		
			Weibull P_{SPAQ}	Hill P_{SPAQ}	Gamma P_{SPAQ}
k	SP+AQ shape parameter	Gamma(4,0.5)	3.40 (2.12-5.40)		
λ	SP+AQ scale parameter	Gamma(10,0.3)	39.34 (31.07-49.86)		
r	SP+AQ shape parameter	Gamma(4,0.5)		3.88 (2.44-6.27)	
l	SP+AQ scale parameter	Gamma(10,0.3)		34.97 (27.28-44.63)	
w	SP+AQ shape parameter	Gamma(2,1.9)			1.86 (1.36-2.44)
θ	SP+AQ scale parameter	Gamma(10,0.3)			31.10 (17.65-51.28)
α_1	Exponential baseline hazard week 1	Gamma(0.001,0.001)	0.021 (0.009-0.045)	0.021 (0.009-0.045)	0.021 (0.009-0.043)
α_2	Exponential baseline hazard week 2	Gamma(0.001,0.001)	0.020 (0.009-0.044)	0.020 (0.009-0.044)	0.019 (0.003-0.042)
α_3	Exponential baseline hazard week 3	Gamma(0.001,0.001)	0.022 (0.010-0.049)	0.022 (0.010-0.049)	0.021 (0.009-0.045)
α_4	Exponential baseline hazard week 4	Gamma(0.001,0.001)	0.017 (0.007-0.039)	0.017 (0.008-0.039)	0.017 (0.007-0.037)
α_5	Exponential baseline hazard week 5	Gamma(0.001,0.001)	0.014 (0.006-0.032)	0.014 (0.006-0.032)	0.014 (0.006-0.032)
α_6	Exponential baseline hazard week 6	Gamma(0.001,0.001)	0.039 (0.017-0.086)	0.039 (0.017-0.086)	0.044 (0.019-0.096)
α_7	Exponential baseline hazard week 7	Gamma(0.001,0.001)	0.061 (0.025-0.140)	0.064 (0.027-0.148)	0.080 (0.044-0.182)
α_8	Exponential baseline hazard week 8	Gamma(0.001,0.001)	0.087(0.035-0.213)	0.093 (0.036-0.224)	0.115 (0.044-0.276)
α_9	Exponential baseline hazard week 9	Gamma(0.001,0.001)	0.172 (0.049-0.514)	0.177 (0.052-0.533)	0.212 (0.060-0.616)
δ_{LLIN}	Bed net modifier	Uniform(0,10)	0.83 (0.60-1.13)	0.83 (0.60-1.13)	0.83 (0.61-1.14)
$\delta_{age\,0-1}$	Age group modifier 0-1 years	Log-normal(0,0.8)	0.72 (0.33-1.56)	0.73 (0.34-1.59)	0.73 (0.35-1.59)
$\delta_{age\,1-2}$	Age group modifier 1-2 years	Log-normal(0,0.8)	1.19 (0.57-2.50)	1.20 (0.58-2.54)	1.18 (0.58-2.49)
$\delta_{age\,2-3}$	Age group modifier 2-3 years	Log-normal(0,0.8)	1.57 (0.75-3.33)	1.58 (0.76-3.34)	1.58 (0.78-3.35)
$\delta_{age\,3-4}$	Age group modifier 3-4 years	Log-normal(0,0.8)	1.13 (0.54-2.42)	1.14 (0.54-2.44)	1.14 (0.55-2.46)
$\delta_{age\,4-5}$	Age group modifier 4-5 years	Log-normal(0,0.8)	0.60 (0.27-1.34)	0.60 (0.27-1.34)	0.59 (0.28-1.31)
$\boldsymbol{\Delta elpd}$	Difference in expected log predictive density relative to the best fitting model		0.0	-0.3	-1.8

3.4 Results

3.4.1 Clinical trial data

In total there were data from 940 individuals with 694 having received SMC with SP+AQ and 246 individuals in the control arm (Table 3.2). Across the two months of follow-up a total of 133 first-malaria events were reported in the SP+AQ arm and 153 in the control arm, and there were no repeat events in this dataset. There were no significant differences in reported characteristics between groups apart from when comparing the age-distributions of children in the SP+AQ arm located in Kadomba with those of the control arm (t-test: p-value = 0.002).

Table 3.2 Summary characteristics of clinical trial data used in model fitting.

Variable	SP+AQ arm	Control arm
Total individuals	690	250
Number (%) of individuals in each study site		
1 (Kadomba)	302 (44)	0
2 (Balla)	140 (20)	250 (100)
3 (Satiri)	248 (36)	0
Number (%) in age group		
<12 months	123 (18)	45 (18)
12-23 months	150 (22)	62 (25)
24-35 months	136 (20)	55 (22)
36-47 months	132 (19)	45 (18)
48-59 months	148 (21)	38 (15)
Missing	1	5 (2)
Number (%) reporting use of ITN	185 (27)	77 (31)
Number (%) malaria events	133 (19)	153 (61)
Median time to malaria event	47 days	17 days

3.4.2 Model fitting

Model fitting revealed that assuming a single constant baseline hazard over the trial follow up resulted in a poor fit to observed clinical malaria patterns (Figure 3.1A). With each successive increase in the number of time intervals used to model the baseline hazard of malaria infection results align more closely with those observed in the trial (Figure 3.1B-D). The estimated mean duration of protection against malaria was found to be similar between the models that partitioned the follow up time, monthly: 34 days (95% CrI 29 days–39 days) bi-weekly: 34.5 days (95% CrI 28 days–42 days) weekly: 35 days (95% CrI 28 days–44 days) compared to the model with a single baseline hazard: 31 days (28 days–36 days). With each increase in the number of intervals to model the baseline hazard there was a corresponding increase in the uncertainty surrounding the SP+AQ efficacy estimates and a slight change in the shape of the waning efficacy over time resulting in slightly slower rate of decline (Figure 3.1E-H). Given the improved fit to the observed times of malaria cases in both trial cohorts the piecewise hazard model with weekly cut points was selected for further model comparisons.

Alternative parameterisations of the functional form of the SP+AQ efficacy over time did not result in significant improvements in model fit to the trial data when compared using leave-one-out cross-validation (Table 3.1). The difference in expected log-posterior density between the three models was <4 (Table 3.1) suggesting the models have very similar predictive performances. The Weibull model was therefore selected for the validation. MCMC trace plots and posterior distributions for the Weibull model parameters are shown in Figure 3.2 and Figure 3.3.

The estimated duration of protection against clinical malaria at a level of 50% or over from the best fitting model was 35 days (95% CrI 28 days–44 days), and the estimated duration of over 90% protection was 20 days (95% CrI 15 days–25 days). The final functional form of SP+AQ efficacy over time is shown in Figure 3.1H.

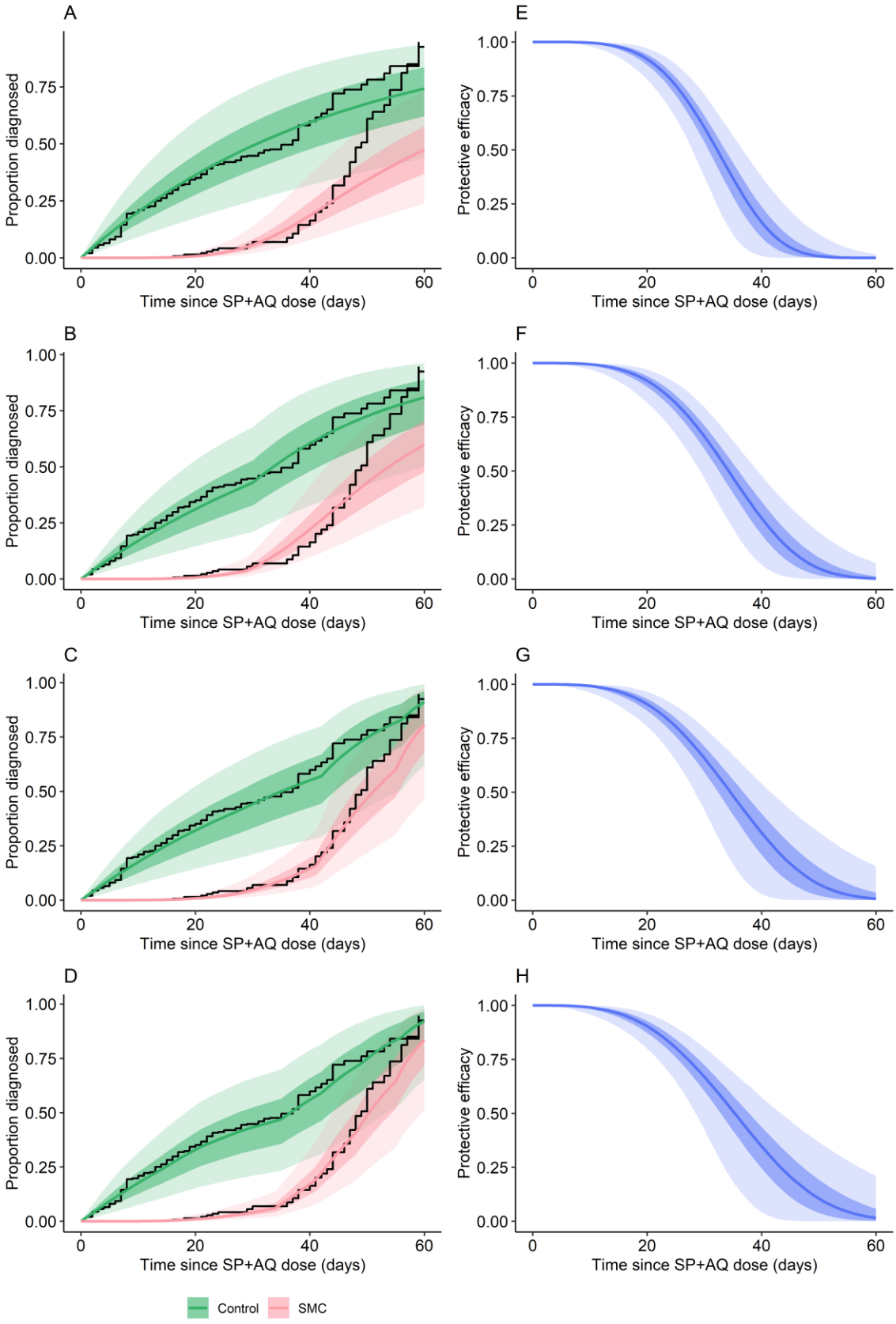

Figure 3.1 Model fitting outcomes. Collum 1 displays the results of the model fitting to the trial data describing the proportion of children infected during follow-up dependent on the number of fitted parameters that describe the baseline hazard of malaria over time **A)** assumes a single parameter, **B)** assumes two separate monthly parameters, **C)** assumes five bi-weekly parameters and **D)** assumes nine weekly parameters. Black stepwise lines show the data from each arm of the trial, and the coloured lines the fits of the model. Column 2 **E-H)** displays the resulting fitted parametric from of SP+AQ efficacy over time from each of the model fittings with each successive increase in parameters modelling the baseline hazard. Solid coloured lines representing the median model estimate and the shaded areas the 50% and 95% Credible Intervals of model estimates.

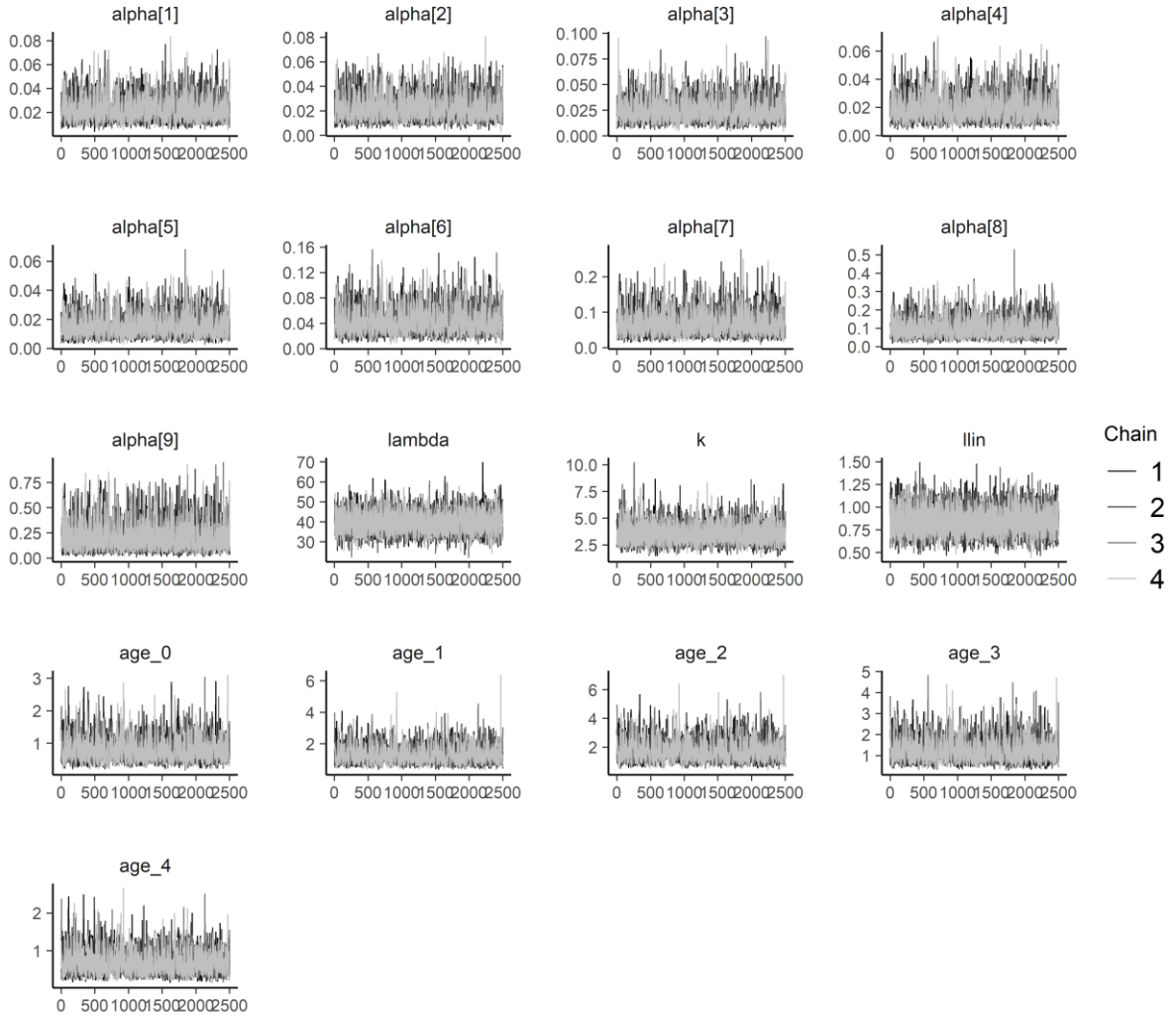

Figure 3.2 MCMC trace plots for all model estimated parameters. Trace plots resulting from the Piecewise baseline hazard with weekly cut points and a Weibull decay model of SP+AQ efficacy.

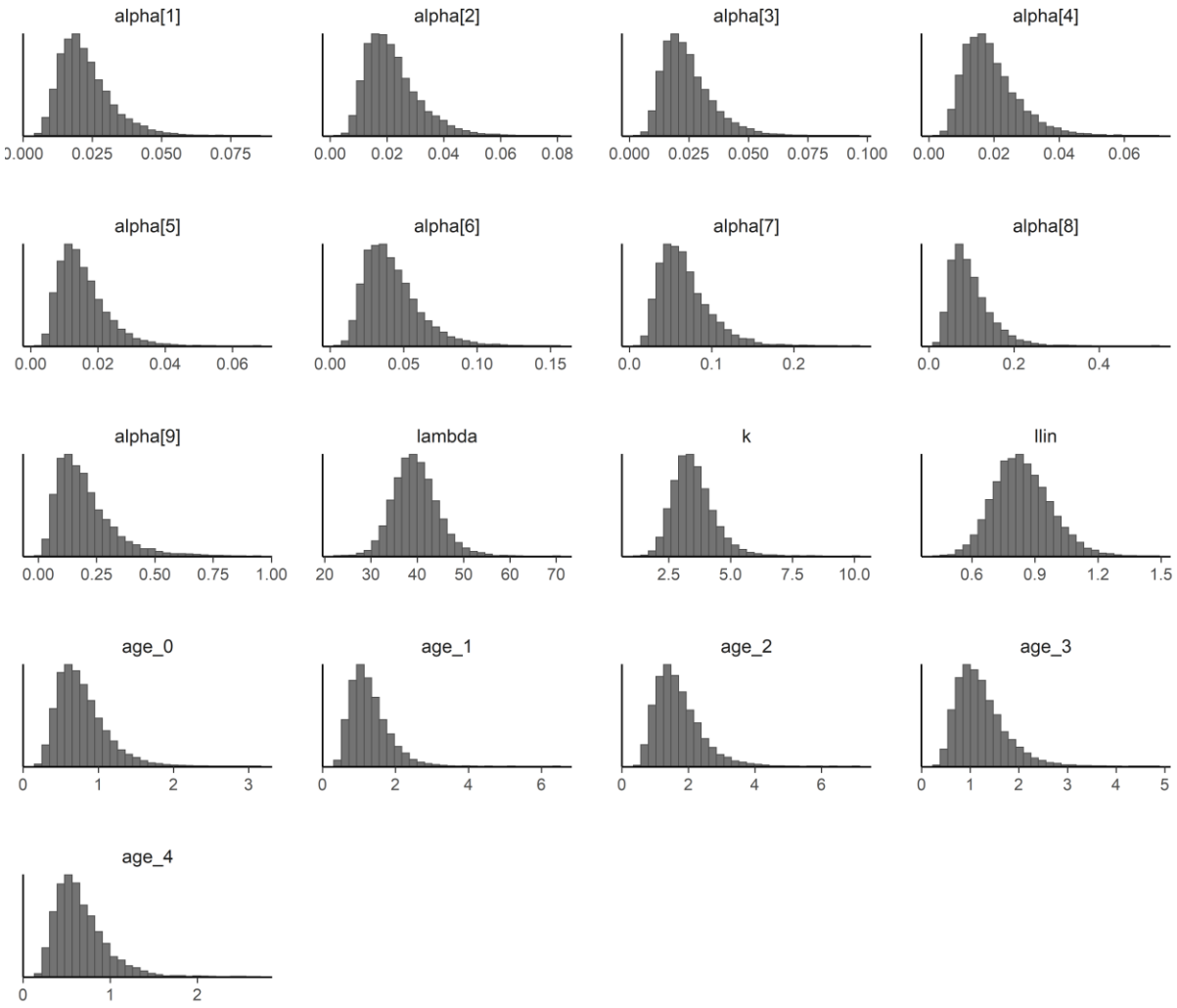

Figure 3.3 MCMC posterior distributions for all model estimated parameters. Trace plots resulting from the Piecewise baseline hazard with weekly cut points and a Weibull decay model of SP+AQ efficacy.

3.4.3 Efficacy validation

Following de-duplication, 108 studies were identified in the literature search. Of these, following title and abstract screening, eight met the inclusion criteria and were included in the validation process (Table 3.3)(Bojang et al., 2010; Dicko et al., 2011; Konaté et al., 2011; Sesay et al., 2011; Zongo et al., 2015; Tine et al., 2011; Ndiaye et al., 2019; Tagbor et al., 2016). These papers reported on SMC clinical trials carried out between 2007 and 2012 in five countries: The Gambia (2), Burkina Faso (2), Senegal (2), Mali (1) and Ghana (1) (Table 3.3 & Figure 3.4A). The locations in which these trials were carried out experience seasonal malaria transmission with seasonality ranging from a short sharp peak in incidence over three months in The Gambia to a longer period with incidence more evenly spread over around five to six months in Ghana (Table 3.3). SMC protocols varied across the studies identified with one trial delivering 2-monthly cycles (Tine et al., 2011), two trials delivering 5-monthly cycles (Ndiaye et al., 2019; Tagbor et al., 2016) and five delivering 3-monthly cycles (Bojang et al., 2010; Dicko et al., 2011; Konaté et al., 2011; Sesay et al., 2011; Zongo et al., 2015) All studies estimated SP+AQ efficacy over the months of delivery. In addition, two trials also delivered ITNs to all trial participants (Dicko et al., 2011; Konaté et al., 2011) and two trials delivered SMC drugs to an extended age-range of children up to 10 years old (Ndiaye et al., 2019; Tagbor et al., 2016). Studies generally had low to moderate levels of bias (defined as the risk that they will overestimate or underestimate the true intervention effect), with a single study *Bojang et al*. having a high risk of bias due to the non-randomised control arm and the non-reporting of control arm baseline characteristics (see Appendix 8.3 for summaries of risk of bias assessments) (Bojang et al., 2010). Model parameterisation for each of these trials is summarised in Table 3.4.

Table 3.3 Characteristics of identified controlled studies and extracted data as reported in publications.

	Bojang et al 2010	Dicko et al 2011	Konaté et al 2011	Sesay et al 2011	Zongo et al 2015	Tine et al 2011	Ndiaye et al 2019	Tagbor et al 2016
Country	The Gambia	Mali	Burkina Faso	The Gambia	Burkina Faso	Senegal	Senegal	Ghana
Trial location	Basse – rural	Kati – rural and urban	Boussé - rural	Farafenni - rural	Lena - rural	Bonconto - rural	Saraya - rural	Ejisu-Juaben - rural
Admin-1	Upper River	Koulikoro	Plateau-Central	North Bank	Haut-Bassins	Kolda	Kedougou	Ashanti
Trial year	2007	2008	2008	2008	2009	2010	2011	2012
Baseline indicator of transmission intensity	1-50‡	7-37‡	11-74‡	<10‡	NR	NR	NR	13.3%-57.6%¥
Average rainy season	July - November	June - October	July - October	July - November	May - October	July - November	May - November	May - October
Peak transmission season	October - November	June - November	July - November	October - November	August - October	October - November	July - December	June - November
Delivery months	September, October, November	August, September, October	July, August, September	September, October, November	August, September, October	October, November	July, August, September, October, November	July, August, September, October, November
SMC drugs	SP+AQ	SP+AQ	SP+AQ	SP+AQ	SP+AQ	SP+AQ	SP+AQ	SP+AQ
Number SMC cycles	3	3	3	3	3	2	5	5
Delivery age range	6-59 months	3-59 months	3-59 months	6-59 months	3-59 months	12-119 months	3-119 months	3-59 months
Control group	Non-randomised arm	Placebo	Placebo	Placebo	Non-randomised arm	Cluster randomised	Cluster randomised	Placebo
Delivery method	Trial staff at health facility and caregivers	Trial staff at health facility and caregivers	Trial staff at health facility and caregivers	Community health workers and caregivers	Trial staff at both health facility and at the home	Community health workers at health huts and at the home	Community health workers and caregivers	Trial staff at central community point, at home and caregivers
Diagnostic method	Microscopy	Microscopy	Microscopy	Microscopy	Microscopy	RDT	RDT	RDT
Coverage with SP+AQ*	94%	96%	83%	97%	98%	100%	91%	75%
ITN usage	66% SMC arm NR control arm	>99% both arms	93% both arms	93% both arms	36% SMC arm, 32% control arm	95% both arms	94% SMC arm, 91% control arm	79% SMC arm, 84% control arm
Reported efficacy	93% (95% CI: 80%-98%)	83% (95% CI 80%-86%)	71% (95% CI 68%-74%)	66% (95% CI -228%-96%)	80% (95% CI 72%-86%)	79% (95% CI 58%-90%)	83% (95% CI 74%-89%)	39% (95% CI 7%-59%)
Risk of bias	High risk	Low risk	Low risk	Some concerns	Some concerns	Some concerns	Low risk	Some concerns

‡EIR = Entomological Inoculation Rate per person per year, ¥ parasite prevalence in study children at baseline, *Average monthly probability of receiving SP+AQ. NR = Not reported, RDT = Rapid diagnostic test

Table 3.4 Site specific input parameterisations for SP+AQ model validation

			Bojang et al 2010	Dicko et al 2011	Konaté et al 2011	Sesay et al 2011	Zongo et al 2015	Tine et al 2011	Ndiaye et al 2019	Tagbor et al 2016
Location			Upper River, The Gambia	Koulikoro, Mali	Plateau-Central, Burkina Faso	North Bank, The Gambia	Haut-Bassins, Burkina Faso	Kolda, Senegal	Kedougou, Senegal	Ashanti, Ghana
Trial year			2007	2008	2008	2008	2009	2010	2011	2012
Vector specification	Proportion of mosquito population that is *Anopheles arabiensis*		84%	57%	39%	81%	10%	35%	48%	0.1%
	Proportion of mosquito population that is *Anopheles funestus*		10%	10%	19%	11%	15%	39%	27%	26%
	Proportion of mosquito population that is *Anopheles gambiae*		6%	33%	42%	8%	75%	26%	25%	74%
Seasonality in transmission	Fourier seasonality coefficients	a0	0.6767322	1.145392	0.7792459	0.9180093	1.528932	1.156818	1.418134	2.91713
		a1	-0.8297394	-1.586955	-1.065362	-1.072643	-1.996974	-1.451055	-1.793909	-1.791489
		b1	-0.7726926	-0.94171	-0.6386744	-1.127164	-1.114417	-1.271983	-1.486858	-0.7057204
		a2	0.03991472	0.4384197	0.2898839	-0.03517421	0.452105	0.1400272	0.206244	-0.9524563
		b2	0.6509968	0.8650493	0.6224794	0.948747	0.961317	1.084821	1.226261	-0.9296798
		a3	0.1403766	0.02352499	0.01776126	0.2789854	0.08475795	0.2079542	0.1972055	-0.1804138
		b3	-0.1635258	-0.2828369	-0.2243274	-0.2362525	-0.2745763	-0.289842	-0.2931582	0.9975769
Transmission intensity	Malaria prevalence in 2–10-year-olds in the year before the trial		14.2%	39.8%	46.1%	4.21%	59.2%	7.1%	14.8%	41.5%
Health systems	Treatment coverage the year before the trial Defined as the proportion of clinical cases that receive treatment		57%	31%	43%	58%	44%	46%	45%	57%
Vector interventions	Insecticide treated net usage in the year before the trial defined as the effective coverage of nets within the population		30% Pyrethroid	29% Pyrethroid	6% Pyrethroid	35% Pyrethroid	7% Pyrethroid	37% Pyrethroid	38% Pyrethroid	30% Pyrethroid

Efficacy estimates from these trials varied and ranged between 39%–93% (Table 3.3 & Figure 3.4B). Model-estimated SP+AQ efficacy was in-line with all the reported trial estimates, falling within trial reported confidence-intervals of all trials (Figure 3.4B-C). When considering the studies with the lowest risk of bias (*Dicko et al., Konate et al., Ndiaye et al*.) model-estimated efficacy was similar to that reported in the trial data. Model and trial results for these studies are highlighted in Table 3.5.

Table 3.5 Comparison of efficacy estimates and model-estimated efficacies for the highest quality studies, assessed as having a 'low risk of bias', identified in the literature search.

Trial	Reported Efficacy	Model estimated efficacy
(Dicko et al., 2011)	83% (95% CI 80%-86%)	80% (95% CrI 76%-84%)
(Konaté et al., 2011)	71% (95% CI 68%-74%)	68% (95% CrI 62%-71%)
(Ndiaye et al., 2019)	83% (95% CI 74%-89%)	80% (95% CrI 76%-81%)

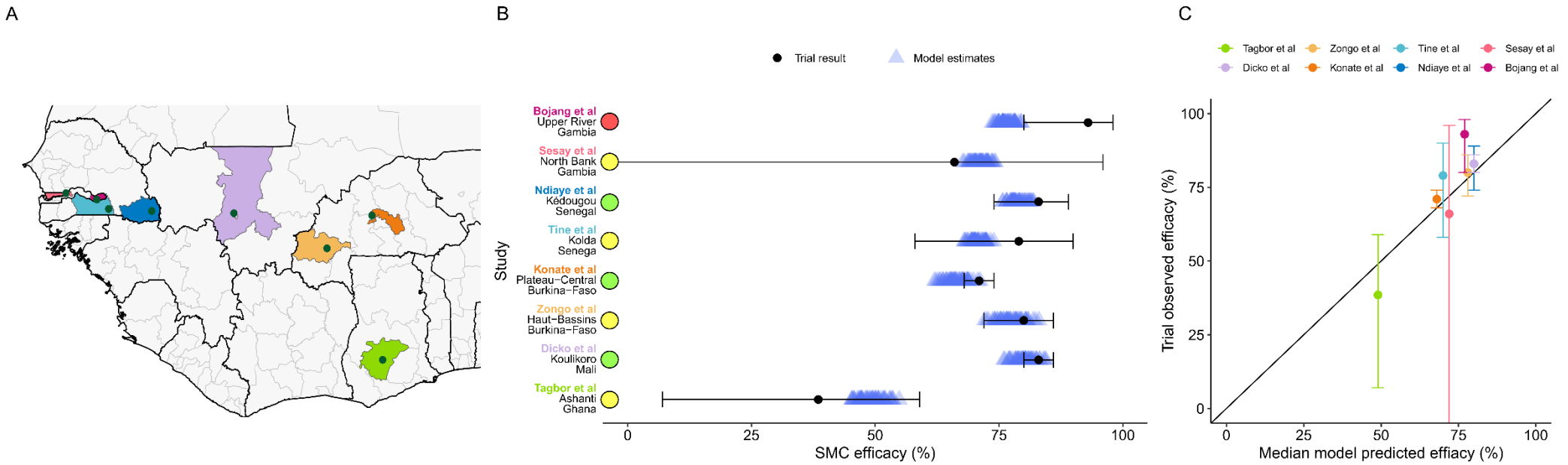

Figure 3.4 SP+AQ efficacy profile validation. A) Map displaying the admin-1 locations of the trials included in the validation with dots indicating the locality within the admin-1 unit. **B)** Summary of trial reported SP+AQ efficacy with 95% Confidence Intervals. Model-estimated efficacy for each trial represented by blue triangles, from 50 posterior draws of the SP+AQ efficacy profile. Coloured circles represent the risk of bias assessment results, red: high risk, yellow: some concerns, green: low risk. **C)** Equivalence comparison between trial observed and median model-predicted efficacy over 50 parameter draws. Trial efficacy is reported with 95% Confidence Intervals. The Confidence Interval for Sesay et al extends to -228% and is cut off for clarity. Diagonal line represents equivalence between observed efficacy and model estimated efficacy.

3.4.4 SMC protocol extension

The trials identified in the above validation process all highlight the potentially high levels of efficacy that can be achieved with SMC in a range of settings and over a range of delivery protocols (2-, 3- or 5- monthly cycles). Under the WHO guidance those countries that have now adopted SMC into their national malaria control programmes (Figure 3.5) have all implemented 4-monthly cycles of SMC (Baba et al., 2020). Using the fitted SP+AQ efficacy profile I wanted to explore the potential impacts of scaling up the number of SMC monthly cycles in countries of the Sahel and West African coastal regions and estimate impact over a full year to account for the variations in transmission seasonality.

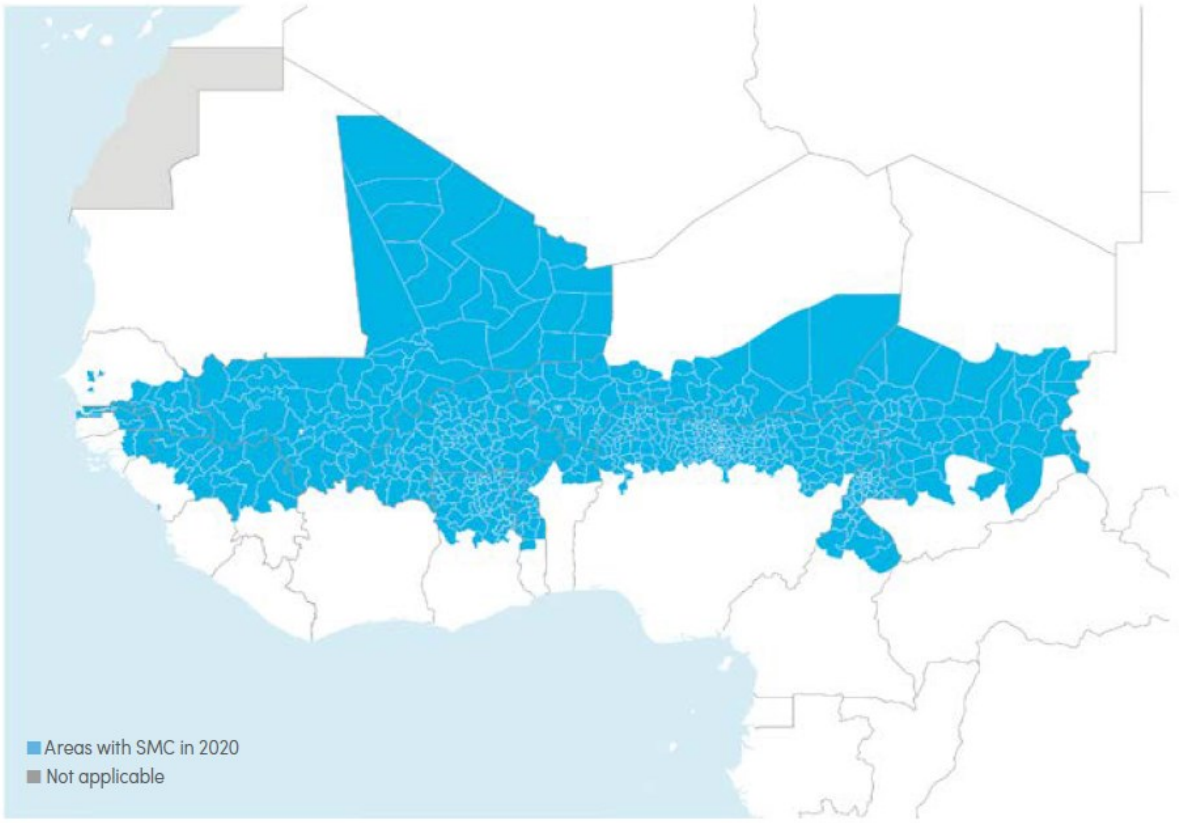

Figure 3.5 Areas that have integrated SMC into their national malaria control programme as of 2020. Map produced by Medicines for Malaria Venture and SMC Alliance and reproduced from the World Malaria Report 2021.

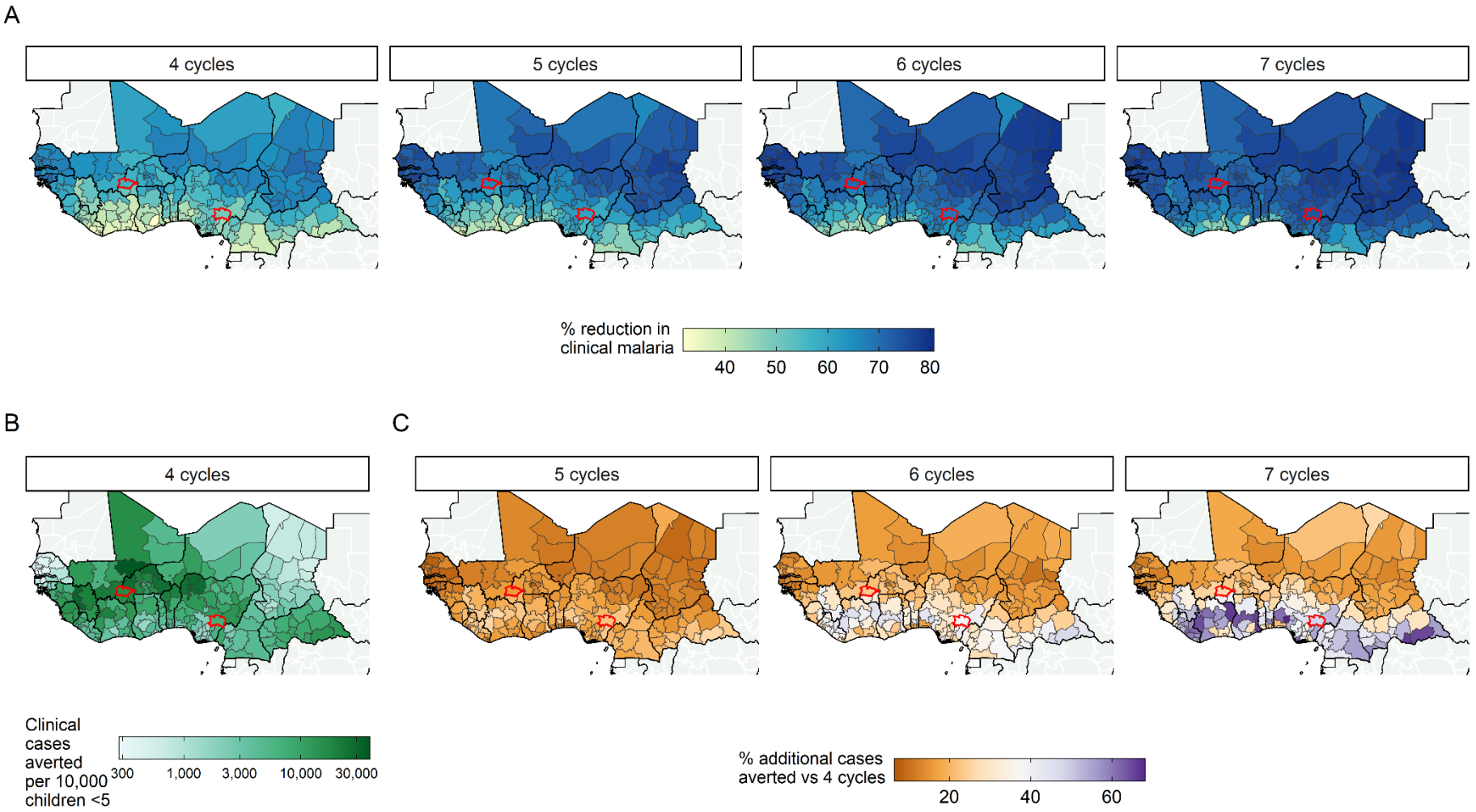

Figure 3.6 Impact of additional cycles of SMC with SP+AQ in Sahel and Sub-Sahel countries. A) % reductions in clinical malaria in children aged 0-5 for 4, 5, 6 or 7 monthly cycles of SMC over the course of 12-months. **B)** The number of clinical malaria cases averted over 12-months following 4 monthly cycles of SMC compared to no SMC. **C)** The percentage additional clinical malaria cases averted in children 0-5 years with 5, 6, or 7 monthly cycles of SMC compared to 4 cycles over the course of 12-months. Outlined in red are the two areas described in the results and in Figure 3.7.

The maps in Figure 3.6 highlight a distinction over the Sahel region in terms of the number of monthly cycles of SMC needed for the largest proportional reductions in malaria burden when measured over a full year. These maps highlight that increasing numbers of monthly cycles were needed to maintain a high level of impact in the southern regions of West Africa where the duration of malaria transmission tends to be longer and less peaked (Figure 3.6). Increasing the number of SMC cycles would enable countries and regions within countries that currently do no implement SMC to potentially integrate this into their control programmes (Figure 3.5, Figure 3.6). For example, many states in Nigeria that experience seasonality in malaria transmission could benefit particularly from extended SMC cycles (Figure 3.6). As an example, the average modelled seasonality in malaria transmission of Benue state in central Nigeria is shown in Figure 3.7A. With each successive increase in the number of monthly cycles an increased proportion of the transmission season could be covered. With 7-monthly cycles in this setting the model estimated a 72% (95% CrI 69%–76%) reduction in clinical incidence in children under five over 12 months compared to a 49% (95% CI 47%–52%) with 4-monthly cycles. This increase to 7-monthly cycles resulted in an additional 3,402 (95% CrI 3,265–3,528) clinical cases averted per 10,000 children aged under five years over 4-monthly cycles.

These maps also highlight that in those locations currently delivering 4-monthly cycles of SMC a further increase in the number of monthly cycles could further enhance malaria burden reductions. For example, using the transmission model parameterised to the Haut-Bassins region of Burkina Faso where 4-monthly cycles of SMC have been routinely administrated since 2015. Each successive

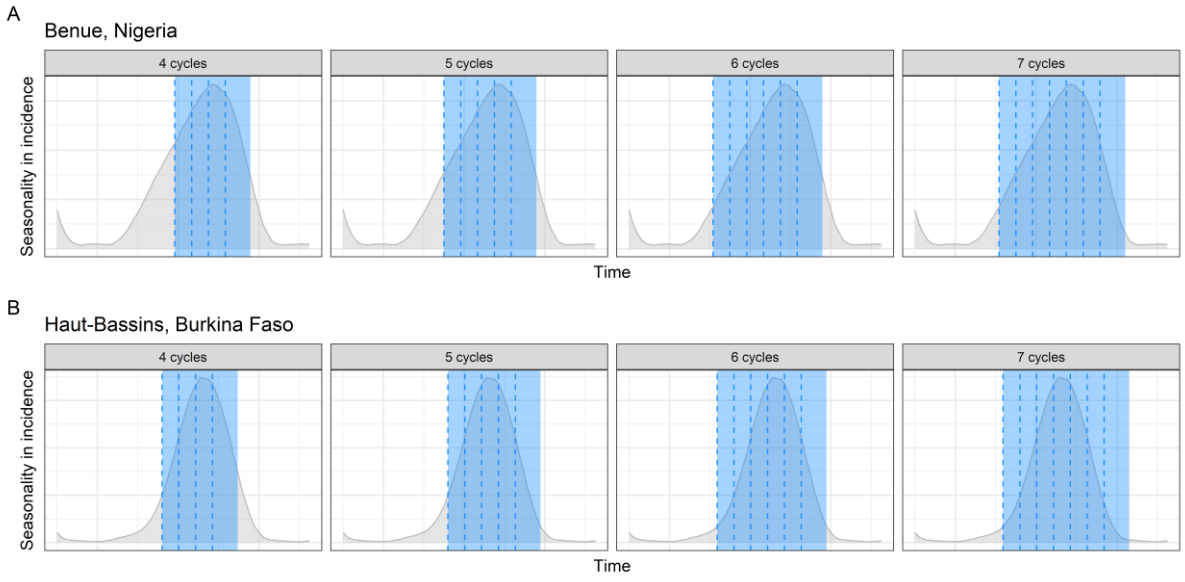

Figure 3.7 Coverage of the transmission season in A) Benue Nigeria and B) Haut-Bassins Burkina Faso with increasing numbers of monthly cycles of SMC. Seasonality in transmission is derived from averaged rainfall time series data Fourier transformed across the geographic boundaries of each administrative unit. Dashed vertical lines represent model delivered SMC cycles and the shaded areas the periods of SP+AQ derived protection.

increase in monthly cycle number reduced clinical cases in children under five by a further 16% (95% CrI 15%–17%), 23% (95% CrI 22%–24%) or 26% (95% CrI 24%–27%) compared with 4-monthly cycles. The modelled seasonality in malaria transmission for this administrative unit and the period of the transmission season covered by each different cycle length is shown in Figure 3.7B, highlighting how increasing the number of monthly cycles provides protection in the months surrounding the peak of the transmission season where residual transmission remains.

3.5 Discussion

In this chapter I present estimates of the functional form of the decay in SP+AQ protection against clinical malaria over time using previously published SMC clinical trial data. I have validated this profile against the results of published randomised controlled trials using an individual based malaria transmission model. Finally, using this same individual based malaria transmission model and deploying this validated model of SP+AQ efficacy, I examine the potential population level public health impacts of increasing the number of monthly cycles of SMC with SP+AQ in Sahel countries that experience varying levels of seasonality in malaria transmission.

Understanding the duration of protection from anti-malarial interventions is critical, especially for drug-based interventions whose effects are time dependent according to the pharmacokinetic and pharmacodynamic properties of the drug or combinations of drugs. Previous estimates of the duration of protection from SMC drugs in clinical trials relied on point estimates of efficacy at set time points (Cairns et al., 2008, 2010) or as time varying effects using smoothed regression splines (Zongo et al., 2015; Lambert & Royston, 2009). However, no previous work has estimated a parametric functional form of protection with associated uncertainty suitable for integration into a transmission modelling framework, to be validated by assessing its ability to replicate overall trial efficacy estimates across different settings. I found that efficacy is best characterised by a Weibull cumulative distribution function and that a high level of protection is maintained for around 20 days before declining to zero protection by day 60. A higher degree of uncertainty was observed in the tail of the distribution was likely a result of the reduction in population at risk at these later time points. Independent estimates of efficacy estimated through the smoothed regression splines we found to fall within the credible intervals of the Weibull distribution estimated here suggesting the results from both analyses are aligned (Figure 3.8). Further to this, previous studies that have estimated SP+AQ efficacy or effectiveness at between 64% to 88% by 28 days (Cairns et al., 2021; Dicko et al., 2011; Konaté et al., 2011; World Health Organization, 2011b; Cairns et al., 2010; Cissé et al., 2006; Cairns et al., 2020; Bojang et al., 2011). Here we estimated protection to be 73% (95% CrI 52%-85%) by day 28. These

results together highlight the importance of ensuring SP+AQ SMC delivery is spaced at monthly intervals to ensure high levels of protection are maintained across the period of greatest malaria risk.

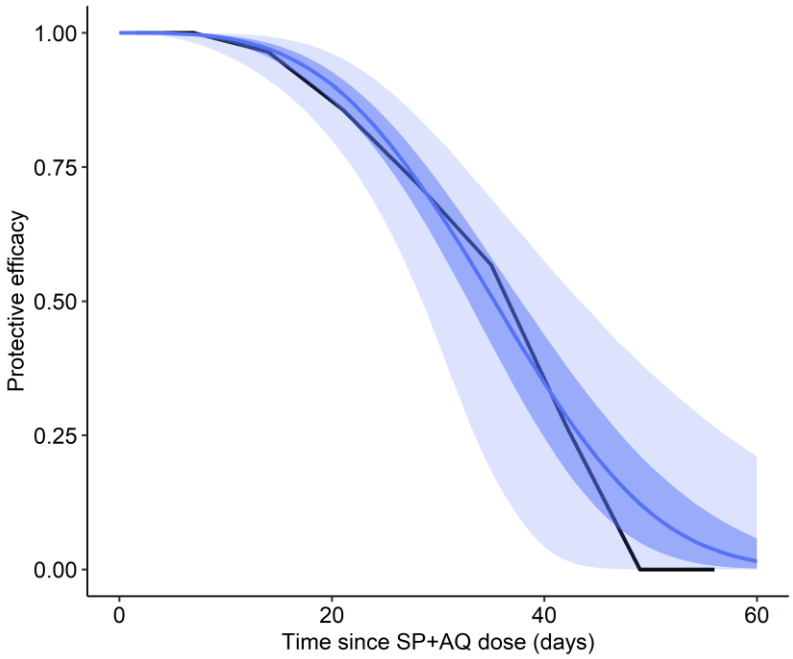

Figure 3.8 Comparison of the best fitting SP+AQ decay function (blue) and a previously published efficacy profile estimated using regression splines (black) in *Zongo et al., 2015*. Shaded areas represent 50% and 95% Credible Intervals.

Results of the validation exercise provide further evidence that the functional form of SP+AQ efficacy estimated in this work replicates that of the dug-combination. This was an important additional step to test given that the trials sites had varying levels of malaria transmission intensity which will have affected the time to infection, dosing rules (weight or age based), detection methods, seasonality, vector control and other individual level variations. Critically the model estimates aligned extremely well with the clinical trials assessed to have the lowest risk of bias which themselves had the narrowest confidence intervals surrounding their efficacy estimates. This validation further supports the notion that the transmission model is also accurately capturing seasonal dynamics and transmission intensity in the Sahel (Cairns et al., 2015).

The model however struggled to replicate trial results from *Bojang et al.* (Upper River, Gambia) (Bojang et al., 2010), which may be a result of the limited reporting of information surrounding the control arm in this trial which itself was non-randomised and not placebo controlled and therefore unlikely captured well by the model. The model also struggled to replicate the results of *Tagbor et al.* (Ashanti, Ghana) (Tagbor et al., 2016) despite the model managing to replicate the standing of this

trial having the lowest efficacy of the trails considered, the model point estimate was significantly higher than that reported. This could potentially be a result of variation that we are not able to capture in the model such as adherence levels to the study drugs, which was reported to be implausibly high. Furthermore, as age-based dosing was used, potential under-dosing in children might have reduced the protective effect in this trial (Barnes, Watkins & White, 2008).

A further important aspect that wasn't captured in this work was the potential levels of parasite resistance to SP+AQ drugs in any of the studies or the fitting process. Significant resistance to both SP and AQ is common in much of Africa, particularly in areas of eastern or southern Africa where a high prevalence of antifolate resistance to SP makes these regimes unsuitable for SMC (Naidoo & Roper, 2011; Okell, Griffin & Roper, 2017; Bwire, Mikomangwa & Kilonzi, 2020; Sitali et al., 2020; Cairns et al., 2012). A small subset sample from the Zongo et al study highlighted that the prevalence of resistance markers to SP and AQ were high in this trial both at baseline and endline but the impact of the prevalence of these resistance markers on the efficacy of the drugs was unknown (Zongo et al., 2015). Given however that efficacy was estimated to remain high for a long time, which was reflected further in the trial validation, it is reasonable to infer that resistance was not having too much of an impact in reducing efficacy in these trials. Especially as these trials were all conducted prior to widescale use of SP+AQ in SMC campaigns. Previous work has shown that high community prevalence of 86Y and 76T AQ resistance markers reduced the duration of post-treatment prophylaxis of AS-AQ significantly and as such would be an important consideration to examine the impact of different resistance markers on SP+AQ efficacy given the widespread use of this drug-combination (Bretscher et al., 2020). While studies have been conducted monitoring the prevalence of SP and AQ resistance markers in communities delivering SMC this is not sufficient to understand the impact of resistance patterns on SMC efficacy and further studies are needed (Baba et al., 2020).

As of 2020, 13 countries have adopted SMC into their national malaria control programmes with around 33.5 million children receiving treatment each month (World Health Organization, 2021g). Despite the complexity of delivering SMC at scale, many countries have achieved high coverage and have seen substantial reductions in malaria cases (Baba et al., 2020). However, despite these benefits, countries where SMC is currently deployed include some of the highest malaria burden countries including Nigeria, Burkina Faso and Mali (World Health Organization, 2021g). Despite the relatively high coverage of SMC in these countries this 'residual burden' of malaria that persists in the presence of current interventions must be tackled by novel methods. This could include for example, increasing the age range of SMC delivery up to 10 years old, combining delivery of other health related interventions (antibiotics, nutritional supplements or vaccination, which I will explore further in Chapters 4 and 5) or delivering more than the currently recommended 4-monthly cycles of SMC to

cover the burden that occurs outside the peak transmission season (Word Health Organization, 2019; Tine et al., 2011; Ndiaye et al., 2019; Sondo et al., 2021; Chandramohan et al., 2019, 2020). The findings in this work indicate that if SMC could be administered over a longer period of 5-, 6- or even 7-months this could optimise SMC maximising reductions in malaria case incidence in countries currently delivering SMC and could also result in the expansion of SMC to geographical areas where the period of highest malaria risk is greater than four months, for example in the southern states of Nigeria or Ghana. This increase in monthly cycles has recently been implemented in Burkina Faso and Mali where 5-monthly cycles are now the standard of care. In addition, increasing the number of monthly cycles of SMC has the operational benefit of potentially making SMC programmes more resilient to annual fluctuations in the arrival or duration of the rainy season. Further recent modelling work has highlighted that increasing the number of cycles of SMC might not increase the selective pressure for resistance as such making it an attractive control method (Cuomo-Dannenburg et al., 2019).

However, increasing the monthly cycles of SMC raises important operational questions, including challenges in the delivery and supply of drug courses and challenges in ensuring high coverage and adherence to regimes. Operationally delivering SMC requires a large commitment of time and resources by National Malaria Control Programmes and their partners especially with the door-to-door campaigns that help to ensure good coverage levels (Baba et al., 2020; Coldiron, Von Seidlein & Grais, 2017). Coverage of routine SMC delivery has shown that only around 50% of all eligible children received all four monthly cycles of SMC in 2016 (Baba et al., 2020), and when five monthly cycles were assessed in a clinical trial only 36% of children received all five cycles (Tagbor et al., 2016). Further to this adherence to the three daily doses is difficult to measure and with reports of both high adherence (>86%) and low adherence (<20%) to the three-day AQ regime in routine settings (Baba et al., 2020; Ding et al., 2020). Therefore, with increased cycles, medicine fatigue could hamper the potential impacts of this intervention. To combat these deployment challenges the development of long acting injectables, such as small molecule drugs or monoclonal antibodies, have been targeted as attractive interventions to replace or complement SMC (Kisalu et al., 2018; Tan et al., 2018; Bakshi et al., 2018). Recent modelling work highlighted that these interventions could make an attractive replacement only if the duration of protection from a single dose covered the duration of the transmission season and that early characterisation of the decay in protection will be essential to improving their impact relative to SMC (Burgert et al., 2022).

3.6 Limitations

There are several limitations to this work. Firstly, from the model fitting for the duration of SP+AQ protection the dataset was relatively small and only covered a single trial, as children are removed

from the dataset clinical cases during the later stages have a large impact on the model estimated hazards. There is therefore uncertainty in the shape of the functional form of protection that increases when we account for the temporal variations in the risk of clinical malaria that occurred over the transmission season. Further to this the data itself has limitations. This dataset only contained data from the final monthly cycle of SMC in this trial. There is the potential therefore for children to have residual levels of SP and AQ concentrations from the two preceding cycles that might impact the levels of observed protection in this trial. With further datasets covering each distribution cycle this could be resolved. The pattern of incidence of symptomatic episodes of clinical malaria will depend on exposure to infectious mosquito bites, with age influencing the acquisition of infection given levels of clinical immunity. Given the small dataset from a single site that was available for this analysis a mathematical model that accounts for the age and exposure dependent acquisition of clinical immunity was considered too complex for the dataset. However, these models have been used previously when combining data from studies across different geographical locations and transmission intensities to estimate both vaccine and anti-malarial efficacy over time and could be used in future studies if estimating efficacy of SP+AQ from individual level data across SMC clinical studies (Bretscher et al., 2020; White et al., 2015). This may also help to resolve some of the uncertainty in the efficacy at later time points with further datasets and model formulations. Finally, when considering the population level modelling of SMC we use average rainfall patterns across first administrative units in the Sahel and Sub-Sahel region to determine the seasonality in malaria transmission within that geographic range. However, seasonality patterns within these geographic units can be highly variable especially when administrative units span large geographical ranges as in Mali for example. Therefore, the results from the protocol extension provide a representative impact estimate for generalised seasonality patterns.

3.7 Conclusions

Seasonal malaria chemoprevention with SP+AQ has been demonstrated to be a highly efficacious tool in preventing clinical malaria across a number of clinical trials and has since shown high levels of effectiveness in routine programmatic conditions. Results of this chapter further our understanding of the decay in SP+AQ efficacy over time and the implications of this decay in ensuring optimised SMC programmes. The formulation of a simple model to estimate the parametric functional form of SP+AQ efficacy enabled quantification of uncertainty in the duration of protection something not previously available in our transmission model structure. With ongoing discussions on the ways to optimise SMC this work has shown that increasing the number of monthly cycles of SMC would enable the expansion of SMC into new geographies but also increases to the public health impact of SMC in those areas already implementing SMC.

Chapter 4

4 Validation and exploration of efficacy models for RTS,S/AS01$_E$ when used in a novel seasonal vaccination schedule

In this chapter I use the efficacy model of Sulfadoxine-Pyrimethamine plus Amodiaquine (SP+AQ) fitted in Chapter 3 and an existing model of first-generation RTS,S/AS01$_E$ (RTS,S) protection to replicate the first large-scale field trial of a novel RTS,S vaccination approach in the Imperial College London malaria transmission model. This replication of the seasonal vaccination trial is used to understand if the first-generation RTS,S efficacy parameterisation is sufficient to describe RTS,S protection in this trial or if a potential re-parameterisation is required.

This work formed part of the evidence packet submitted to the Malaria Policy Advisory Committee meeting in October 2021: http://terrance.who.int/mediacentre/data/malaria/documents/mpag-october2021-session5-rtss-malaria-vaccine.pdf?sfvrsn=9507a63b_10

This work is under review at The Lancet Global Health.

4.1 Background

First-generation RTS,S/AS01$_E$ was assessed in Phase 3 field trials between 2009-2014, in 11 sites of varying transmission intensity across sub-Saharan Africa (RTSS Clinical Trials Partnership, 2015). Efficacy against clinical malaria was 43.9% (95% CI 39.7%–47.8%) in children aged 5–17 months who received four doses of the vaccine after 32 months of follow up. Efficacy was shown to wane significantly over the course of the trial with efficacy of 67.6% in 5–17-month-old children in the 6 months following the third dose which declined to extremely low levels (~4%) by seven years despite the delivery of a fourth dose (Olotu et al., 2016).

The trial provided estimates of efficacy against clinical malaria but did not provide direct estimates of the underlying protection against infection, defined as the probability that the vaccine-induced immune responses prevent infection with pre-erythrocytic stages of malaria. This measure of vaccine efficacy against infection can be characterised directly in human challenge trials as described in Chapter 2, whereas in large scale field trials vaccine efficacy against clinical malaria is calculated as a relative measure of the incidence of malaria between control and vaccination groups. However, reductions in malaria infections in the vaccination group might reduce naturally acquired blood-stage immunity over time compared to in the control group, which will influence the levels of observed efficacy against clinical malaria, causing efficacy against clinical malaria to wane at a faster rate than efficacy against infection (White et al., 2015). This effect may be a factor in the observation in the longer term follow up where efficacy against clinical malaria during the fifth year of follow up was negative (i.e higher numbers of malaria cases in vaccinated than in the control group) among children in high transmission sites (Olotu et al., 2016)).

Mathematical models of malaria transmission can be used to characterise vaccine efficacy against infection and the duration of this protection over time, by accounting for the dynamics of local malaria transmission and the acquisition of natural immunity that can be related to observed levels of efficacy against clinical malaria in trial cohorts. Previously different malaria transmission models with their underlying differences in model assumptions have been used for the purpose of characterising infection blocking efficacy (Penny et al., 2016). Despite the underlying differences in transmission models and fitting procedures, all groups came to qualitatively similar conclusions that RTS,S has a high initial efficacy following the third dose which declines rapidly in the first 12 months, with slower rates of decline after one year post dose three. All models again predicted lower efficacy of the fourth dose compared to the primary series with variable estimates in the rates of waning in protection following this fourth dose (see *Penny et al. 2016* for full review).

Given this high initial protection against malaria infection and disease that occurs following the first three doses of RTS,S and the associated decline over the ensuing months, a novel approach to RTS,S vaccination has garnered interest as a way to more effectively deploy RTS,S. It has been suggested that RTS,S could be used as a seasonal vaccine in areas of highly seasonal malaria transmission such as the Sahel or other areas with high seasonality including East Africa (Greenwood et al., 2017). This seasonal vaccination strategy would use the same first-generation formulation of RTS,S but primary doses (doses 1–3) would be delivered to young children (5–17 months old) immediately prior to the onset of the transmission season with subsequent additional doses delivered to these children annually prior to the transmission season (Chandramohan et al., 2020). With this approach the hope

is that the peak in RTS,S efficacy would align with the period of peak risk in malaria annually in these seasonal settings, with lower levels of protection during the intervening dry season.

As discussed in Chapter 3, seasonal malaria chemoprevention (SMC) with SP+AQ is currently deployed in settings of seasonal malaria transmission to provide prophylactic cover to children aged 0–5 years old for the four months of highest malaria burden. Despite effective delivery of SMC there are areas of the Sahel where malaria morbidity and mortality remain high, including Burkina Faso for example, which is one of the six countries that accounts for almost half the global malaria burden (World Health Organization, 2021g). Additional control tools are needed in these counties. Previous studies have examined the additional benefit of adding azithromycin to the SMC drug course but found that this did not reduce hospital admissions or deaths from non-traumatic causes (Chandramohan et al., 2019). Thus, determining whether seasonally targeted RTS,S could be an alternative or additive tool to SMC is warranted.

To evaluate seasonal RTS,S vaccination, an individually randomised Phase 3b clinical trial was conducted in children aged 5–17 months old in two sites of high intensity highly seasonal malaria transmission in Burkina Faso and Mali. This study was designed to assess whether vaccination was non-inferior to SMC in preventing clinical malaria and/or whether when combined the interventions were superior to either alone in preventing clinical and severe malaria (Chandramohan et al., 2020). This is the first large-scale RTS,S field trial of a novel delivery schedule to be completed. Approximately 6,000 children were randomised and over three-years of follow up, seasonally targeted RTS,S vaccination alone was found to be non-inferior to SMC in preventing clinical malaria, the hazard ratio was 0.92, (95% CI: 0.84–1.01), which excluded the pre-specified noninferiority margin of 1.20. And the combination of interventions resulted in reductions in the incidence of clinical malaria, severe malaria, and deaths from malaria substantially greater than either intervention alone (Chandramohan et al., 2021).

4.2 Aims

In this Chapter I use the Imperial College London malaria transmission model to replicate the Phase 3b seasonal vaccination clinical trial to understand if the previously fitted and validated models of RTS,S/AS01 and SP+AQ are able to replicate the results observed under this novel schedule. Validating models against clinical trial data is a valuable way to ensue the model is accurately capturing the magnitude and duration of impact of these interventions across multiple clinical outcomes. This is useful for confirming the mechanistic relationships between interventions and health outcomes in the model and the predictive ability of the transmission model as a whole, which is important for increasing confidence in future predictions. Any changes to the underlying intervention efficacy

models as a result of this validation will then be used in Chapter 5 to examine the population-level public health impacts of this novel seasonal vaccination schedule.

4.3 Methods

4.3.1 Clinical trial data

The seasonal malaria vaccination Phase 3b clinical trial occurred in two locations in southern Burkina Faso and Mali over the years 2017–2020 (Figure 4.1) (Chandramohan et al., 2021). There were three trial arms: seasonal-vaccination (SV) alone; SMC alone; and SV and SMC combined. Children aged 5–17 months of age on April 1st, 2017, were enrolled into the trial and all children received a long-lasting insecticide treated bed net at enrolment. Children in the vaccine alone or combined group were scheduled to receive three doses of the RTS,S/AS01$_E$ vaccine in April, May and June 2017 followed by a fourth and fifth dose in June 2018 and 2019. Children in the SMC-only arm received a rabies vaccine in 2017 (Rabipur) and a single dose of hepatitis A vaccine (Havrix) in 2018 and 2019. Children in the SMC-alone or combined group received four courses of SP+AQ at monthly intervals during the transmission season each year whilst children in the vaccine-alone group received SP+AQ placebo at the same time points. SP+AQ doses were age dependent with children 12 months or older receiving 500mg of sulfadoxine, 25mg of pyrimethamine and 150mg of amodiaquine, and with infants younger than 12 months receiving half of these doses. Administration of SP+AQ or placebo was directly observed by trial staff at distribution points in the village. Children who presented with malaria symptoms were tested for malaria at health facilities with the use of a rapid diagnostic test and

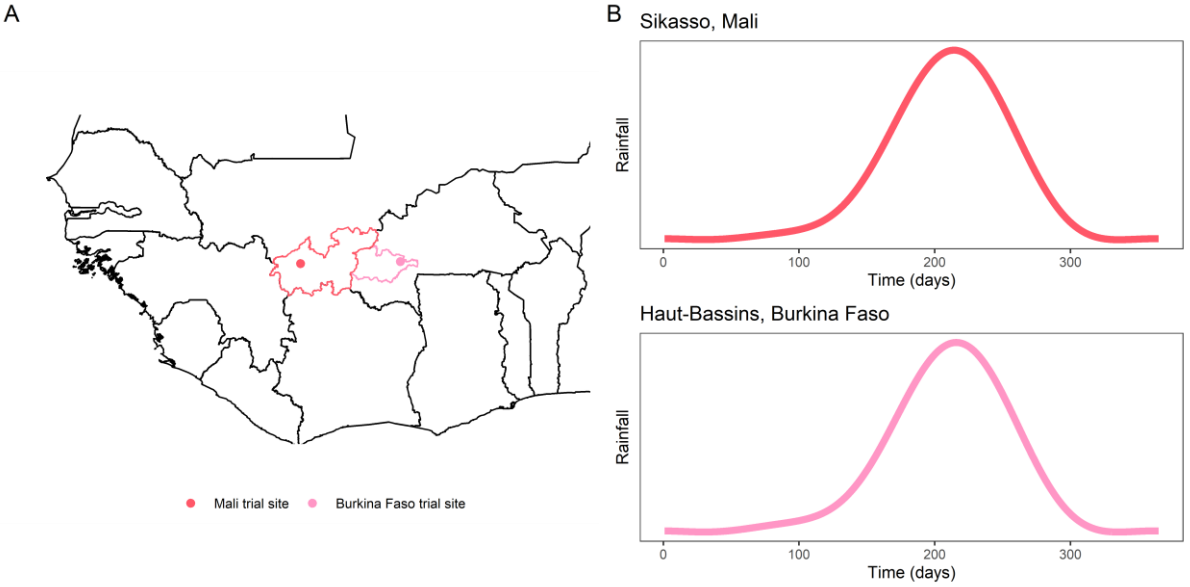

Figure 4.1 Geographical locations of trial sites and their corresponding modelled seasonality profiles. **A)** Geographical locations of trial sites (dots) and their respective administrative level one boundaries (outline) used in model simulations. **B)** The associated seasonality patterns in rainfall that drive seasonal patterns in malaria incidence in these administrative level one locations used in model simulations.

children who were positive were treated with artemether-lumefantrine and a blood film was taken for subsequent reading. The primary trial outcome was uncomplicated clinical malaria, defined as a measured temperature ≥37.5^{o}C or a history of fever within the previous 48 hours and parasite density ≥5000 per cubic millimetre. In addition, sera from a randomly selected subset of children collected before and one-month after the third, fourth and fifth doses of RTS,S were collected each year. Anti-CSP IgG antibody titres were measured using standardised ELISA (Sagara et al., 2021).

4.3.2 Model methods

Results from the clinical trial were compared to model predictions to determine whether the current intervention parameterisations of RTS,S and SMC satisfactorily matched the observed trial data. As in previous chapters, I use the Imperial College London malaria transmission model for this validation. Details of the full model are presented in Appendix 8.1 and I briefly describe the intervention models here.

4.3.2.1 SMC SP+AQ intervention model

The fitted SP+AQ SMC efficacy model from Chapter 3 was taken forward into this analysis. Briefly, to describe SP+AQ action within the model, I assume that SP+AQ directly treats any existing infections with a given clearance probability which then moves all infected individuals to a state of prophylaxis. SP+AQ also provides a period of drug-dependent prophylaxis to individuals who weren't infected. The period of prophylactic protection for all individuals is captured by the fitted Weibull distribution from Chapter 3:

$$P_{SPAQ}(t) = \exp^{-(t/\lambda)^k} \quad (4.1)$$

Parameters describing the duration of protection are shown in Table **4.1** and the corresponding efficacy profile in Figure 4.2.

Table 4.1 SMC parameters used in the analysis.

Parameter & description	Value
Probability of successfully clearing infection	0.95
Shape parameter of Weibull distribution (k)	3.40
Scale parameter of Weibull distribution (λ)	39.34

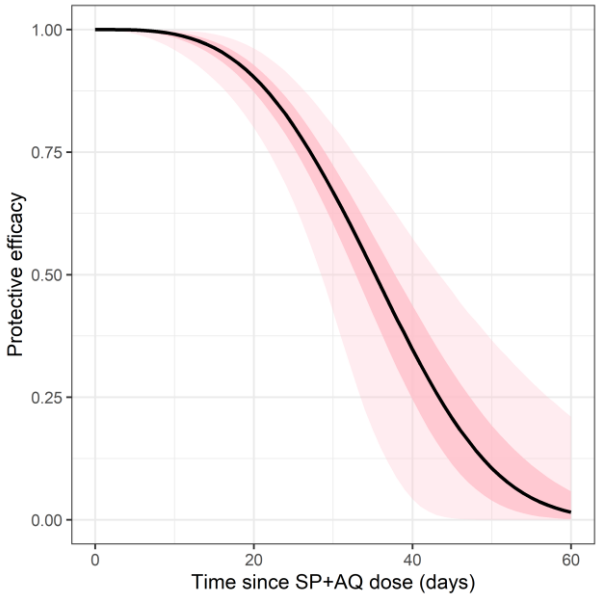

Figure 4.2 Efficacy profile of SP+AQ over time. Solid line representing the median model estimate and the shaded areas the 50% and 95% Credible Intervals.

4.3.2.2 RTS,S antibody model of vaccine efficacy

To establish the relationship between anti-circumsporozoite antibodies and vaccine efficacy, *White et al.* analysed immunogenicity data following primary vaccination with RTS,S/AS01$_E$ with and without a booster dose from the 11 Phase 3 clinical trial sites, and assessed how efficacy against clinical malaria depended on the rate of waning of vaccine induced antibodies in these study sites of varying transmission intensity (White et al., 2015). The model is composed of two parts: the antibody dynamics compartment and the association between antibodies and efficacy against clinical malaria.

Antibody dynamics

This part of the model takes the form of a biphasic exponential decay function. This function simulates the initial increase, and then decay, of antibody titres induced by the combined short-lived and long-lived B cell responses to vaccination. Following three doses of RTS,S, antibody titres peak $\left(CSP_{peak}\right)$ and then decline over time such that the antibody titre at time (t) is given by:

$$CSP(t) = CSP_{peak}\left(\rho_{peak}e^{-r_s t} + \left(1 - \rho_{peak}\right)e^{-r_l t}\right) \quad (4.2)$$

This function contains two components to the immune response: the short-lived and long-lived antibody-secreting cells (ASC), with separate decay rates included in the equations as (r_s) and (r_l) respectively where $r_s = log_e(2)/d_s$ and $r_l = log_e(2)/d_l$, where d_s and d_l are the half-lives of each respective component. The proportion of antibodies generated from short-lived and long-lived ASC are incorporated here as: ρ_{peak} and $1 - \rho_{peak}$ respectively.

Following a fourth dose at time (t_{four}) titres will peak again to their new level $\left(CSP_{four}\right)$ and then decline. I assume that the decay rates of these post-dose four antibodies remain the same as above but that the proportion of the response that is generated from short-lived ASC (ρ_{four}) is allowed to decrease to represent the priming that has been achieved following the first three doses. For any time-point post dose four, the antibody titre will therefore be described by the equation:

$$CSP(t) = CSP_{four}\left(\rho_{four}e^{-r_s\left(t-t_{four}\right)} + \left(1 - \rho_{four}\right)e^{-r_l\left(t-t_{four}\right)}\right) \quad (4.3)$$

To account for an additional fifth dose of RTS,S at time (t_{five}), I assume the same relationship as in equation 4.3 to describe the peak and decline in antibodies over time:

$$CSP(t) = CSP_{five}\left(\rho_{five}e^{-r_s\left(t-t_{five}\right)} + \left(1 - \rho_{five}\right)e^{-r_l\left(t-t_{five}\right)}\right) \quad (4.4)$$

The fitted parameters describing the antibody dynamics model are shown in Table 4.2. The observed peak antibody titre following the primary series (CSP_{peak}) was taken as the median value of the 11 sites in the Phase 3 trial which was 621 EU/ml and for CSP_{four} and CSP_{five} this was 277 EU/ml (White

et al., 2015). Variation between individuals is captured by drawing antibody dynamics parameters from a Normal distribution on a natural log-scale.

Table 4.2 Fitted parameters used for the antibody dynamics and dose response relationship between antibody titres and vaccine efficacy against infection. Median parameter **v**alues from *White et al.* 2015.

Parameter		**Value**
Half-life of antibody component generated from short-lived ASC (mean in days)	d_s	45 days
Half-life of antibody component generated from long-lived ASC (days, sampled from log-normal distribution)	d_s	591 days
Proportion of antibodies generated from short-lived ASC following primary schedule	ρ_{peak}	0.88
Proportion of antibodies generated from short-lived ASC following dose four and dose five	ρ_{four} ρ_{five}	0.70
Shape parameter of the dose-response curve	α	0.74
Scale parameter of the dose-response curve	β	99.2 EU/mL
Maximum efficacy against infection	$Vmax$	0.93

Relating antibody dynamics to efficacy against clinical malaria

To relate declining antibody titres to vaccine efficacy against infection over time, I use a Hill function to represent the dose-response curve:

$$V(t) = V_{max}1 - \left(\frac{1}{1 + \left(\frac{(CSP(t)}{\beta}\right)^{\alpha}}\right), \quad (4.5)$$

where V_{max} (maximum efficacy against infection), α (shape parameter – initial efficacy) and β (scale parameter – duration of protection) were estimated during fitting and their values are given in Table 4.2. For a clinical episode of malaria to be prevented, antibodies act to prevent or reduce the probability that sporozoites are successful at initiating blood stage infection.

Figure 4.3 shows the resulting decay in antibody titres and vaccine efficacy over time for a five dose RTS,S schedule with the fourth and fifth doses given 12 months after the primary series using the fitted parameters from the Phase 3 trial analysis in Table 4.2.

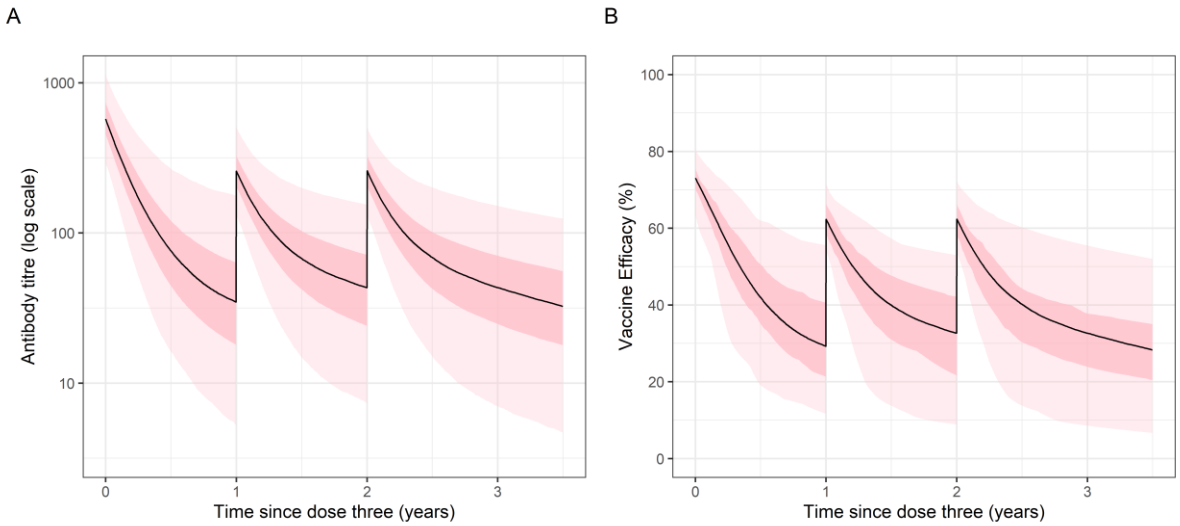

Figure 4.3 Antibody titre (A) and efficacy profile (B), for a five-dose RTS,S schedule assuming the same parameters as in White et al. (2015). The heavy black line is the median of 5000 simulations, and the dark and light pink shaded regions represent the 50% and 95% predictive intervals respectively.

4.3.2.3 Validation

For each trial the first administrative unit (admin-1) of the trial location was used for simulations with admin-1 boundaries sourced from GADM (GADM, 2021) (Figure 4.1A). To capture key characteristics of the trial cohorts, the Imperial College London malaria transmission model described in Appendix 8.1 was calibrated to Malaria Atlas Project (MAP) prevalence estimates and to clinical malaria cases obtained from the World Malaria Report for the trial admin-1 locations for 2016 the year before the trial interventions were delivered (Pfeffer et al., 2018; World Health Organization, 2020b) (Table 4.3). Location-specific seasonality profiles (Figure 4.1B) were derived from the first three frequencies of a Fourier transformation to historic rainfall patterns from the Climate Hazards Group InfraRed Precipitation with Station (CHIRPS) data (Table 4.3) (Funk et al., 2015; CHIRPS, 1999; Garske, Ferguson & Ghani, 2013). Historic treatment coverage levels were derived from the Demographic and Health Surveys and insecticide-treated bed-net usage from MAP (Table 4.3)(The DHS Program, 2019; Pfeffer et al., 2018). Vector species compositions were determined from MAP datasets (Table 4.3)(Sinka et al., 2016; Pfeffer et al., 2018; Wiebe et al., 2017) Population growth in each location was incorporated into the model using data from the United Nations Population Prospects (United Nations, 2019). Parameterisation of the model was cross-checked with pre-intervention survey prevalence data from the trial sites and showed good alignment (Table 4.4).

Study interventions were simulated according to the country-specific delivery timings, coverages and age-cohorts reported in the trial publication (Table 4.5) (Chandramohan et al., 2021). When modelling the combined intervention arm, interventions were correlated to be delivered to the same person

within the model structure. Model-predicted clinical malaria incidence rate ratios (IRR) between intervention arms after three years of delivery were compared with the trial-reported modified intention-to-treat hazard ratios for efficacy against clinical episodes of malaria. If model estimates fell outside the reported confidence intervals (CI) of the trial results, I explored changes to the underlying parameterisation of intervention efficacy models to improve model alignment with the trial results. To do so the model validation runs were repeated over 100 draws of the parameters that describe the initial efficacy and the duration in protection from RTS,S (V_{max}, α, β) and SP+AQ (λ, κ). Once validated against the clinical malaria endpoints I also performed model comparisons to the secondary outcome measures of hospitalisations and deaths from malaria reported in the trial publication.

Table 4.3 Site specific input parameterisations

		Burkina Faso	**Mali**
Vector specification	Proportion of mosquito population that is *Anopheles arabiensis*	9%	8%
	Proportion of mosquito population that is *Anopheles funestus*	15%	27%
	Proportion of mosquito population that is *Anopheles gambiae*	76%	65%
Seasonality in transmission	Fourier seasonality coefficients	a0: 1.528932 a1: -1.996974 b1: -1.114417 a2: 0.425105 b2: 0.961317 a3: 0.08475795 b3: -0.2745763	a0: 1.789623 a1: -2.375921 b1: -1.272668 a2: 0.5633152 b2: 1.092725 a3: 0.06611265 b3: -0.3077321
Transmission intensity	Malaria prevalence in 2–10-year-olds 2016	38.1%	45.7%
Health systems	Treatment coverage 2016 First line treatment: Artemether-Lumefantrine Defined as the proportion of clinical cases that receive treatment	47%	30%
Vector interventions	Insecticide treated net usage 2017 defined as the effective coverage of nets within the population	59% pyrethroid	58% pyrethroid

Table 4.4 Model calibration checks to pre-intervention survey data

	Burkina Faso	Mali	Reference
Parasite prevalence in primary school children aged 6-12 living in trial areas and who did not receive SMC in 2016	50%	54%	(Chandramohan et al., 2019)
Model value of parasite prevalence in primary school children aged 6-12	47%	55%	-

Table 4.5 Baseline characteristics and model validation inputs from the seasonal vaccination trial cohorts.

	Burkina Faso			Mali		
	SMC	**SV**	**Combined**	**SMC**	**SV**	**Combined**
Total number of children	936	911	930	1029	1077	1037
Number in each age group (%)						
< 8 months	210 (22.4)	202 (22.2)	218 (23.4)	261 (25.4)	246 (22.8)	245 (23.6)
8-11 months	253 (27.0)	240 (26.3)	233 (25.1)	279 (27.1)	287 (26.6)	298 (28.7)
12-15 months	215 (23.0)	230 (25.2)	229 (24.6)	271 (26.3)	322 (29.9)	308 (29.7)
≥16 months	258 (27.6)	239 (26.2)	250 (26.9)	218 (21.2)	222 (20.6)	186 (17.9)
Number (%) reporting use of LLIN	721 (77.0)	671 (73.7)	705 (75.8)	872 (84.7)	899 (84.4)	898 (84.1)
Delivery months*						
Year 1	Aug, Sep, Oct, Nov	Dose 3: July		Jul, Aug, Sep, Oct	Dose 3: July	
Year 2	Jul, Aug, Sep, Oct	Dose 4: June		Jul, Aug, Sep, Oct	Dose 4: June	
Year 3	Jul, Aug, Sep, Oct	Dose 5: June		Jul, Aug, Sep, Oct	Dose 5: June	
Intervention coverage‡						
Year 1	69.4%	94.0%	SMC: 81.7% SV: 93.9%	74.7%	93.2%	SMC: 75.7% SV: 93.7%
Year 2	62.8%	95.6%	SMC: 78.5% SV: 95.8%	79.9%	94.8%	SMC: 80.7% SV: 95.4%
Year 3	73.8%	93.8%	SMC: 82.1% SV: 95.1%	86.7%	94.8%	SMC: 84.5% SV: 95.0%

*Median date of study contact for vaccination and administration of chemoprevention.

‡Intervention coverage defined as percentage of children who received each intervention at each yearly contact.

4.3.2.4 Extension of trial results

Further to the exploration of the number of rounds of SMC required for maximal impact in seasonal settings in Chapter 3, I explored the potential results of this trial had five monthly courses of SMC been delivered. I additionally utilise the ability of the model to include a baseline counterfactual scenario without any of the interventions considered to understand how the protective efficacy of interventions compares over time in this modelled cohort. Protective efficacy is defined as $1 - Incidence\ Rate\ Ratio$ comparing interventions with the baseline counterfactual.

4.4 Results

4.4.1 Seasonal malaria vaccine Phase 3b clinical trial data

A total of 5,920 children received the first dose of trial vaccine or placebo and were included in the trial analysis. Baseline characteristics for each trial arm in each location are shown in Table 4.5 and characteristics were well-balanced between the trial intervention groups. In total there were 3,825 events of clinical malaria among the trial cohorts over the three years of follow up. The trial found that SV alone was non-inferior to SMC alone with a reported hazard ratio for the protective efficacy of SV relative to SMC of 0.92 (95% CI 0.84–1.01) (Chandramohan et al., 2021). The combined intervention delivery arm resulted in substantially lower incidence of clinical malaria compared to SMC or SV alone with a reported hazard ratio for the protective efficacy of the combined intervention compared to SMC alone of 0.37 (95% CI 0.03–0.42) and compared to SV alone of 0.40 (95% CI 0.36–0.45) (Chandramohan et al., 2021).

In total 202, 279 and 291 pre- and post-vaccination paired blood samples were obtained in 2017, 2018 and 2019 respectively. High levels of anti-CSP antibody titres were reported following each vaccination. Following dose three, geometric mean antibody titres (GMT) reached 368.9 EU/ml (95% CI: 317.7–428.4). The GMT reduced to 257.5 EU/ml (95% CI 234.5–282.8) following the fourth dose and 177.4 EU/ml (95% CI 161.4–195.0) following the fifth dose. Large variation was reported across children over the three years of the trial Figure 4.4A. When comparing the observed antibody titres to the antibody decay model used within the model structure there is good alignment between the two (Figure 4.4B). However, the confidence intervals in Panel A are significantly wider than the range presented in panel B as A includes sample variation as a result of the relatively small population size in addition to the true population variance. Despite the observed reductions in antibody titre following each annual dose this was not associated with large reductions in efficacy against clinical malaria and this is explored further in the following results sections (Sagara et al., 2021; Chandramohan et al., 2021).

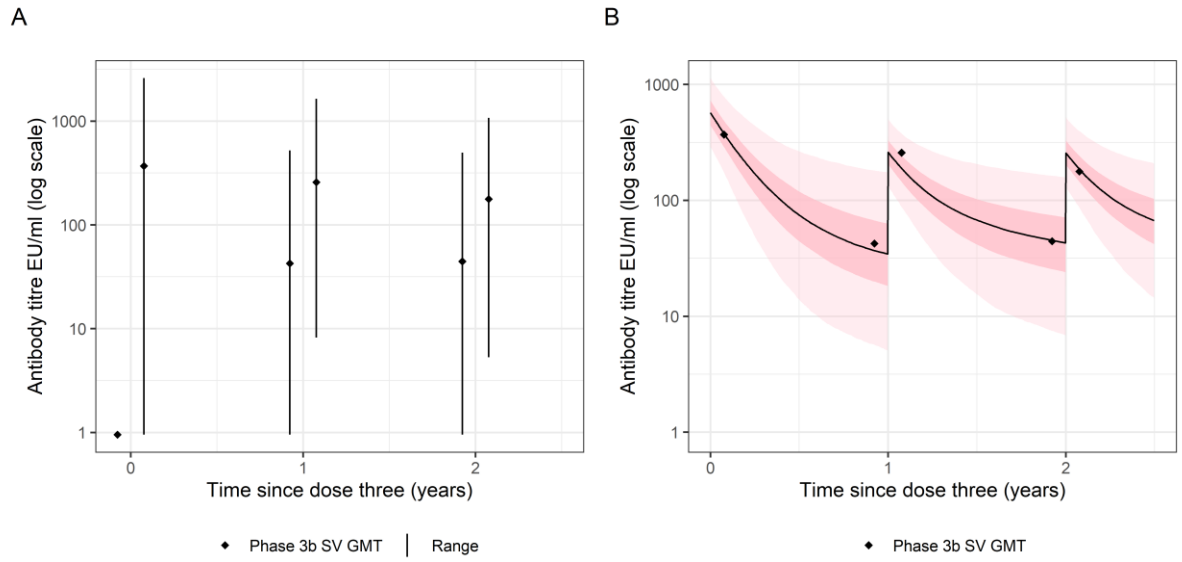

Figure 4.4 Seasonal RTS,S vaccination schedule antibody dynamics. A) Trial observed anti-CSP IgG antibody titres pre- and post- vaccination from a subset of around 200-300 children who received RTS,S/AS01$_E$ in Sagara et al. (2020). Data presented are the Geometric Mean antibody titres (GMT) and the minimum and maximum range reported. **B)** Comparison of the trial observed GMT pre and post vaccination (points) and the modelled antibody decay dynamics model fitted to data from the first-generation RTS,S Phase 3 clinical trial. The heavy black line is the median of 5000 simulations, and the dark and light shaded regions represent the 50% and 95% predictive intervals respectively using parameters from *White et al*., (2015).

4.4.2 Model validation

Preliminary model validation using the existing intervention efficacy models detailed in the methods and shown in Figure 4.5A-B revealed several inconsistencies between the trial and model estimates of intervention impact. Figure 4.5C compares the model-estimated IRRs aggregated over both countries at the four different time points reported in the trial against the observed results. While the model-estimated IRR between SV and SMC fell within the 95% CI of the trial results for Year 1, the model underestimated the remaining IRR across all comparison arms and time points (Figure 4.5C). To address these shortfalls in the model estimates I explored several alterations to the RTS,S model parameterisation.

Firstly, the existing RTS,S efficacy profile (Figure 4.5A) assumes that antibody titre and thus efficacy following the fourth and fifth doses does not reach the same levels as after the primary series, as was demonstrated in the Phase 3 RTS,S vaccine clinical trial analysis (White et al., 2015; RTSS Clinical Trials Partnership, 2015). As shown in Figure 4.4, the observed antibody titres in the Phase 3b trial of SV follow this same trend, however yearly non-inferiority results suggest that efficacy following doses four and five does not decline significantly and is comparable with that of the protection following the primary series (Sagara et al., 2021; Chandramohan et al., 2021). Comparing the hazard ratios for SV against SMC for each year shows this trend: 1.01 (95% CI 0.85–1.21), 0.90 (95% CI 0.79–1.01), 0.87 (95% CI 0.77–0.99).

Therefore, I explored a modified efficacy profile for the fourth and fifth doses that returns peak efficacy to the same level as after the primary series (Figure 4.5D). To do this, the underlying antibody decay model had to be modified such that CSP_{four} and CSP_{five} values were set to the same 621 EU/ml value as CSP_{peak}. The results from this updated efficacy profile fell within or on the edge of the 95% CI of the IRR between SV and SMC across all time points (Figure 4.5F).

However, the model still underestimated the impact of the combined intervention arms when compared to each single intervention alone (Figure 4.5F). I hypothesise that this could potentially result from potential synergies that occur when interventions are combined that are not currently captured in the model. To test this, I repeated the model validation across 100 sets of parameter draws that describe the uncertainty in the RTS,S efficacy model (V_{max}, α, and β) and SP+AQ efficacy model (k and λ). Visualising results across all combinations of parameter draws (Figure 4.6) highlights that through sampling over the uncertainty in the intervention models it is possible to capture the observed trial results. I then selected parameters that resulted in IRR values most similar to those reported in the trial. The resulting efficacy models from this selection are shown in blue in Figure 4.5G-H, the parameters are reported in Table 4.6. The newly selected parameters altered the SP+AQ model more significantly that the RTS,S model. This modification resulted in a slower decline in efficacy and an increase in the mean duration of protection from SP+AQ (Figure 4.5H, Table 4.6). The mean duration of protection increased from 35 days to 41 days under the updated parameters. This slightly slower decline in efficacy was also captured with the updated parameters describing RTS,S efficacy (Figure 4.5G, Table 4.6). With these changes the model validation results captured the observed combined arm impact (Figure 4.5I).

Table 4.6 Parameter updates to intervention efficacy models when interventions are delivered in combination in the model structure.

Intervention model	Parameter		Single arm intervention value	Combined arm intervention value
RTS,S	Shape parameter of the dose-response curve (related to initial efficacy of the vaccine)	α	0.74	0.87
	Scale parameter of the dose-response curve (related to the duration of protection)	β	99.2 EU/mL	70.9 EU/mL
	Maximum efficacy against infection	$Vmax$	0.93	0.84
SP+AQ	Shape parameter of Weibull distribution	k	3.40	2.87
	Scale parameter of Weibull distribution	λ	39.34	45.76

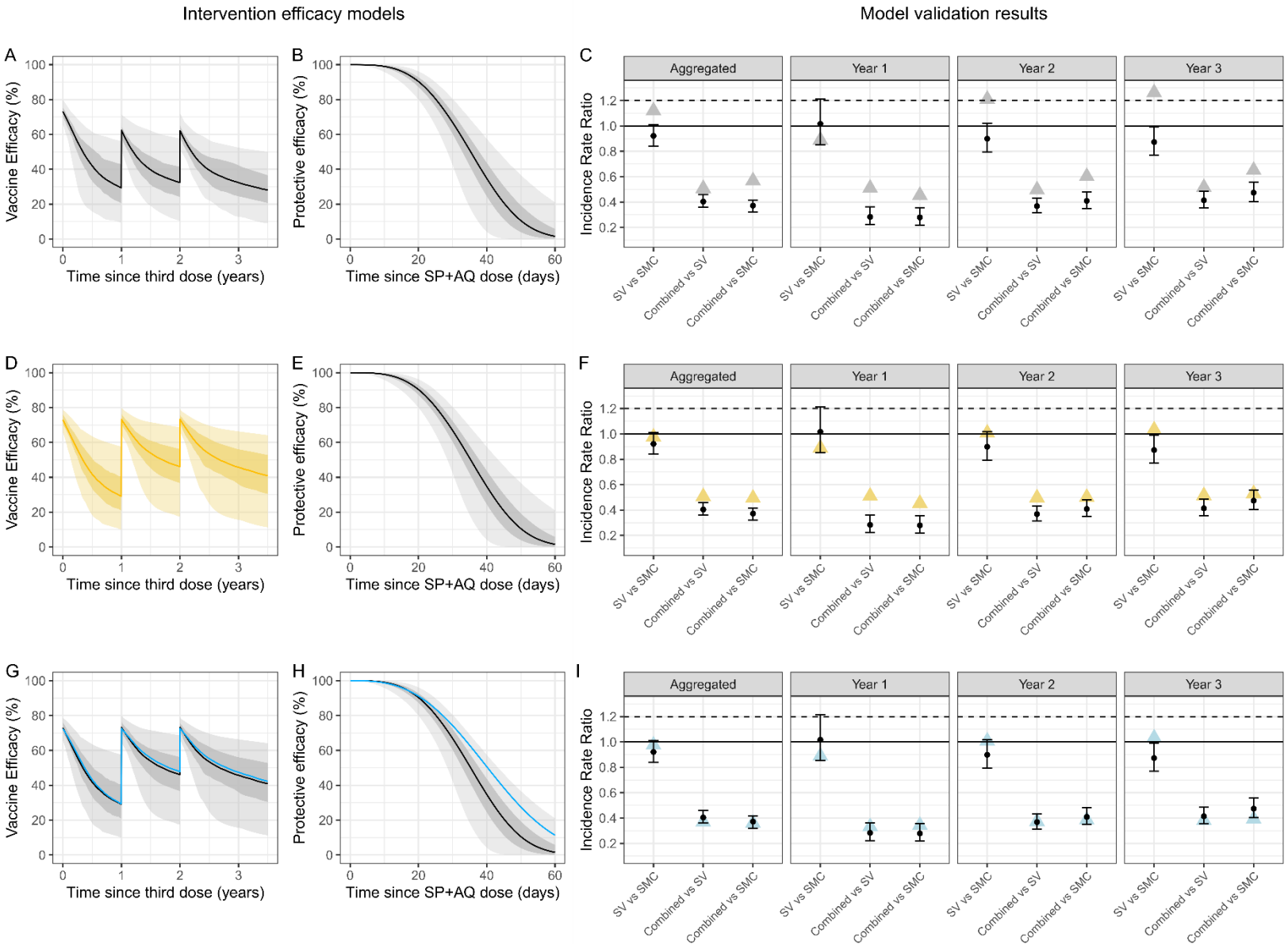

Figure 4.5 Intervention efficacy models and corresponding validation results. A) Efficacy profile for the SV 5-dose schedule based on the parameters from fitting to Phase 3 RTS,S trial data. **B)** SP+AQ efficacy profile resulting from the fitting in Chapter 3. Used to produce the model estimates of intervention comparisons in **C**. **D)** Updated efficacy profile for the SV 5-dose schedule whereby the efficacy following the fourth and fifth doses return to the same level as following the primary series but wanes at the rate described by the Phase 3 fitted model of the fourth dose and **E)** SP+AQ efficacy profile from the fitting in Chapter 3. Used to produce the model estimate of intervention comparisons in **F. G, H)** the blue line in these plots depicts the resulting efficacy profiles selected from the uncertainty sampling to represent intervention synergies and **I)** the resulting model estimated intervention comparisons. **C,F,I)** The datapoints in black are the trial reported pairwise Hazard Ratios (and 95%CIs) for the intervention comparisons (modified Intention-to-treat) listed on the x-axis and the coloured triangles the model predictions. Dashed horizontal line represents the trial specified non-inferiority margin at 1.2 for RTS,S SV compared to SMC alone and the solid line the equivalence limit at IRR = 1.

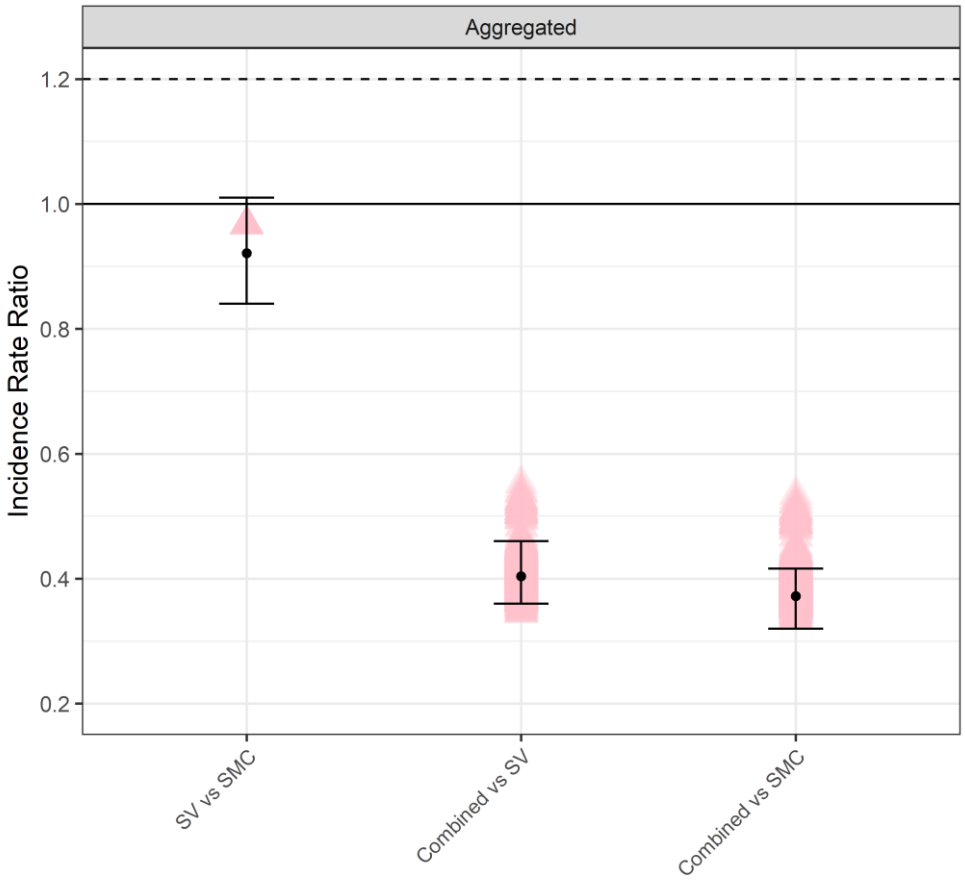

Figure 4.6 Combined arm sampling results. The datapoints in black are the trial reported pairwise Hazard Ratios (and 95%CIs) for the intervention comparisons (modified Intention-to-treat) listed on the x-axis and the coloured triangles the model predictions for each set of parameter draws for RTS,S and SMC. Dashed horizontal line represents the trial specified non-inferiority margin at 1.2 for RTS,S SV compared to SMC alone and the solid line the equivalence limit at IRR = 1.

4.4.3 Secondary outcomes

I also explored the modelled results with the secondary data outcomes reported in the trial publication. These were defined as hospital admission with any malaria, hospital admission with any severe malaria (according to the WHO classification (World Health Organization, 2014)) and deaths from malaria. Using the previously validated intervention efficacy profiles detailed above, incidence rate ratios comparing model estimates to the trial data are shown in Figure 4.7. Model predictions show good alignment with these secondary outcome measures, with a slight overestimate of the combined arm impact when considering hospitalisations with any malaria (Figure 4.7).

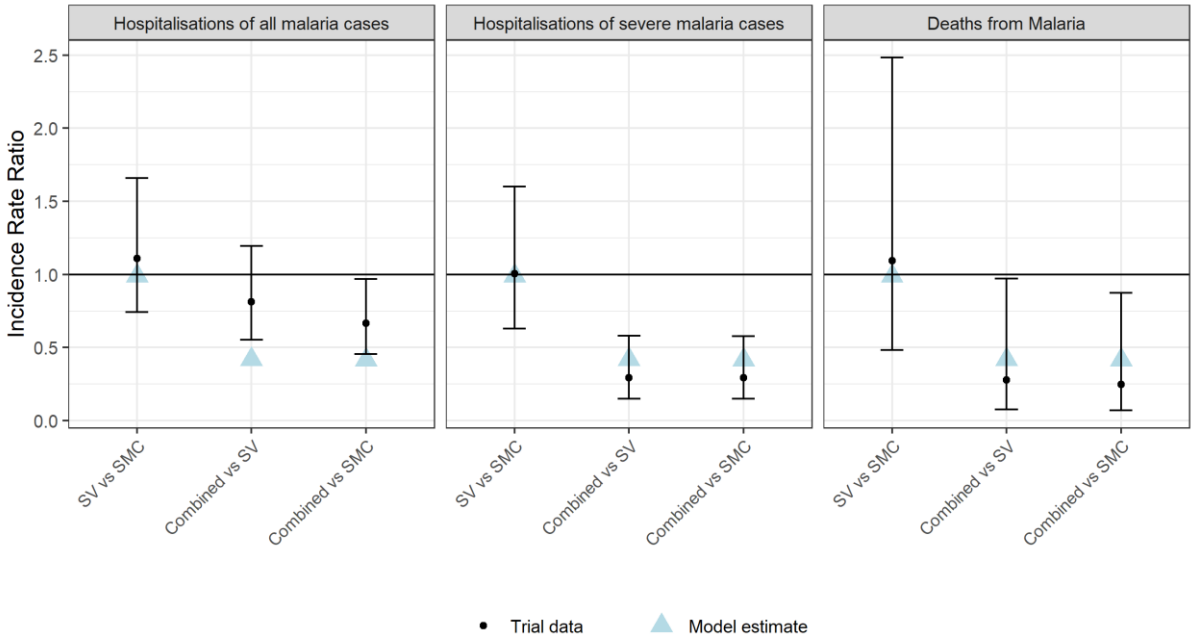

Figure 4.7 Secondary outcome model validation. The datapoints in black are the trial reported pairwise Hazard ratios for the intervention comparisons (modified Intention-to-treat) listed on the x-axis and the coloured triangles the model predictions. The solid line represents the equivalence limit at IRR = 1.

4.4.4 Extension of non-inferiority comparison with five SMC cycles

The trial finding of SV non-inferiority to SMC depends not only on the performance of the vaccine under seasonal conditions but also the performance of SMC. SMC programmes with four monthly cycles have been shown to be too short for the seasonality patterns in these two trial locations and five monthly cycles are now being delivered as the standard of care in Hounde, Burkina Faso. This can be seen in the plots in Figure 4.8 whereby the modelled SMC cohorts received an additional fifth monthly cycle which provided protection in the final month of the transmission season which wasn't achieved with four cycles. If five cycles of SMC had been delivered, modelling suggest that the results comparing SV to SMC alone would have been more favourable for SMC than for SV with an estimated IRR of 1.1 but that the trial results would have remained within the non-inferiority margin (Figure 4.9).

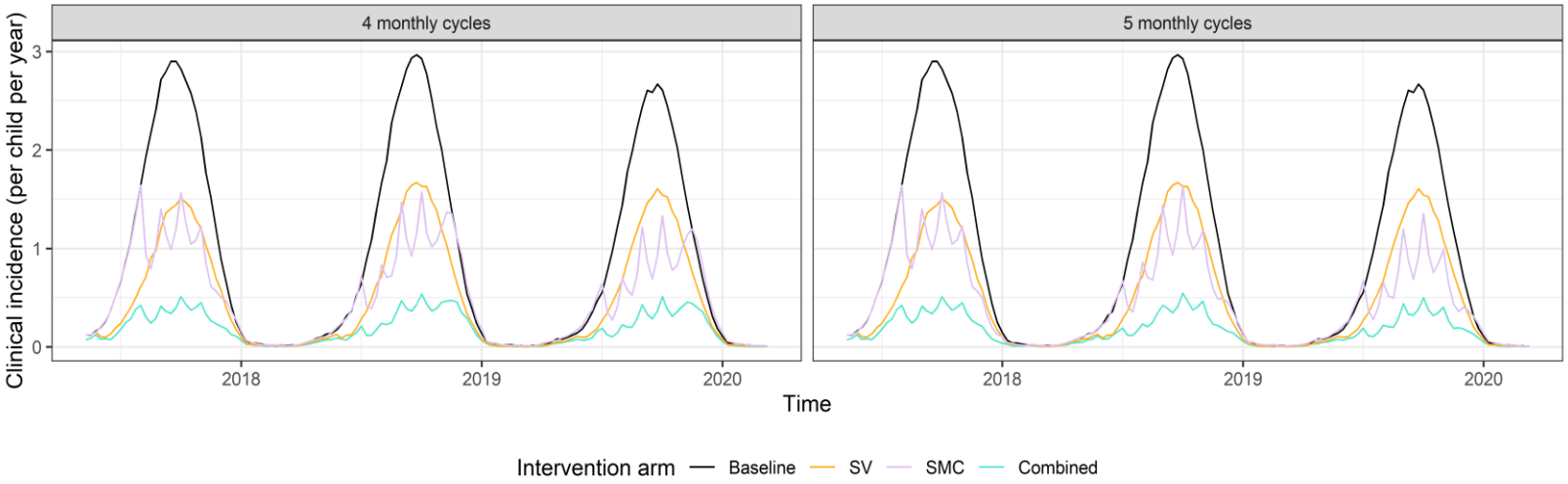

Figure 4.8 Model simulated clinical incidence in trial cohorts. With the inclusion of a simulated baseline control cohort that wasn't included in the trial for ethical reasons.

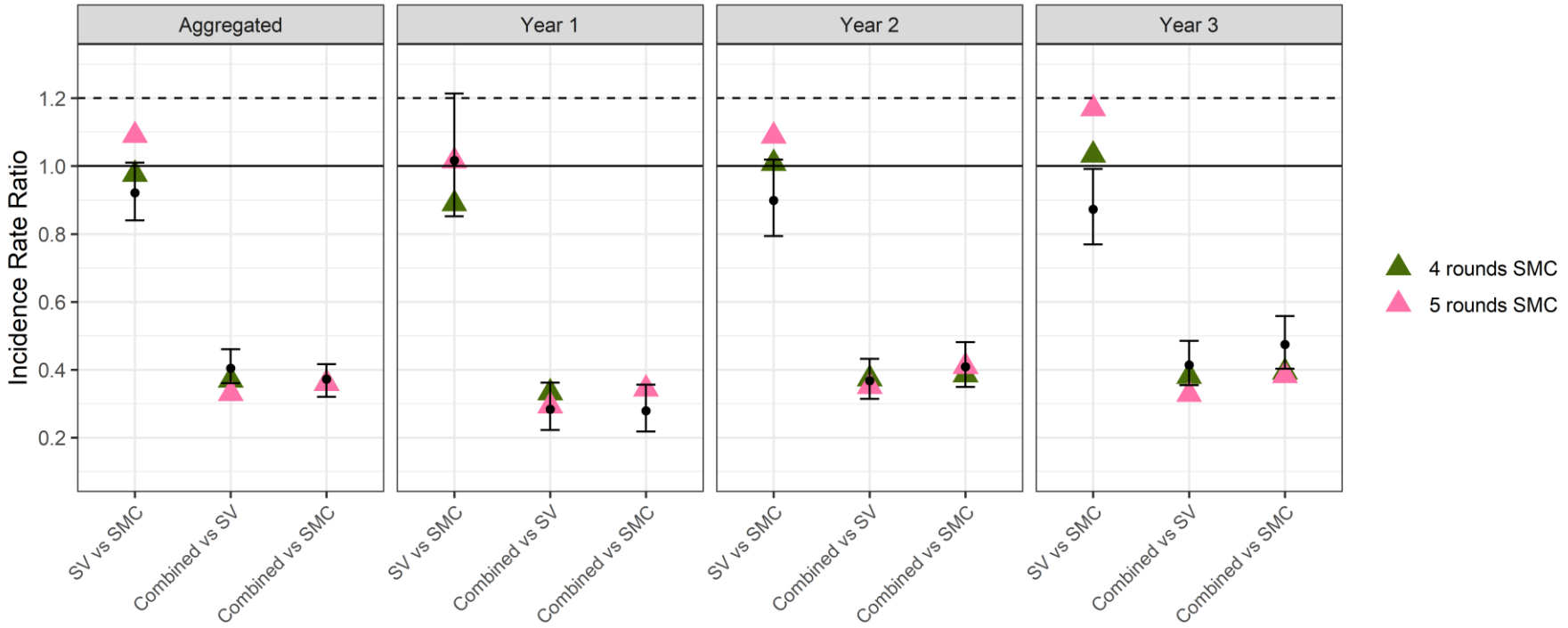

Figure 4.9 Sensitivity analysis of trial comparisons when a fifth monthly cycle of SMC is included. The datapoints in black are the trial reported pairwise Hazard ratios for the intervention comparisons (modified Intention-to-treat) listed on the x-axis and the coloured triangles the model predictions. The dashed horizontal line represents the trial specified non-inferiority margin at 1.2 for RTS,S compared to SMC alone and the solid line the equivalence limit at IRR = 1.

4.4.5 Baseline counterfactual

The inclusion of a control cohort in this study, whereby children did not receive any protective intervention would have been unethical. Within a modelling framework we can include a baseline counterfactual cohort to understand the expected transmission dynamics without any interventions under investigation, and how this might influence intervention efficacy in these settings. The incidence curves in Figure 4.8 highlight the temporal reductions in incidence across both countries in this trial compared to this counter-factual baseline scenario.

When estimating the efficacy of interventions in this modelled cohort over time, it is clear that SMC with SP+AQ was more protective than RTS,S alone during the peak in the transmission season when it was delivered (Figure 4.10). However, RTS,S provided significant protection outside this period ensuring that the interventions were non-inferior over the entire trial period. The combination of interventions ensure protection against clinical malaria remained high both during the peak transmission season and in the intervening months (Figure 4.10). With the addition of the fifth monthly cycle of SMC the levels of protection in these cohorts remains high for a further month, reducing the period of time these children spent with no protection (Figure 4.10).

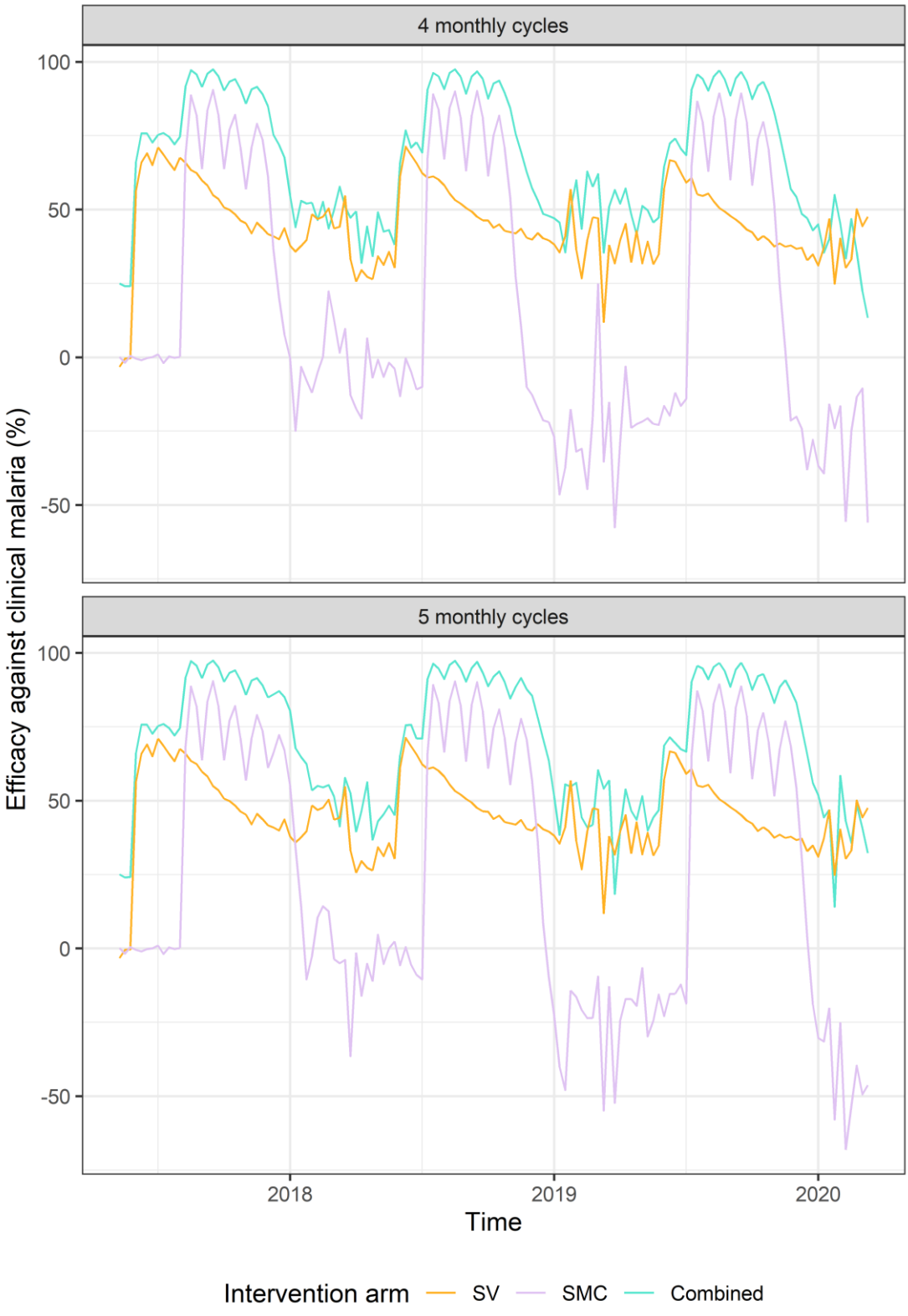

Figure 4.10 Intervention efficacy in modelled trial cohorts comparing four and five monthly cycles of SMC. Efficacy measurements are calculated in an according to protocol population, assuming 100% coverage of interventions. Efficacy is defined as the proportional reduction in weekly incidence between a modelled baseline cohort and each intervention arm. High levels of variation in efficacy are seen during the period between the peak transmission season as a result of model stochasticity in these low transmission intensity months.

4.5 Discussion

In this chapter I used a mathematical model of malaria transmission to replicate the recent Phase 3b seasonal vaccination clinical trial. In this I aimed to understand if the current parameterisations of RTS,S and SP+AQ intervention models satisfactorily match observed trial outcomes. Current efficacy models were unable to satisfactorily match the trial results, underestimating the results for all trial endpoints. With biologically motivated reparameterizations to the efficacy models I was able to align the model and trial results. These results suggest that the alterations to the RTS,S vaccination schedule in this trial and the novel delivery of RTS,S and SMC combined could have potentially enhanced the protection of both interventions provided above and beyond that seen in trials previously.

Our existing RTS,S vaccine model previously fitted to antibody titre measurements and clinical disease endpoints from the RTS,S Phase 3 clinical trial (White et al., 2015), underestimated the impact of seasonal vaccination alone. The first change that was required to capture SV results was to return antibody levels and thus efficacy following fourth and fifth doses to the same level as after the third dose. This change was required despite the fact that antibody titres decreased with each successive vaccine dose in line with the decay model fitted to the original Phase 3 trial data. Despite these observed decreases in the immunogenicity of each additional dose of RTS,S in the SV trial this was not associated with large reductions in efficacy (Sagara et al., 2021; Chandramohan et al., 2021). Previously, as demonstrated in Chapter 2, dose spacing changes have been shown to influence the achievable efficacy of RTS,S without alterations to antibody titres (Regules et al., 2016). The reduction between the primary RTS,S series and additional doses from 18 to 12 months may have impacted humoral immune responses outside of antibody titres including the avidity of antibodies, antibody subclass, a breaching of the immunodominance resulting in a more balanced response to CSP target regions or other qualitative humoral response features (Kurtovic et al., 2020; Chaudhury et al., 2017; Pallikkuth et al., 2020; Das et al., 2021; Suscovich et al., 2020; Seaton et al., 2021). Further research into the potential changes to the RTS,S induced immune response dynamics observed in the Phase 3b clinical trial is required to explore this hypothesis further along with ongoing 12-month RTS,S dose spacing challenge studies in malaria naïve adults (Moon et al., 2020).

Further to this SV study a recent Phase 2 clinical trial of a novel pre-erythrocytic malaria vaccine candidate R21, which was delivered seasonally in Nanoro, Burkina Faso with a 12-month dose spacing between the third and fourth dose, also demonstrated high vaccine efficacy in the six to 12 months following vaccination (Datoo et al., 2021). In this trial it is currently unclear whether the additional fourth dose will also retain these high levels of efficacy as following the primary series, but antibody titres were comparable to those measured after the third dose, suggesting efficacy could be high

(Datoo et al., 2021). The evidence from both of these clinical trials provides strong evidence for the advantage of providing seasonally targeted malaria vaccination to maximise efficacy in these settings.

The second biologically-motivated change that was required to align model estimates with the trial results was to select parameters that significantly increased the duration of SP+AQ protection when the two interventions were delivered in combination, alongside a more modest increase in the duration of RTS,S protection. This change could reflect a potential synergistic relationship between anti-malaria vaccines and drugs when delivered in combination. This could potentially result from RTS,S induced reduction in the liver-to-blood inoculum of parasites which was demonstrated in Chapter 1 to be as high as 99%. This reduction in parasite load would then result in smaller initial generations of blood-stage parasites that could result in more efficient clearance of parasites by SP+AQ. This type of synergistic interaction has previously been shown in a mouse model between transmission blocking and pre-erythrocytic vaccine candidates (Sherrard-Smith et al., 2018). In this study the authors demonstrated that the presence of transmission blocking antibodies reduced the sporozoite parasite density inoculated by infected mosquitoes which allowed pre-erythrocytic antibodies to achieve greater efficiency at clearing sporozoites. Further research into any potential synergistic relationships between pre-erythrocytic vaccines and anti-malarial drugs is required.

Understanding the temporality in efficacy of these interventions over time allows for a deeper exploration of the drivers of non-inferiority observed in this trial. Protection from SP+AQ was higher during the peak in transmission annually, but protection waned quickly in the final month after delivery as described in Chapter 3. The modelled SMC cohort is therefore left with no protection from clinical malaria in the months following the final delivery cycle. As a result, efficacy measured as the percentage reduction in clinical incidence between the intervention and baseline cohort falls below zero as residual transmission increases in these children who have had minimal exposure to malaria parasites during the transmission season. The model takes into account the impact of this reduced exposure on the delay in the acquisition of natural immunity in this cohort which is why efficacy falls below zero relative to the baseline cohort who develop immunity more rapidly. However, despite RTS,S efficacy being slightly lower during the transmission season, the vaccine provides protection across the course of the whole year which resulted in sustained reductions to clinical incidence between transmission seasons in this modelled cohort. These different dynamics of protective action of interventions resulted in the non-inferiority over the course of the full trial period. When SMC and RTS,S were combined this resulted in each intervention providing a protective safety-net to counterbalance the other weaknesses .

The use of a modelling framework to understand the potential non-inferiority relationship between SMC and RTS,S had five monthly cycles of SMC been administrated in these trial locations is important given the recent suggestions to allow greater flexibility in the number of monthly cycles of SMC that can be adopted in different locations as discussed in Chapter 3. Given the seasonality profile of these two trial locations, I predict that a fifth cycle of SMC would have resulted in an overall IRR that favoured SMC over RTS,S but that still remained within the non-inferiority margin specified in the trial protocol. When considering the potential utility of these seasonal interventions in locations that experience longer less peaked transmission seasons, the number of potential monthly cycles of SMC that could be implemented will be an important determinant of whether or not burden reductions with seasonally targeted RTS,S would be equivalent or exceed those with SMC.

4.6 Limitations

This work has several limitations. Firstly, the reparameterizations to the intervention efficacy models are driven by the discordance between the model and trial results and biologically motivated hypotheses for the reasons between these differences. However, these assumptions have yet to be shown in further research. As such, when examining the population level impacts of this novel RTS,S schedule in Chapter 5, I will use all parameterisations of RTS,S and SP+AQ efficacy to represent the current levels of uncertainty. Secondly, there was no direct baseline prevalence survey in this trial which meant parameterisations of the transmission intensity in the modelling framework were informed by overall first administrative unit trends in the two trial sites based on Malaria Atlas Project estimates. This parameterisation showed good alignment with a survey completed following the azithromycin plus SMC clinical trial conducted in the same study areas prior to the start of this trial in 2016 (Chandramohan et al., 2019). However, in the prevalence surveys conducted again in school aged children at the end of each malaria transmission season during the current trial there appears to be a significant decrease in parasite prevalence in this population in Mali: 54% in 2016 compared to 17% in 2018 and 22.5% in 2019, with no measured levels in 2017 (Chandramohan et al., 2021). The same trends were not observed in the study areas in Burkina Faso. While the population size for these surveys decreased in the current trial from 500 to 250 children, real reductions in transmission intensity in these communities could have also occurred over this time and could potentially impact the observed relationships between intervention impacts, which is something we could not account for in this analysis. Notably the clinical trial results were observed under high levels of vector control (~80% of children reported using an insecticide treated bed-net at baseline and all children received a bed-net as per trial protocol) highlighting the maximum benefit that could be achieved under high coverage and usage levels of combined intervention packages.

4.7 Conclusions

While models of malaria transmission are useful tools for setting realistic expectations for intervention programmes, they cannot replace high quality empirical field trials. I have shown in this work how modelling validation studies can complement ongoing field trials, particularly prior to extensive immunological data analysis being available to help to characterise and understand the potential efficacy changes as a result of novel second-generation delivery approaches. In this work I adapted an established model of RTS,S/AS01 efficacy within a transmission model framework to account for additional doses and changes in delivery structure. The required reparameterizations of intervention models provides early evidence for potential underlying immunological changes in the relationship between antibody titre and efficacy along with potential synergistic relationships between pre-erythrocytic vaccines and anti-malarial drugs which have not previously been studied. These validation results are important for making population level public health impact assessments in a modelling framework which will be examined in Chapter 5.

Chapter 5

5 Mathematical modelling of a seasonal use-case for the RTS,S/AS01$_E$ malaria vaccine

In this chapter, I combine the results of all previous chapters to characterise the seasonal use-case for the RTS,S/AS01$_E$ malaria vaccine. Using the Imperial College London model of malaria transmission parameterised to capture West African seasonality archetypes I estimate the potential population-level public health impacts of age-based and alternative seasonally-targeted RTS,S vaccination approaches in both the absence and presence of seasonal malaria chemoprevention.

This work formed part of the evidence packet submitted to the Malaria Policy Advisory Committee meeting in October 2021: http://terrance.who.int/mediacentre/data/malaria/documents/mpag-october2021-session5-rtss-malaria-vaccine.pdf?sfvrsn=9507a63b_10

This work is under review at The Lancet Global Health.

5.1 Background

The Phase 3b clinical trial of seasonal RTS,S/AS01$_E$ (RTS,S) vaccination described in the previous Chapter showed that vaccination was non-inferior to seasonal malaria chemoprevention (SMC) in preventing clinical malaria in two locations in southern Burkina Faso and Mali (Chandramohan et al., 2021). In addition, the combination of these two interventions provided significant additional protection against clinical and severe malaria outcomes in the trial. Impact projections of this novel approach to RTS,S vaccination in different epidemiological settings to those of the trial sites and for longer time frames at the population level are needed to inform policy recommendations surrounding seasonal vaccination approaches.

Mathematical models of malaria dynamics have previously been useful for estimating the public health impact of first-generation RTS,S beyond the impact estimates obtained from clinical trials

(Penny et al., 2016). Transmission models that have been fitted to data from malaria-endemic settings have particular utility for understanding the potential impacts of vaccinations as models can account for the potential epidemiological changes in the age-burden of cases and deaths as a result of exposure reducing interventions that can delay the acquisition of naturally acquired immunity that is often not captured in trial follow up periods. In addition, by incorporating realistic assumptions about access to malaria treatment, models can be used to understand the impact of interventions on malaria mortality outside a trial setting where access to care can be higher than a real-world setting as was seen in the RTS,S Phase 3 trial (RTSS Clinical Trials Partnership, 2015).

In 2015, following the Phase 3 RTS,S trial that provided estimates of vaccine impact in the 11 trial sites, four modelling groups were brought together to provide full population-level impact estimates of routine age-based RTS,S administration in settings of perennial transmission (Penny et al., 2016). This work found that RTS,S could have a substantial public health impact, averting on average 116,480 (range 31,450–160,410) clinical cases per 100,000 fully vaccinated children across settings of perennial malaria transmission ranging from 10% to 60% parasite prevalence in 2–10 -year-olds ($PfPR_{2\text{-}10}$) over a 15 year time horizon. Results also showed that RTS,S could be highly cost-effective with a median incremental cost-effectiveness ratio of $30 (range $18–$211) per clinical case averted in settings with >10% $PfPR_{2\text{-}10}$ (Penny et al., 2016). This work made an important contribution to the broader policy assessment process related to RTS,S. However, this work did not consider the potential impact of first-generation RTS,S in seasonal settings of malaria transmission and was conducted prior to the alternative RTS,S delivery approaches of delayed-fractional doses and seasonally targeted vaccination.

These two approaches have since been assessed in modelling studies. *Hogan et al.* used the Imperial College London malaria transmission model to estimate the potential impact of an age-based four-dose RTS,S regime that captured the potential delayed-fractional RTS,S efficacy increases observed in *Regules et al.* (Hogan et al., 2018; Regules et al., 2016). Modelling first-generation and delayed-fractional RTS,S immunization *Hogan et al.* found that the delayed-fractional regime averted 21%–25% more clinical cases per 1,000 children 0–5 years old over 10 years in perennial settings of malaria transmission with baseline levels of $PfPR_{2\text{-}10}$ between 5%–45% (Hogan et al., 2018). Again, however, this work did not consider the impact of these schedules in settings of seasonal malaria transmission.

Prior to the Phase 3b seasonal RTS,S vaccination trial, *Camponovo et al.* used the *OpenMalaria* transmission model to assess the potential of mass seasonal RTS,S vaccination and/or seasonal malaria chemoprevention (SMC) delivered to individuals older than six months in settings with a three month seasonal transmission season and low baseline parasite prevalence between 1%–20%, to reach malaria elimination and prevent resurgence (Camponovo et al., 2019). This work showed that, RTS,S

vaccination alone in these settings was a less effective intervention compared to chemoprevention strategies. However, when combined these interventions, deployed in a seasonal schedule, had the potential to achieve dramatic reductions in parasite prevalence levels and in some settings the interruption of transmission, due to the resulting synergistic interactions between RTS,S and SMC (Camponovo et al., 2019). This work however, only looked at mass seasonal vaccination/chemoprevention in near elimination settings and did not compare age-based or seasonally-targeted vaccination in settings of variable seasonality and high transmission intensity.

These previous population-level modelling studies highlight the utility of transmission models to help inform guide decision making surrounding RTS,S. Following the evidence provided by the Phase 3b clinical trial of the impact of seasonally-targeted vaccination with or without seasonal malaria chemoprevention, I employ this population-level mathematical modelling approach to generalise these findings by estimating the public health impact of these approaches over a longer time frame and across a range of epidemiological transmission settings. These estimates can help national governments and international agencies to systematically evaluate the potential impact of seasonally-targeted RTS,S compared to age-based administration, as well as the value of combining this intervention with seasonal malaria chemoprevention.

5.2 Aims

Mathematical models of malaria dynamics have been useful for estimating the public health impact of malaria vaccination beyond the impact estimates obtained from clinical trials previously. In this chapter, I aim to evaluate if RTS,S is more impactful if delivered seasonally compared to an age-based approach, and if RTS,S delivers additional benefit on top of SMC. I aim to understand how seasonality and transmission intensity influence these relationships and I explore how impact varies by wider health system and operational factors.

5.3 Methods

The Imperial College London transmission model as described in Appendix 8.1 along with the intervention models described in Chapter 3 and Chapter 4 formed the basis of the modelling work presented in this Chapter.

5.3.1 Transmission settings

Two seasonality archetypes were considered in this analysis: a highly seasonal setting with a single strong peak in rainfall annually and a relatively short transmission season of around four to five months; and a seasonal setting with a less strong peak in rainfall annually with a transmission season of around seven to eight months (Figure 5.1). The seasonality archetypes are based on the averaged Fourier transformed rainfall data from the first administrative units of Fatik in Senegal and Upper East in Ghana (CHIRPS, 1999; Garske, Ferguson & Ghani, 2013). Several baseline prevalence rates were considered ranging from 10%–65%, representative of the range of transmission intensities across sub-Saharan Africa (Pfeffer et al., 2018). Baseline prevalence levels are intended to reflect existing levels of malaria vector control interventions and access to treatment (first line Artemisinin Combination Therapy at 45%) with no change in coverage over the time horizon considered. Population size is assumed to remain constant.

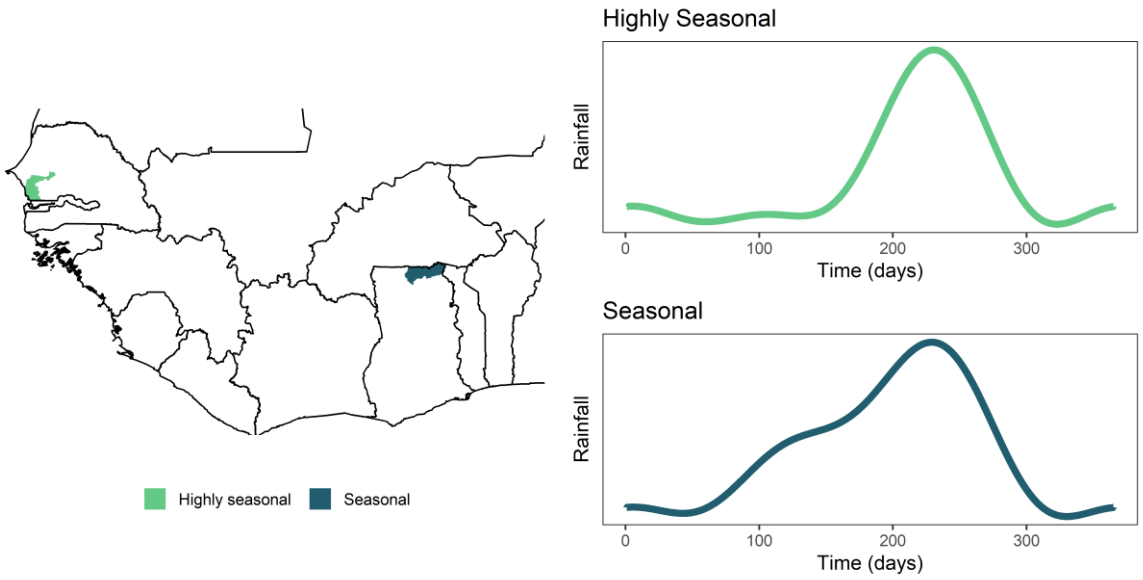

Figure 5.1 Geographical locations of seasonality archetypes and their corresponding modelled seasonality profiles. The associated seasonality patterns in rainfall drive seasonal patterns in malaria incidence in these administrative level one locations used in model simulations.

5.3.2 Intervention Delivery

Aged-based RTS,S (AB-RTS,S) doses were delivered to children aged at 6-, 7- and 9-months of age for the first three doses, with a fourth dose at 24-months of age as per the schedules adopted in the

Malaria Vaccine Implementation Programme (MVIP) in Ghana and Kenya (World Health Organization, 2021b). RTS,S efficacy under this schedule is shown in Figure 5.2A**.** For seasonally targeted vaccination (SV-RTS,S), all children aged 5–17 months at the time of first vaccination received the first three doses in the three months preceding the transmission season, with any subsequent doses delivered annually (Chandramohan et al., 2020). I considered two SV-RTS,S schedules: a four-dose schedule to make a direct comparison to the four-dose age-based schedule and a five-dose schedule as was delivered in the seasonal trial. Three models of RTS,S efficacy under SV-RTS,S schedules were used to reflect the uncertainty in the dynamics of RTS,S efficacy as a result of the earlier model validation in Chapter 4 (Figure 5.2B-E). Full vaccine coverage of 64% was assumed resulting from 80% coverage of the first three doses with a 20% drop off between the third and fourth dose in four-dose schedules and a 10% drop off for both dose four and dose five in a five-dose schedule.

SMC was implemented in the model as both four- and five-monthly courses of SP-AQ given in consecutive months to children aged between three months to five years old with the doses timed to coincide with the months of highest transmission intensity. The decay in efficacy of SMC was modelled according to the Weibull survival function fitted in Chapter 3 and shown in Figure 5.2F, with a median duration of protection ≥50% of 35 days. A secondary efficacy profile was considered when SMC was combined with SV-RTS,S as a result of the model validation in Chapter 4 (Figure 5.2F dashed line). Coverage of 75% was assumed based on coverage levels observed in routine SMC use and defined as the proportion of eligible children who received all four or five cycles (Baba et al., 2020). I did not model the effect of incomplete adherence to the three-day course. When interventions were delivered in combination, interventions were distributed randomly to individuals in the model structure.

Table 5.1 summarises key modelling parameterizations.

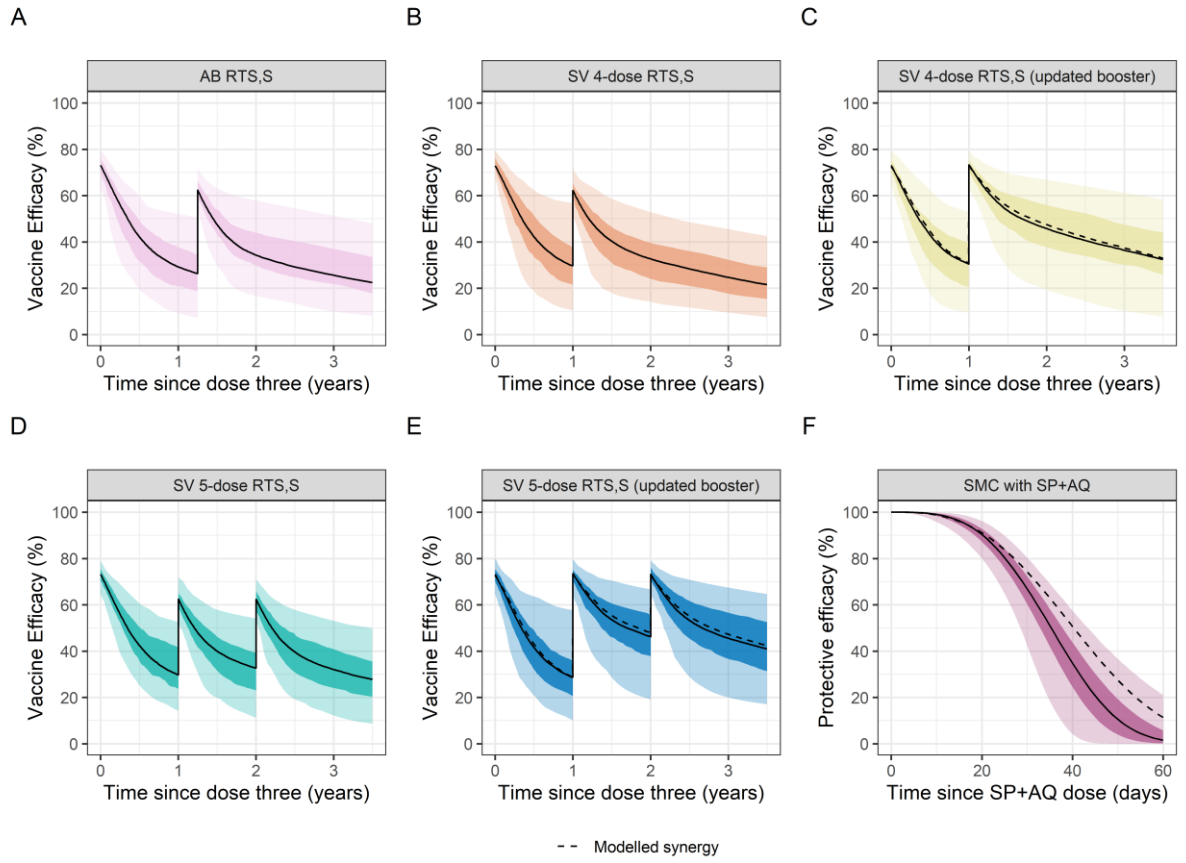

Figure 5.2 Intervention efficacy models used in the population modelling simulations. A) RTS,S efficacy profile for age-based delivery resulting from fitting to the Phase 3 RTS,S clinical trial where the dose four is delivered 15 months following dose three as per the recent MVIP. **B)** Efficacy profile for the SV-RTS,S 4-dose schedule based on the parameters from fitting to the Phase 3 RTS,S clinical trial where dose four is delivered 12 months following dose three. **C)** Efficacy profile for the SV-RTS,S 4-dose schedule with the updated fourth dose peak efficacy based on the results from the model validation runs in Chapter 4. Dashed line represents the efficacy assuming synergy between RTS,S and SMC when delivered in combination resulting from the model validation runs in Chapter 4. **D)** Efficacy profile for the SV-RTS,S 5-dose schedule based on the parameters from fitting to the Phase 3 RTS,S clinical trial where dose four is delivered 12 months and dose five 24 months following dose three. **E)** Efficacy profile for the SV-RTS,S 5-dose schedule with the updated fourth and fifth dose efficacy resulting from the model validation runs in Chapter 4. Dashed line represents the efficacy assuming potential synergy between RTS,S and SMC when delivered in combination resulting from model validation runs in Chapter 4. **F)** SP+AQ efficacy profile from fitting to clinical trial data described in Chapter 3. Dashed line represents the efficacy assuming potential synergy between RTS,S and SMC when delivered in combination resulting from model validation runs in Chapter 4. Solid lines in all plots correspond to the model medians with the shaded areas the 50% and 95% Credible Intervals.

Table 5.1 Parameterization and set-up of the malaria transmission model.

Model parameterization	Description
Transmission intensity	Baseline $PfPR_{2-10}$ 10%, 15%, 20%, 25%, 35%, 45%, 55%, 65%. Assume that $PfPR_{2-10}$ reflects current levels of ITN, IRS and access to treatment which remain at static levels following vaccine introduction in all scenarios.
Seasonality	"Highly Seasonal" archetype based on seasonality patterns in Fatik, Senegal. "Seasonal" archetype based on seasonality patterns in Upper East, Ghana.
Demographics	Constant population size and demography based on the life table for Butajira, Ethiopia, with an average life expectancy at birth of 46.6 years.
Case management	Effective coverage with ACT for clinical malaria at 45%.
Vaccine scenarios	Two main vaccination scenarios are considered: 1. Routine age-based immunization with RTS,S (AB-RTS,S), with primary doses given at 6, 7.5 and 9 months of age with a fourth dose at 24 months of age as per the schedules adopted in the MVIP in Ghana and Kenya. 2. Seasonally targeted vaccination approach (SV-RTS,S) where primary doses are delivered to all children aged between 5-17 months old in the three months preceding the transmission season with a fourth dose delivered 12 months after the third dose and a fifth dose 24 months after the third dose. A four-dose SV-RTS,S and five-dose SV-RTS,S are considered.
Vaccine efficacy and waning	Model estimates of RTS,S efficacy are based on fitting to Phase 3 trial data (White et al., 2015). Both vaccination schedules are run assuming this fitted profile. In addition, given the results of the model validation in Chapter 4, several additional changes to the RTS,S efficacy profile are considered for seasonal campaigns to represent uncertainty in the potential vaccine efficacy under this schedule: 1. Improved fourth and fifth dose efficacy matching that of the third dose. 2. Slight reduction in the rate of decay of RTS,S efficacy when combined with SMC.
Vaccine coverage	80% coverage of the first three doses is assumed with a 20% drop off in coverage of the fourth dose in four-dose schedule and a 10% drop off for each of the fourth and fifth doses in a five-dose schedule. This gives a full vaccine coverage for all doses of 64%.
Seasonal Malaria Chemoprevention	Seasonal Malaria Chemoprevention with SP+AQ is explicitly modelled when assessing the impact of vaccination and SMC combined. This was modelled as 4 or 5 monthly cycles of SMC delivered to children aged 3 months to 5 years old during the peak in transmission season, with a coverage of 75% (Baba et al., 2020).
Time horizon	15 years.

5.3.3 Outcome measures

I summarise outputs as cumulative events averted over a 15-year time horizon. I assess the impact of intervention strategies on clinical cases and deaths from malaria and report these health outcomes per 100,000 children aged 0–5 years, and for one-year age groupings up to 20 years of age per 100,000 population. Unless otherwise stated, events averted are calculated relative to a no-vaccine or no-SMC baseline scenario. Outputs are presented as median estimates from 25 parameter draws describing the fitted parameter uncertainty in the transmission model.

5.3.4 Health systems considerations sensitivity analysis

I conducted two sensitivity analysis to understand the impact of several health system operational factors on health outcomes in these settings. The first was a sensitivity analysis whereby I varied coverage levels of the primary series of all RTS,S schedules between 50%–90% and SMC coverage levels between 50%–90%. Secondly, within the model framework, delivery of interventions can easily be aligned to the peak in malaria transmission, in order to maximise impact. To understand each intervention's robustness to delivery challenges I performed a sensitivity analysis by including +/- 1- and 2-month adjustments in delivery from those identified as optimal (Figure 5.3 **SMC and SV-RTS,S timings relative to the underlying seasonality in transmission.** Vertical lines in the top row of figures

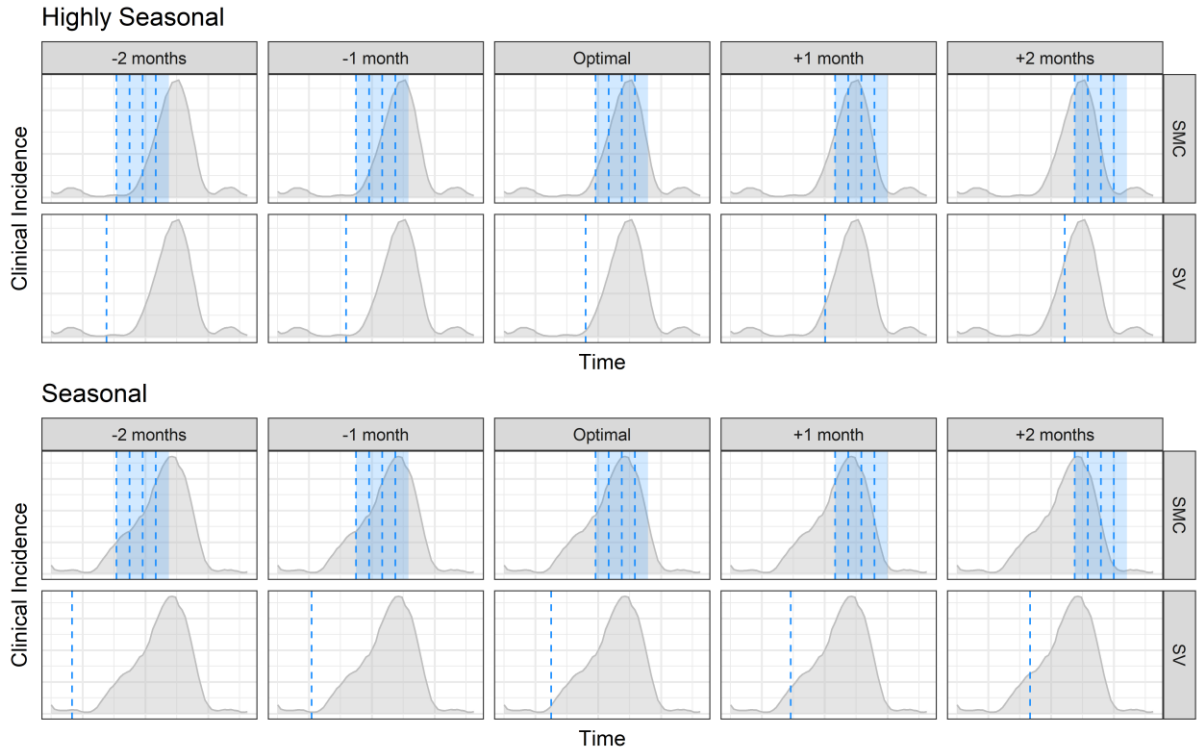

Figure 5.3 SMC and SV-RTS,S timings relative to the underlying seasonality in transmission. Vertical lines in the top row of figures represent the 4 monthly cycles of SMC and the lower panels the timing of the third, fourth and fifth vaccine doses. Shaded area represents the months over which SMC then provides protection with no protection provided outside these months. This is not shown for SV-RTS,S as the vaccine provides protection across the whole year following delivery.

represent the 4 monthly cycles of SMC and the lower panels the timing of the third, fourth and fifth vaccine doses. Shaded area represents the months over which SMC then provides protection with no protection provided outside these months. This is not shown for SV-RTS,S as the vaccine provides protection across the whole year following delivery.).

5.3.5 Delayed-fractional AB-RTS,S vs first-generation AB-RTS,S

Finally, I include a sensitivity analysis with a focus on simulating a potential delayed-fractional age-based RTS,S schedule (DF RTS,S). In the *Regules et al.* study that formed the basis of the work in Chapter 2, efficacy against malaria infection following dose three which was delivered with a five month delay and at one-fith of the standard dose resulted in an increase in efficacy to 86.7% (95% CI 66.8–94.6%) compared to 62.5% (95% CI 29.4–80.1%) for volunteers on the standard 0-, 1-, 2-month full dose regime (Regules et al., 2016). Current delayed-fractional RTS,S field trials are not yet completed and as such I include an efficacy profile assuming a higher peak efficacy following the primary schedule, as in the work by *Hogan et al.* (Hogan et al., 2018). Protection following the fourth vaccine dose is assumed to peak at the same level as first-generation AB-RTS,S as no data is yet available to parameterise this. Due to the nature of the delayed-fractional schedule doses were delivered to children at 6-, 7- and 12- months of age for the first three doses to account for the five-month delay between doses two and three and the fourth dose at 24 months of age to maintain the same vaccine contact for this dose as the prior analysis. RTS,S efficacy under this schedule in comparison to the first-generation AB-RTS,S is shown in Figure 5.4 **Sensitivity modelling of a delayed-fractional RTS,S efficacy profile.** First-generation RTS,S efficacy is shown on the left (Vmax: 0.93, α: 0.74, β: 99.2) with the modified potential second-generation delayed fractional efficacy shown on the right. This profile assumes a higher peak efficacy following the primary series which is captured by altering the underlying efficacy model parameters (Vmax: 0.93, α: 0.95, β: 70.0 as per Hogan et al 2018). The peak efficacy of the booster remains unchanged as there is currently no data to parameterise this but the dose spacing between the third and fourth dose is reduced as a result of the delayed third dose but maintaining the fourth dose age contact at 24 months old. Solid line corresponds to the model median with the shaded areas the 50% and 95% Credible Intervals..

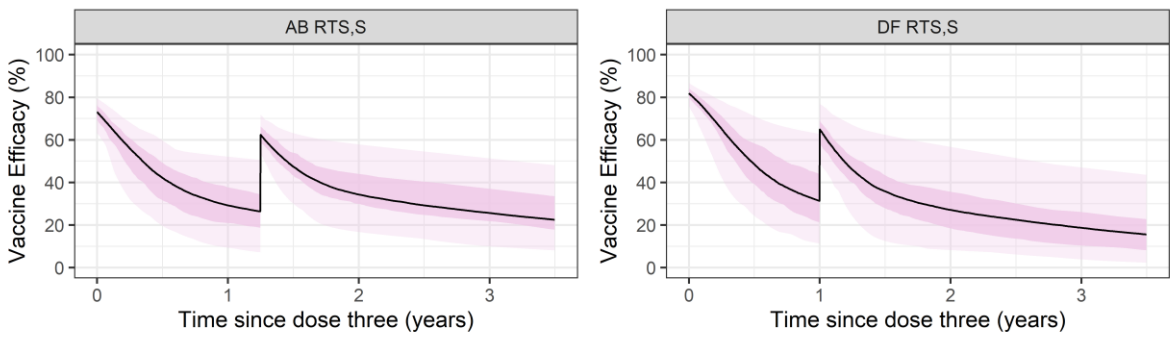

Figure 5.4 Sensitivity modelling of a delayed-fractional RTS,S efficacy profile. First-generation RTS,S efficacy is shown on the left (Vmax: 0.93, α: 0.74, β: 99.2) with the modified potential second-generation delayed fractional efficacy shown on the right. This profile assumes a higher peak efficacy following the primary series which is captured by altering the underlying efficacy model parameters (Vmax: 0.93, α: 0.95, β: 70.0 as per Hogan et al 2018). The peak efficacy of the booster remains

5.4 Results

5.4.1 RTS,S impact in seasonal transmission settings: seasonally targeted compared to age-based

The introduction of RTS,S either through age-based delivery or seasonal-vaccination campaigns was predicted to result in a significant reduction in clinical malaria cases and deaths in children under five years old in seasonal settings, with the absolute impact of vaccination increasing with higher transmission intensity (Figure 5.5, Table 5.2). Model simulations showed that SV-RTS,S resulted in greater reductions in cases and deaths than AB-RTS,S vaccination across all endemicity settings in both seasonal and highly seasonal settings over 15 years (Figure 5.5, Table 5.2). An additional fifth dose and/or higher fourth and fifth dose efficacy against infection increased this impact (Figure 5.5, Table 5.2). AB-RTS,S vaccination was predicted to avert a median of between 11,000–80,000 clinical cases in children aged 0–5 years dependent on seasonality and transmission intensity whereas SV-RTS,S was predicted to avert between 15,000–152,000 with a four-dose schedule and 17,000–172,000 with a five-dose schedule, dependent on seasonality, transmission intensity and the underlying efficacy model (Table 5.2).

Considering the effect of seasonality, the incremental benefit of SV-RTS,S over AB-RTS,S (defined as the proportion of additional events averted with SV-RTS,S versus AB-RTS,S) was slightly larger in highly seasonal settings compared to seasonal settings. On average, across all baseline $PfPR_{2\text{-}10}$ levels SV-RTS,S averted an additional 40–100% of cases and 42–94% of deaths in children under five years old in highly seasonal transmission settings and 32–88% and 31%–83% of cases and deaths in seasonal transmission settings depending on dose number and efficacy model.

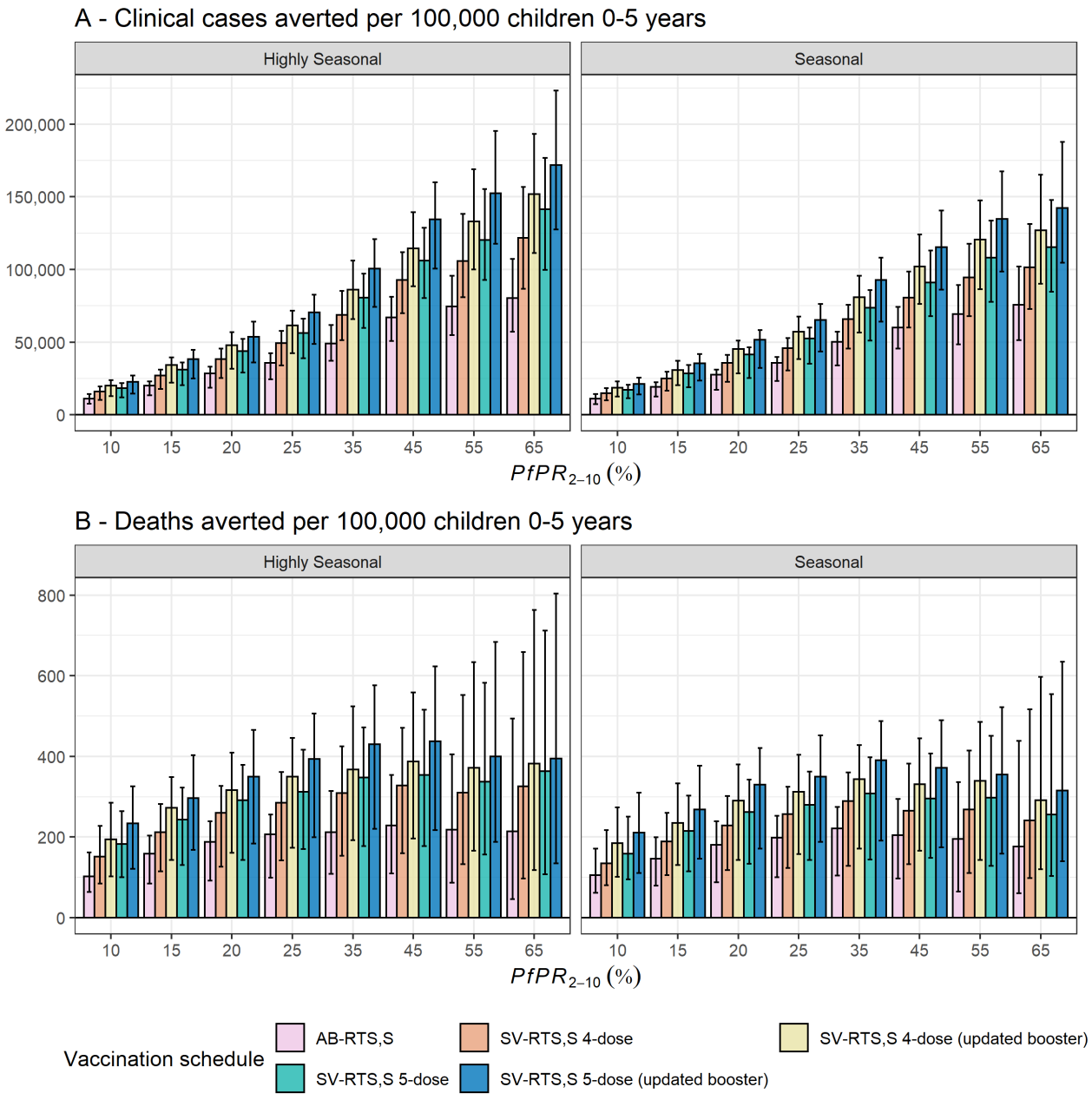

Figure 5.5 Population impacts of different RTS,S vaccination strategies in seasonal settings. A) Cumulative clinical cases averted and **B)** deaths averted over 15 years as a function of baseline *PfPR*$_{2\text{-}10}$ and seasonality in children aged 0-5 years old. Coverage is fixed at 80% for the first three doses with a 20% drop off (from the third dose) for the fourth and fifth doses. AB-RTS,S - is the four-dose age-based strategy, SV-RTS,S 4 & 5-dose is the seasonal strategy assuming the original vaccine efficacy profile from the Phase 3 RTS,S trials, SV-RTS,S 4 & 5-dose (updated booster) is the seasonal strategy assuming the updated higher efficacy against infection for the fourth and fifth dose based on the validation to the seasonal malaria vaccination Phase 3b clinical trial in Chapter 4. Model median values are presented in the coloured bars with error bars representing the 95% Credible Intervals.

Table 5.2 Predictions of public health impact of different RTS,S vaccination strategies at 15 years of follow-up in children aged 0-5 years, in regions with a parasite prevalence in 2–10 year olds of 10–65%. Cases and deaths averted are reported as cumulative over 15 years relative to a baseline no vaccination scenario per 100,000 population. Median value model predictions with 95% Credible Intervals in brackets.

			***PfPR*$_{2-10}$**							
Vaccination delivery	**Seasonality**	**Outcome Measure**	**10%**	**15%**	**20%**	**25%**	**35%**	**45%**	**55%**	**65%**
AB-RTS,S	Highly seasonal	Clinical cases averted	11,000 (8,000 - 14,000)	20,000 (14,000 - 23,000)	28,000 (19,000 - 33,000)	36,000 (24,000 - 42,000)	49,000 (37,000 - 62,000)	67,000 (51,000 - 81,000)	75,000 (55,000 - 96,000)	80,000 (57,000 - 107,000)
		Deaths averted	100 (70 - 160)	160 (90 - 200)	190 (90 - 240)	210 (100 - 250)	210 (100 - 310)	230 (110 - 350)	220 (90 - 410)	210 (50 - 490)
	Seasonal	Clinical cases averted	11,000 (7,000 - 14,000)	19,000 (13,000 - 23,000)	28,000 (17,000 - 31,000)	36,000 (23,000 - 40,000)	50,000 (34,000 - 57,000)	60,000 (46,000 - 74,000)	69,000 (48,000 - 89,000)	76,000 (51,000 - 102,000)
		Deaths averted	100 (60 - 170)	150 (80 - 200)	180 (90 - 240)	200 (100 - 250)	220 (100 - 270)	200 (100 - 290)	200 (60 - 330)	180 (60 - 440)
SV-RTS,S 4-dose	Highly seasonal	Clinical cases averted	16,000 (10,000 - 20,000)	27,000 (18,000 - 31,000)	38,000 (26,000 - 45,000)	49,000 (34,000 - 58,000)	69,000 (52,000 - 85,000)	93,000 (70,000 - 112,000)	106,000 (81,000 - 138,000)	122,000 (86,000 - 157,000)
		Deaths averted	150 (80 - 230)	210 (120 - 280)	260 (130 - 320)	280 (140 - 360)	310 (150 - 420)	330 (160 - 470)	310 (130 - 550)	330 (100 - 660)
	Seasonal	Clinical cases averted	15,000 (10,000 - 18,000)	25,000 (17,000 - 30,000)	36,000 (23,000 - 41,000)	46,000 (31,000 - 52,000)	66,000 (45,000 - 75,000)	80,000 (60,000 - 98,000)	94,000 (68,000 - 118,000)	101,000 (73,000 - 131,000)
		Deaths averted	140 (80 - 220)	190 (110 - 260)	230 (120 - 300)	260 (120 - 330)	290 (130 - 360)	260 (130 - 390)	270 (110 - 420)	240 (100 - 520)
SV-RTS,S 4-dose updated booster	Highly seasonal	Clinical cases averted	20,000 (13,000 - 24,000)	34,000 (22,000 - 39,000)	48,000 (32,000 - 57,000)	61,000 (42,000 - 71,000)	86,000 (66,000 - 106,000)	114,000 (88,000 - 140,000)	133,000 (100,000 - 169,000)	152,000 (111,000 - 193,000)
		Deaths averted	190 (100 - 280)	270 (140 - 340)	320 (160 - 410)	350 (170 - 450)	370 (190 - 520)	390 (200 - 560)	370 (160 - 630)	380 (120 - 760)
	Seasonal	Clinical cases averted	19,000 (13,000 - 23,000)	31,000 (21,000 - 37,000)	45,000 (28,000 - 51,000)	57,000 (38,000 - 67,000)	81,000 (57,000 - 96,000)	102,000 (76,000 - 124,000)	121,000 (87,000 - 148,000)	127,000 (90,000 - 165,000)
		Deaths averted	180 (100 - 270)	240 (130 - 330)	290 (140 - 380)	310 (160 - 400)	340 (170 - 430)	330 (170 - 450)	340 (140 - 480)	290 (120 - 600)
SV-RTS,S 5-dose	Highly seasonal	Clinical cases averted	18,000 (12,000 - 22,000)	31,000 (21,000 - 36,000)	44,000 (29,000 - 52,000)	56,000 (39,000 - 66,000)	80,000 (60,000 - 97,000)	106,000 (80,000 - 128,000)	120,000 (93,000 - 155,000)	141,000 (100,000 - 177,000)
		Deaths averted	180 (100 - 260)	240 (130 - 320)	290 (140 - 380)	310 (170 - 410)	350 (180 - 470)	350 (180 - 520)	340 (150 - 580)	360 (110 - 710)
	Seasonal	Clinical cases averted	17,000 (12,000 - 21,000)	29,000 (19,000 - 34,000)	41,000 (25,000 - 46,000)	53,000 (35,000 - 60,000)	74,000 (51,000 - 86,000)	91,000 (68,000 - 113,000)	108,000 (77,000 - 133,000)	115,000 (84,000 - 148,000)
		Deaths averted	160 (100 - 250)	220 (120 - 300)	260 (130 - 340)	280 (140 - 360)	310 (140 - 390)	300 (150 - 410)	300 (130 - 450)	260 (110 - 560)
SV-RTS,S 5-dose updated booster	Highly seasonal	Clinical cases averted	23,000 (15,000 - 27,000)	38,000 (25,000 - 44,000)	54,000 (36,000 - 64,000)	70,000 (48,000 - 82,000)	101,000 (75,000 - 121,000)	135,000 (101,000 - 160,000)	152,000 (117,000 - 196,000)	172,000 (128,000 - 223,000)
		Deaths averted	230 (120 - 330)	300 (170 - 400)	350 (180 - 460)	390 (200 - 510)	430 (220 - 580)	440 (220 - 630)	400 (190 - 680)	400 (130 - 800)
	Seasonal	Clinical cases averted	21,000 (14,000 - 26,000)	35,000 (24,000 - 42,000)	52,000 (32,000 - 58,000)	65,000 (43,000 - 76,000)	93,000 (64,000 - 108,000)	115,000 (86,000 - 141,000)	135,000 (99,000 - 167,000)	142,000 (104,000 - 188,000)
		Deaths averted	210 (110 - 310)	270 (150 - 380)	330 (170 - 420)	350 (190 - 450)	390 (190 - 480)	370 (170 - 490)	360 (160 - 520)	320 (140 - 640)

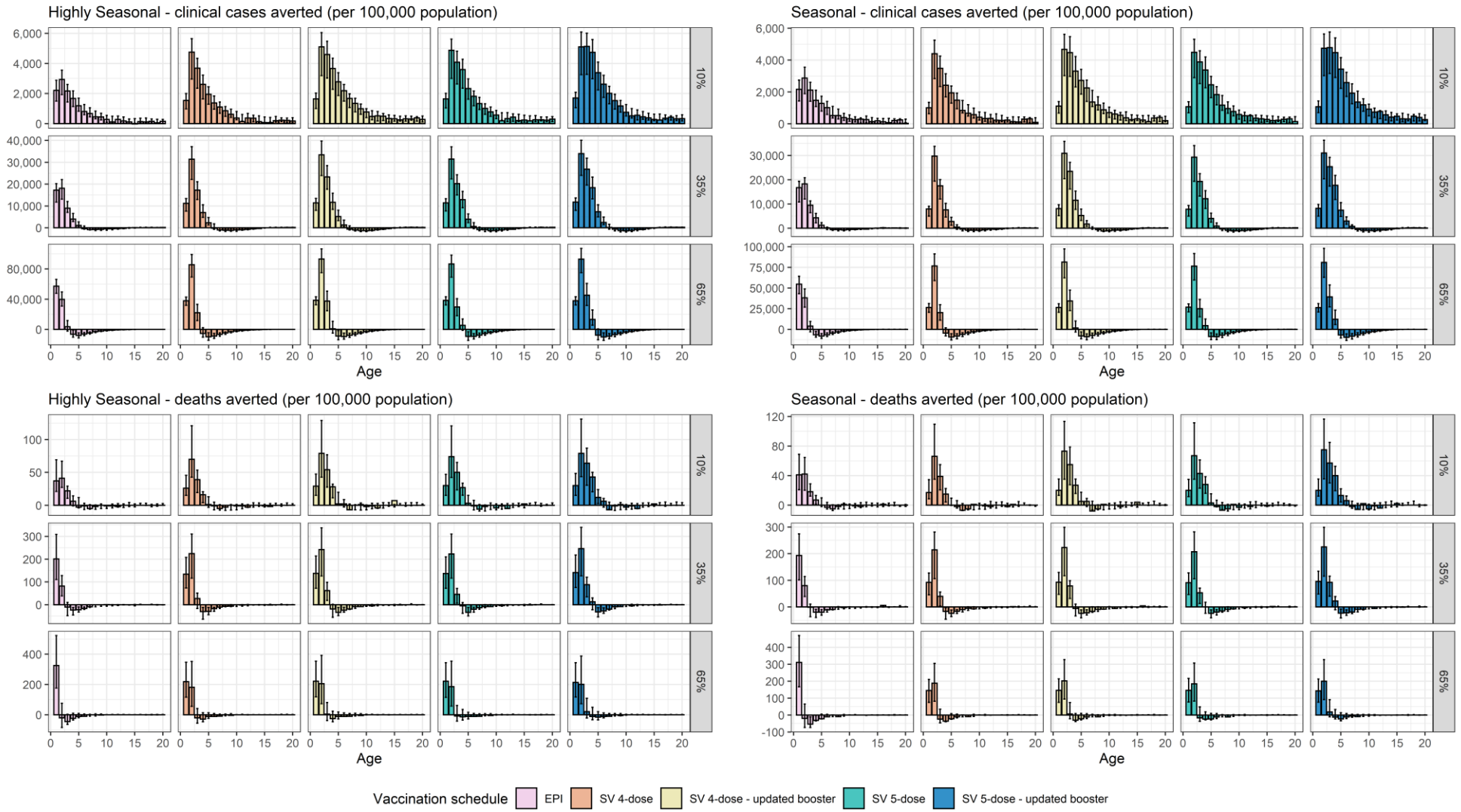

Figure 5.6 Modelled population impacts of different RTS,S vaccination strategies in seasonal settings across 1 year age groupings. Cumulative number of clinical cases (top row) and deaths (bottom row) averted over 15 years for individuals up to 20 years old in 1-year age bands. The total cases averted are shown per 100,000 population for both seasonality settings. Results are presented for three baseline $PfPR_{2\text{-}10}$ settings representative of low, medium and high transmission intensity. As expected with partially effective malaria control interventions, the model predicts a shift in cases to older ages due to the reduction in malaria exposure leading to a delay in the development of naturally acquired immunity. Therefore, the model predicts higher relative incidence at older ages resulting in slightly higher numbers of cases in the intervention groups compared to baseline shown here as the negative case reductions for older ages. Despite this overall cumulative impact of RTS,S on clinical cases and mortality over 15 years remains positive. Error bars represent the 95% Credible Intervals. Vaccine coverage is fixed at 80% for the first three doses with a 20% drop off (relative to the third dose) for the fourth and fifth doses.

Stratifying impact by age there was evidence of a shift in cases to older ages in high transmission settings as expected with this partially protective malaria intervention due to reduced malaria exposure leading to delays in the development of natural immunity (Figure 5.6)(Olotu et al., 2016). This effect was delayed with the introduction of a fifth dose in the SV-RTS,S schedule and was of similar magnitude across all vaccination scenarios and seasonality profiles (Figure 5.6). Despite this, the overall cumulative impact of all schedules and intervention models remained positive over this 15-year horizon in all settings.

Further, when stratifying impact by age there was evidence of some disparities between AB-RTS,S and SV-RTS,S in very young children. AB-RTS,S had a greater impact over 15 years in terms of reducing clinical cases and deaths in the first year of life compared to SV-RTS,S where impact was greater and sustained in children older than two (Figure 5.6). I examined this further in a single birth cohort of children, born over the course of a calendar year and followed from birth in the model simulation. This birth cohort is depicted in Figure 5.7 below where the shaded area represents the months at risk for a birth cohort born between months 1 and 12 on the x-axis, and the corresponding ages of children in the cohort at each time point. This disparity resulted in a slightly higher cumulative numbers of cases in the first 20 months of follow up in the SV-RTS,S cohort (reflecting the age range when all children would have received three doses under an age-based EPI schedule, but not all children would have received three doses under SV-RTS,S, depending on their birth month) (Figure 5.8). This is most marked when SV-RTS,S is compared to AB-RTS,S in seasonal settings. In highly seasonal settings, the disadvantage of SV-RTS,S (due to potential higher age at vaccination) was partly offset due to the shorter transmission season (Figure 5.8).

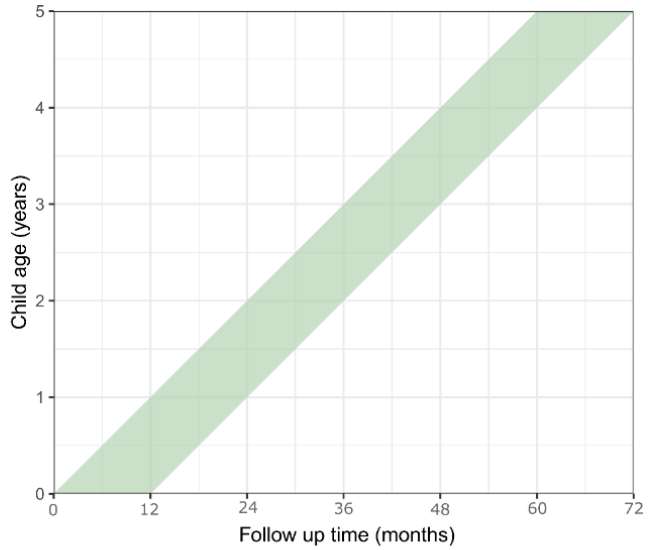

Figure 5.7 Lexis plot of the age of a birth cohort over calendar time. The shaded green area shows months at risk for a birth cohort born between months 1 and 12 on the x-axis, and the corresponding ages of children in the cohort at each follow up time point.

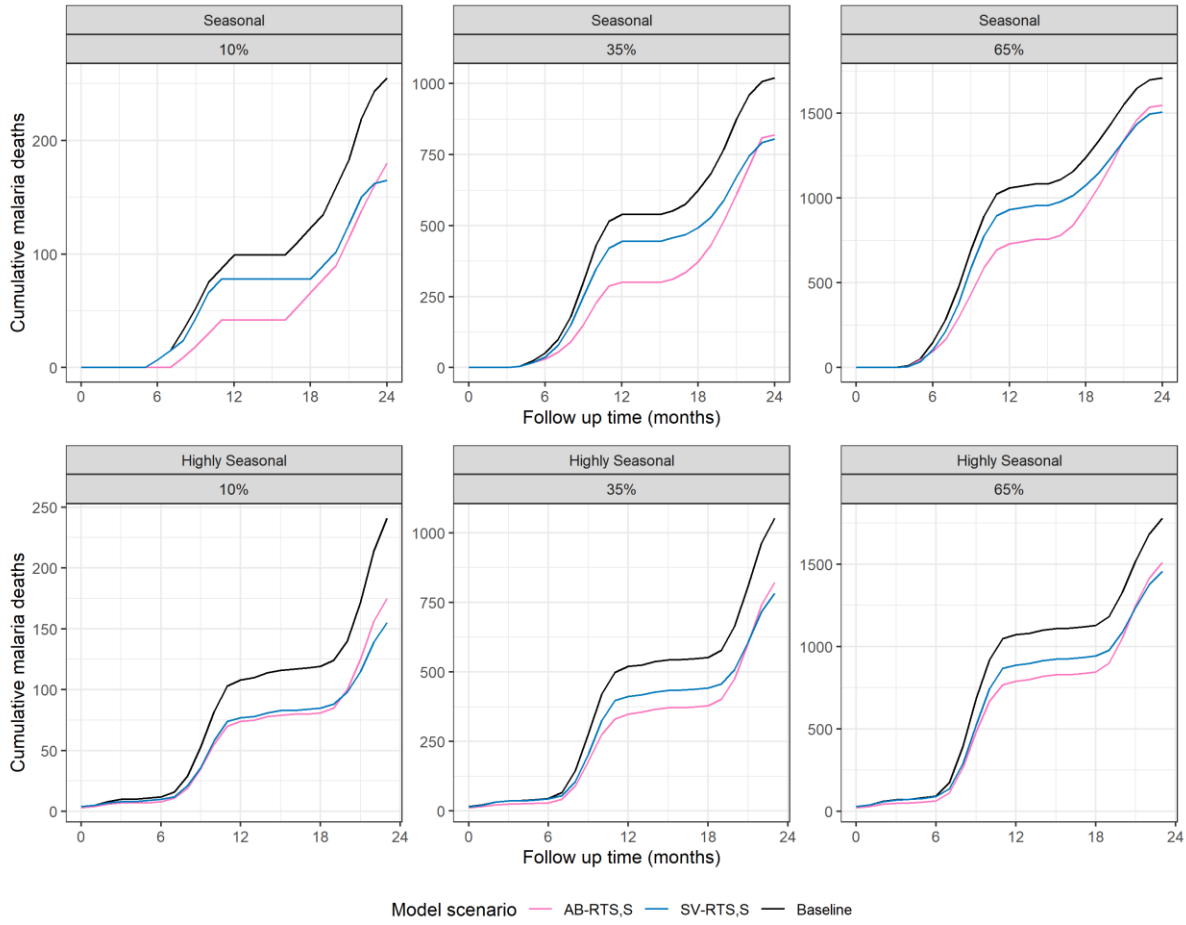

Figure 5.8 Impact of RTS,S vaccination schedules for a birth cohort of children over two years. Cumulative malaria deaths as a function of baseline $PfPR_{2\text{-}10}$ (three settings representative of low, medium and high transmission intensity are shown) and seasonality. All SV-RTS,S scenarios are represented by the blue lines as impact is consistent for all schedules following the primary series prior to any additional doses. Results are presented for a seasonal setting (top row) and a highly seasonal setting (bottom row). Vaccine coverage is fixed at 80% for the first three doses with a 20% drop off (relative to the third dose) for the fourth and fifth doses. Baseline cumulative incidence assuming no vaccination is shown in the black line.

5.4.2 RTS,S impact in seasonal transmission settings in combination with seasonal malaria chemoprevention: seasonally targeted compared to age-based

The combination of RTS,S and SMC was predicted to have substantially more impact than either intervention given alone in seasonal transmission settings. The combination of SV-RTS,S + SMC resulted in a greater number of cases and deaths averted in children 0-5 years old compared to AB-RTS,S + SMC across all transmission levels and intervention models (Figure 5.9, Table 5.3). SV-RTS,S + SMC averted a median of between 37,000–407,000 clinical cases over 15 years with a four-dose schedule or between 38,000–421,000 with a five-dose schedule, dependent on seasonality, transmission intensity and efficacy model (Table 5.3). AB-RTS,S + SMC averted between 35,000–340,000 clinical cases over 15 years dependent on seasonality and transmission intensity (Table 5.3). SMC alone was predicted to avert between 28,000–270,000 clinical cases over 15 years dependent on seasonality and transmission intensity.

The combination of RTS,S vaccination on top of SMC therefore was predicted to avert up to an additional 151,000 clinical cases with a five-dose seasonal schedule over 15 years in this target age group or with an age-based approach up to an additional 63,000 cases dependent on efficacy model, seasonality and transmission intensity. Seasonally targeting RTS,S averted up to 2.4 times more clinical cases than an age-based RTS,S schedule when combined with SMC.

A difference noted when RTS,S was combined with SMC, rather than when considered alone, was that the additional modelled impact of vaccination over SMC was higher in seasonal transmission settings than in highly seasonal settings. On average, across all baseline $PfPR_{2\text{-}10}$ levels, the addition of SV-RTS,S to SMC was predicted to reduce clinical cases and deaths in children 0-5 years old by a further 36%–56% and 46%–70% respectively in seasonal settings and by 26%–41% and 32%–49% respectively in highly seasonal settings, depending on dose number and efficacy model. The incremental impact of AB-RTS,S over SMC was smaller across both seasonality profiles averting an additional 26% of cases and 35% of deaths in seasonal settings and 19% of cases and 23% of deaths in highly seasonal settings.

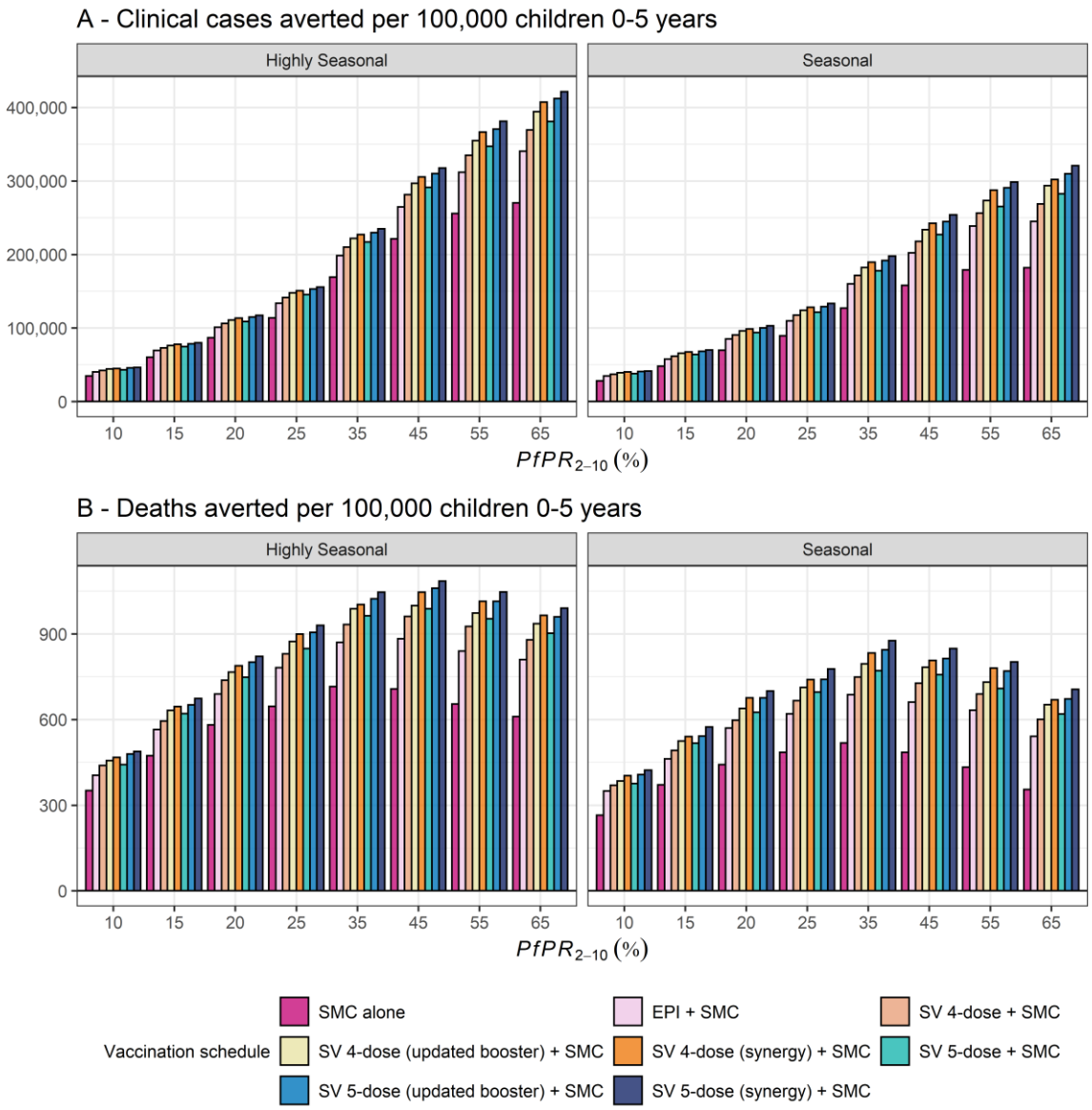

Figure 5.9 Population impacts of different RTS,S vaccination strategies in seasonal settings when combined with SMC. A) Cumulative clinical cases averted and **B)** deaths averted over 15 years as a function of baseline $PfPR_{2\text{-}10}$ and seasonality in children aged 0-5 years old. Vaccine coverage is fixed at 80% for the first three doses with a 20% drop off (from the third dose) for the fourth and fifth doses and SMC coverage of 75%. AB-RTS,S - is the four-dose age-based strategy, SV-RTS,S 4 & 5-dose is the seasonal strategy assuming the original vaccine efficacy profile from the Phase 3 RTS,S trials, SV-RTS,S 4 & 5-dose (updated booster) is the seasonal strategy assuming the updated higher efficacy against infection for the fourth and fifth dose, SV-RTS,S 4 & 5-dose (synergy) assumes higher SP+AQ efficacy both based on the validation to the seasonal malaria vaccination Phase 3b clinical trial data in Chapter 4 and. Model median values are presented in the coloured bars Credible Intervals are not shown on the plot for clarity but can be seen in Table 5.3. Vaccine coverage is fixed at 80% for the first three doses with a 20% drop off (from the third dose) for the fourth and fifth doses, SMC coverage of 75%. RTS,S 4 & 5-dose (synergy) assumes higher SP+AQ efficacy both based on the validation to the seasonal malaria vaccination Phase 3b clinical trial data in Chapter 4 and. Model median values are presented in the coloured bars Credible Intervals are not shown on the plot for clarity but can be seen in Table 5.3. Vaccine coverage is fixed at 80% for the first three doses with a 20% drop off (from the third dose) for the fourth and fifth doses, SMC coverage of 75%.

Table 5.3 Predictions of public health impact of different RTS,S vaccination strategies when combined with SMC at 15 years of follow-up in children aged 0-5 years, in regions with a parasite prevalence in 2–10 year olds ($PfPR_{2-10}$) of 10–65%. Cases and deaths averted are reported as cumulative over 15 years relative to a baseline no vaccination no SMC scenario, per 100,000 population. Median value model predictions with 95% Credible Intervals in brackets.

			$PfPR_{2-10}$							
Vaccination delivery	**Seasonality**	**Outcome Measure**	**10%**	**15%**	**20%**	**25%**	**35%**	**45%**	**55%**	**65%**
AB-RTS,S + SMC	Highly seasonal	Clinical cases averted	40,000 (26,000 - 49,000)	69,000 (45,000 - 82,000)	101,000 (67,000 - 120,000)	134,000 (90,000 - 157,000)	198,000 (145,000 - 240,000)	265,000 (200,000 - 321,000)	312,000 (233,000 - 392,000)	340,000 (248,000 - 433,000)
		Deaths averted	400 (220 - 620)	560 (310 - 780)	690 (360 - 910)	780 (410 - 1,020)	870 (450 - 1,160)	880 (460 - 1,270)	840 (410 - 1,400)	810 (280 - 1,620)
	Seasonal	Clinical cases averted	35,000 (23,000 - 42,000)	58,000 (38,000 - 70,000)	85,000 (54,000 - 98,000)	110,000 (73,000 - 129,000)	160,000 (112,000 - 189,000)	202,000 (151,000 - 248,000)	239,000 (173,000 - 295,000)	245,000 (175,000 - 322,000)
		Deaths averted	350 (180 - 520)	460 (250 - 650)	570 (290 - 740)	620 (330 - 810)	690 (340 - 880)	660 (330 - 900)	630 (250 - 960)	540 (220 - 1,140)
SV-RTS,S 4-dose + SMC	Highly seasonal	Clinical cases averted	42,000 (27,000 - 51,000)	73,000 (47,000 - 86,000)	106,000 (70,000 - 126,000)	141,000 (94,000 - 165,000)	210,000 (153,000 - 252,000)	281,000 (214,000 - 341,000)	335,000 (250,000 - 418,000)	369,000 (271,000 - 472,000)
		Deaths averted	440 (240 - 650)	600 (320 - 830)	740 (390 - 970)	830 (440 - 1,080)	930 (480 - 1,240)	960 (490 - 1,350)	930 (450 - 1,490)	880 (310 - 1,710)
	Seasonal	Clinical cases averted	37,000 (24,000 - 44,000)	62,000 (40,000 - 74,000)	91,000 (57,000 - 105,000)	117,000 (78,000 - 137,000)	172,000 (119,000 - 201,000)	218,000 (163,000 - 268,000)	256,000 (186,000 - 317,000)	269,000 (193,000 - 351,000)
		Deaths averted	370 (190 - 550)	490 (270 - 690)	600 (310 - 780)	670 (350 - 860)	750 (380 - 940)	730 (360 - 980)	690 (310 - 1,030)	600 (260 - 1,210)
SV-RTS,S 4-dose updated booster + SMC	Highly seasonal	Clinical cases averted	44,000 (28,000 - 53,000)	76,000 (49,000 - 90,000)	111,000 (73,000 - 132,000)	148,000 (99,000 - 174,000)	222,000 (161,000 - 265,000)	297,000 (226,000 - 360,000)	355,000 (266,000 - 444,000)	394,000 (290,000 - 502,000)
		Deaths averted	460 (250 - 680)	630 (350 - 880)	770 (410 - 1,020)	870 (470 - 1,140)	990 (510 - 1,300)	1,000 (520 - 1,430)	970 (490 - 1,570)	940 (330 - 1,770)
	Seasonal	Clinical cases averted	39,000 (26,000 - 47,000)	66,000 (42,000 - 78,000)	96,000 (61,000 - 111,000)	124,000 (83,000 - 147,000)	182,000 (126,000 - 215,000)	234,000 (174,000 - 284,000)	273,000 (201,000 - 341,000)	293,000 (209,000 - 379,000)
		Deaths averted	380 (200 - 580)	520 (290 - 740)	640 (350 - 840)	710 (380 - 930)	800 (400 - 1,010)	780 (390 - 1,040)	730 (340 - 1,100)	650 (290 - 1,280)
SV-RTS,S 4-dose synergy + SMC	Highly seasonal	Clinical cases averted	45,000 (29,000 - 54,000)	78,000 (51,000 - 92,000)	113,000 (74,000 - 135,000)	151,000 (101,000 - 177,000)	227,000 (164,000 - 270,000)	305,000 (232,000 - 369,000)	366,000 (273,000 - 453,000)	407,000 (298,000 - 514,000)
		Deaths averted	470 (250 - 700)	640 (360 - 900)	790 (420 - 1,040)	900 (470 - 1,160)	1,000 (530 - 1,340)	1,050 (530 - 1,460)	1,010 (510 - 1,610)	960 (340 - 1,820)
	Seasonal	Clinical cases averted	40,000 (26,000 - 48,000)	67,000 (44,000 - 81,000)	99,000 (63,000 - 114,000)	128,000 (85,000 - 150,000)	190,000 (131,000 - 222,000)	242,000 (179,000 - 295,000)	287,000 (209,000 - 355,000)	302,000 (217,000 - 391,000)
		Deaths averted	400 (220 - 610)	540 (300 - 770)	680 (350 - 860)	740 (390 - 970)	830 (430 - 1,060)	810 (410 - 1,090)	780 (350 - 1,130)	670 (300 - 1,320)
SV-RTS,S 5-dose + SMC	Highly seasonal	Clinical cases averted	43,000 (28,000 - 52,000)	75,000 (49,000 - 89,000)	109,000 (72,000 - 130,000)	145,000 (98,000 - 170,000)	217,000 (157,000 - 260,000)	291,000 (220,000 - 351,000)	347,000 (261,000 - 431,000)	381,000 (285,000 - 489,000)
		Deaths averted	440 (240 - 670)	620 (340 - 860)	750 (400 - 1,000)	850 (460 - 1,110)	960 (500 - 1,280)	990 (500 - 1,390)	950 (480 - 1,530)	900 (320 - 1,750)
	Seasonal	Clinical cases averted	38,000 (25,000 - 46,000)	64,000 (41,000 - 77,000)	94,000 (59,000 - 108,000)	122,000 (81,000 - 142,000)	178,000 (124,000 - 209,000)	227,000 (169,000 - 277,000)	265,000 (195,000 - 329,000)	282,000 (200,000 - 366,000)
		Deaths averted	380 (200 - 570)	520 (280 - 720)	620 (320 - 820)	700 (360 - 890)	770 (390 - 980)	760 (380 - 1,010)	710 (330 - 1,060)	620 (260 - 1,240)

SV-RTS,S 5-dose updated booster + SMC	Highly seasonal	Clinical cases averted	46,000 (29,000 - 55,000)	78,000 (51,000 - 93,000)	115,000 (76,000 - 136,000)	153,000 (102,000 - 179,000)	230,000 (166,000 - 275,000)	310,000 (235,000 - 372,000)	370,000 (278,000 - 463,000)	412,000 (303,000 - 525,000)
		Deaths averted	480 (250 - 710)	650 (360 - 910)	800 (420 - 1,050)	900 (470 - 1,180)	1,020 (530 - 1,360)	1,060 (550 - 1,470)	1,010 (510 - 1,620)	960 (350 - 1,820)
	Seasonal	Clinical cases averted	41,000 (27,000 - 49,000)	68,000 (44,000 - 81,000)	100,000 (64,000 - 115,000)	129,000 (86,000 - 152,000)	192,000 (132,000 - 224,000)	245,000 (183,000 - 299,000)	291,000 (212,000 - 359,000)	310,000 (221,000 - 400,000)
		Deaths averted	410 (220 - 610)	540 (300 - 780)	680 (350 - 880)	740 (390 - 960)	840 (420 - 1,060)	810 (400 - 1,090)	770 (360 - 1,130)	670 (300 - 1,320)
SV-RTS,S 5-dose synergy + SMC	Highly seasonal	Clinical cases averted	46,000 (30,000 - 55,000)	80,000 (52,000 - 95,000)	117,000 (77,000 - 139,000)	156,000 (104,000 - 183,000)	235,000 (170,000 - 281,000)	318,000 (240,000 - 382,000)	381,000 (286,000 - 473,000)	421,000 (312,000 - 538,000)
		Deaths averted	490 (250 - 720)	670 (360 - 940)	820 (430 - 1,090)	930 (500 - 1,210)	1,050 (550 - 1,390)	1,080 (560 - 1,500)	1,050 (520 - 1,640)	990 (370 - 1,860)
	Seasonal	Clinical cases averted	41,000 (27,000 - 50,000)	70,000 (45,000 - 83,000)	103,000 (66,000 - 118,000)	133,000 (89,000 - 158,000)	198,000 (137,000 - 231,000)	254,000 (189,000 - 308,000)	298,000 (219,000 - 370,000)	321,000 (230,000 - 413,000)
		Deaths averted	420 (230 - 630)	570 (320 - 800)	700 (360 - 900)	780 (410 - 1,000)	880 (440 - 1,100)	850 (430 - 1,140)	800 (370 - 1,170)	710 (320 - 1,350)
SMC	Highly seasonal	Clinical cases averted	35,000 (23,000 - 42,000)	60,000 (39,000 - 71,000)	87,000 (57,000 - 104,000)	114,000 (77,000 - 136,000)	169,000 (122,000 - 203,000)	221,000 (168,000 - 270,000)	256,000 (192,000 - 321,000)	270,000 (201,000 - 344,000)
		Deaths averted	350 (180 - 520)	470 (270 - 650)	580 (310 - 760)	650 (340 - 830)	720 (360 - 960)	710 (360 - 1,040)	650 (330 - 1,120)	610 (200 - 1,260)
	Seasonal	Clinical cases averted	28,000 (19,000 - 34,000)	48,000 (31,000 - 57,000)	70,000 (44,000 - 81,000)	89,000 (60,000 - 104,000)	127,000 (88,000 - 150,000)	158,000 (118,000 - 197,000)	179,000 (131,000 - 225,000)	182,000 (129,000 - 238,000)
		Deaths averted	260 (160 - 400)	370 (200 - 500)	440 (230 - 570)	480 (260 - 610)	520 (250 - 660)	480 (250 - 670)	430 (190 - 700)	360 (160 - 820)

Again, there was a shift in cases to older ages that was of a slightly higher magnitude than when only vaccination was modelled (Figure 5.11). But again, despite this age shift in cases, the overall cumulative impact of all schedules and intervention models remained positive over this 15-year horizon in all settings. In addition, the inclusion of SMC alongside SV-RTS,S marginally reduced the effect of disparity in age at first vaccination between SV- and AB-RTS,S strategies described above, since SMC provided protection from the age of three months (Figure 5.10).

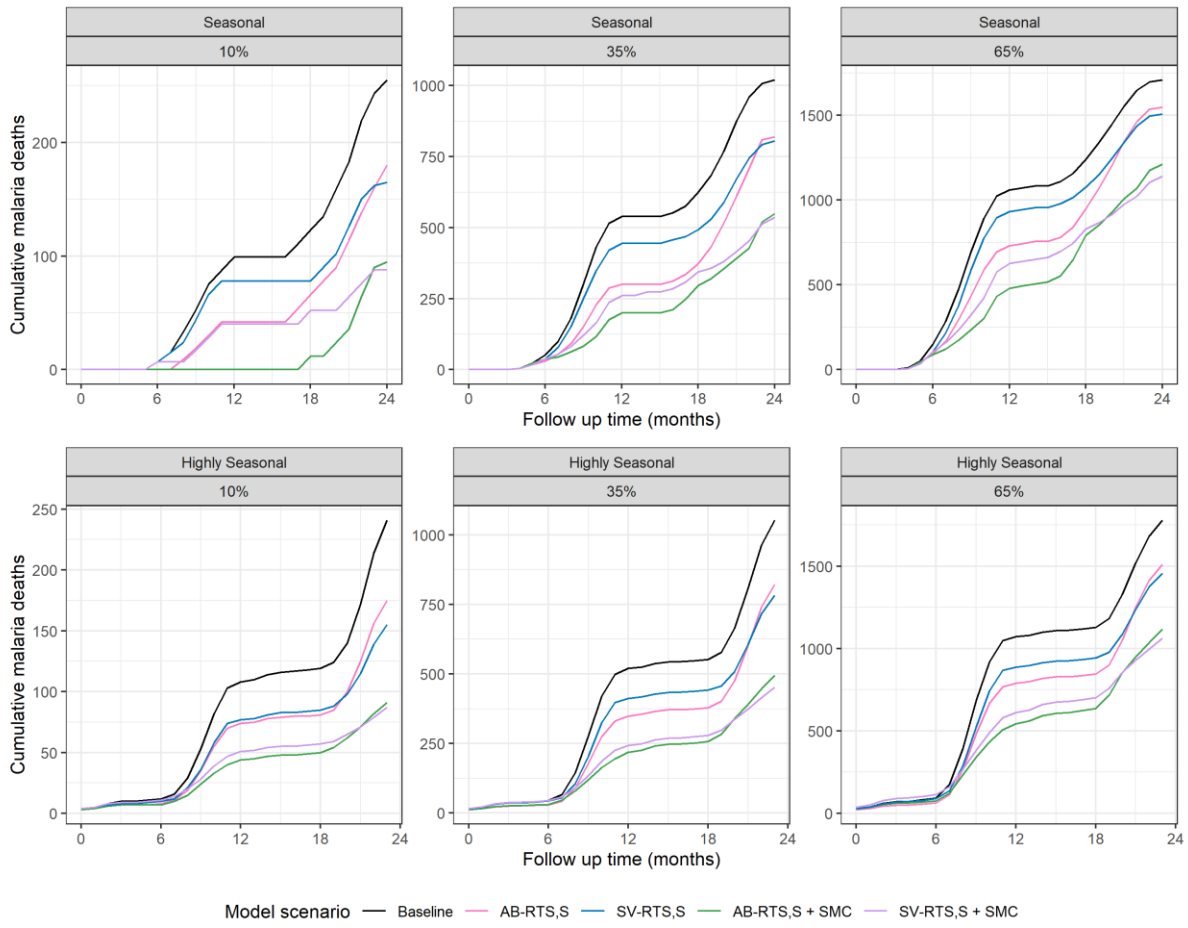

Figure 5.10 Modelled impact of RTS,S vaccination schedules for a birth cohort of children over two years in the presence of SMC or without SMC. Cumulative malaria deaths as a function of baseline $PfPR_{2\text{-}10}$ (three settings representative of low, medium and high transmission intensity are shown) and seasonality. All SV-RTS,S scenarios are represented by the blue lines as impact is consistent for all schedules following the primary series. Results are presented for a seasonal setting (top row) and a highly seasonal setting (bottom row). Vaccine coverage is fixed at 80% for the first three doses with a 20% drop off (from the third dose) for the fourth and fifth doses, SMC coverage of 75%.

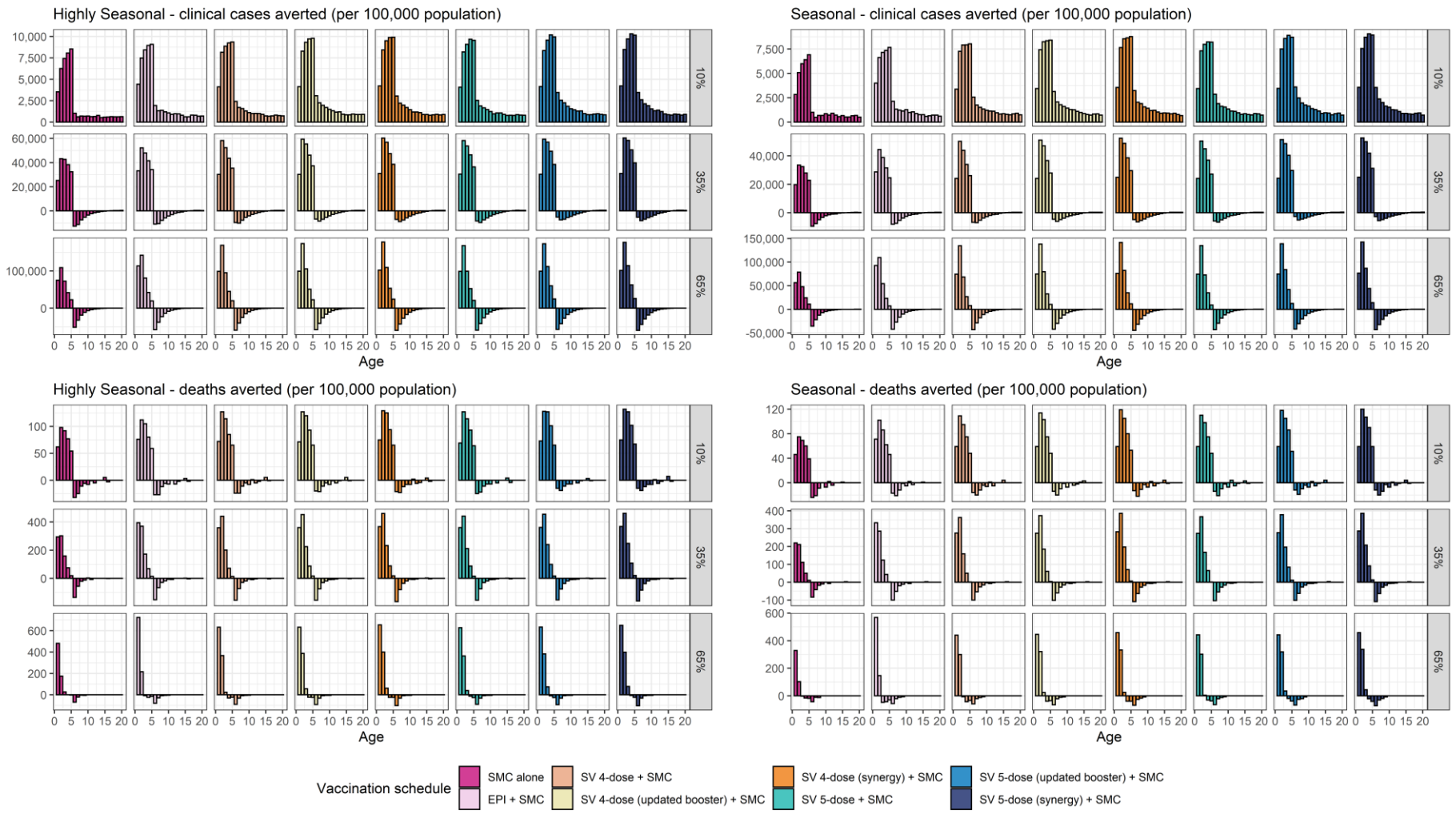

Figure 5.11 Modelled population impacts of different RTS,S vaccination strategies when combined with SMC in seasonal settings across 1 year age groupings. Cumulative number of clinical cases (top row) and deaths (bottom row) averted over 15 years for individuals up to 20 years old in 1-year age bands. The total cases averted are shown per 100,000 population for both seasonality settings. Results are presented for three baseline *PfPR*$_{2\text{-}10}$ settings representative of low, medium and high transmission intensity. As expected with partially effective malaria control interventions, there was a shift in cases to older ages due to the reduction in malaria exposure leading to a delay in the development of naturally acquired immunity. Therefore, the model predicts higher relative incidence at older ages resulting in slightly higher numbers of cases in the intervention groups compared to the baseline groups shown here as the negative case reductions for older ages. Despite this overall cumulative impact of seasonal interventions on clinical cases and mortality over 15 years remains positive. Bars represent the median of model simulations, error bars with 95% Credible Intervals are not shown for clarity. Vaccine coverage is fixed at 80% for the first three doses with a 20% drop off (relative to the third dose) for the fourth and fifth doses, SMC coverage of 75%.

As discussed in Chapter 3, four monthly cycles of SMC are considered too short for the transmission seasons of much of the southern Sahel and an increase in the number of monthly cycles are increasingly being considered in the region. Exploring the modelled impact of RTS,S vaccination when combined with 5-monthly cycles of SMC in these characteristic seasonal settings, there was an increase in the overall number of cases averted in both seasonal settings with a greater increase in case reductions in seasonal compared to highly seasonal settings (Figure 5.12A). The incremental impact of any RTS,S vaccination schedule on top of SMC however was reduced as a larger proportion of the peak transmission season was covered by SMC (Figure 5.12B). Despite this, seasonally targeted

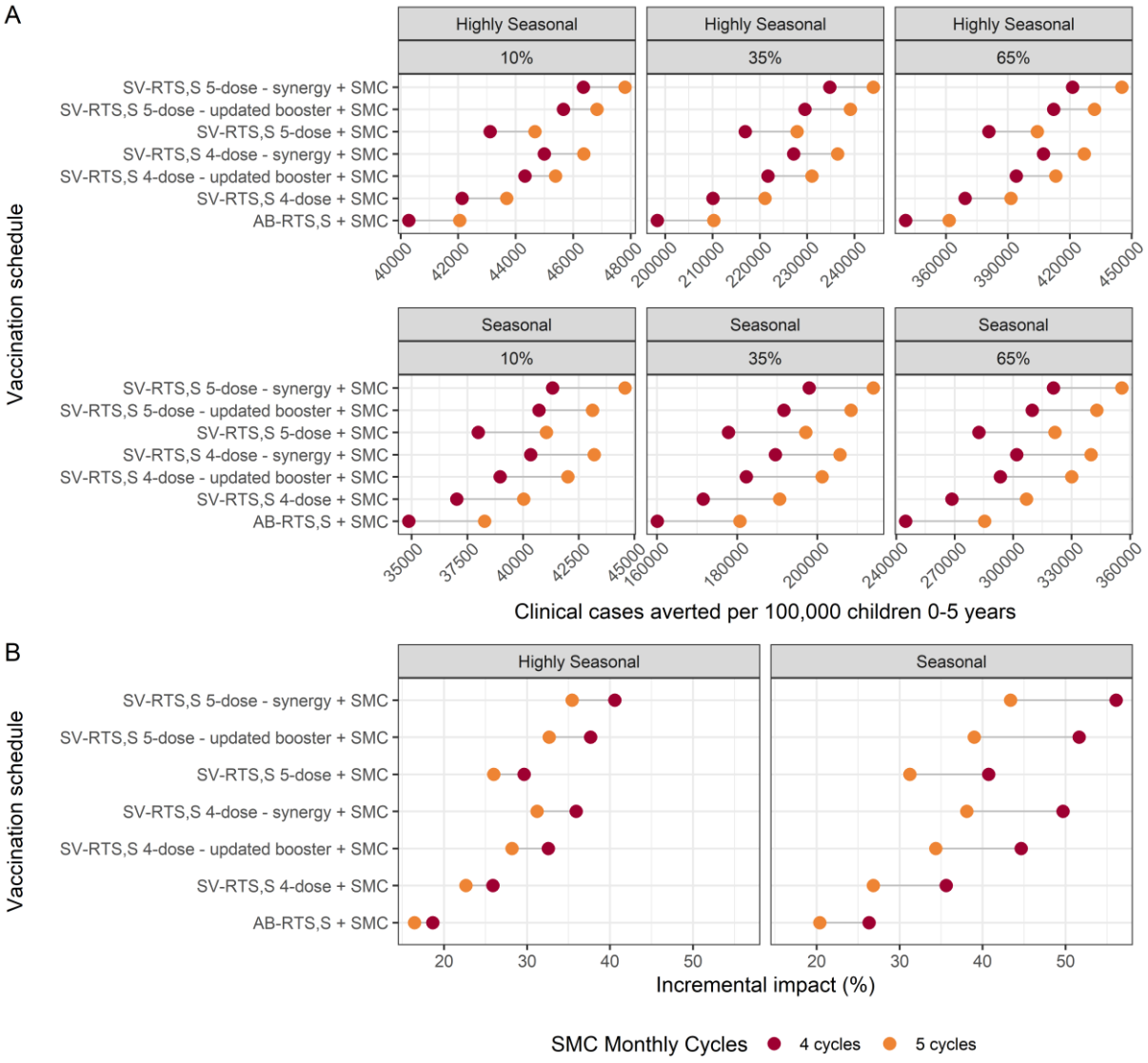

Figure 5.12 Impact of an additional SMC monthly cycle. A) The impact of an additional round of SMC on cumulative clinical cases averted in children 0-5 years old over 15 years as a function of baseline $PfPR_{2\text{-}10}$ (three settings representative of low, medium and high transmission intensity) and each vaccination schedule. **B)** Average change in the incremental impact (defined as the proportion of additional events averted with RTS,S combined with SMC over SMC alone) of combined arms simulations relative to SMC alone averaged over all baseline $PfPR_{2\text{-}10}$ levels (10%-65%). Vaccine coverage was fixed at 80% for the first three doses with a 20% drop off (relative to the third dose) for the fourth and fifth doses with SMC coverage of 75%.

RTS,S vaccination could still avert on average, across all baseline $PfPR_{2-10}$ levels, up to an additional 43% more cases than SMC alone B).

5.4.3 Health Systems considerations - sensitivity analysis

For all the previous analyses, assumed vaccine coverage was the same for both vaccine schedules at 80% coverage with the first three doses with a 20% drop-off for the subsequent doses. Testing the sensitivity of results to the coverage of the first three doses I found that RTS,S vaccine impact for all schedules scaled approximately linearly with coverage (Figure 5.13).

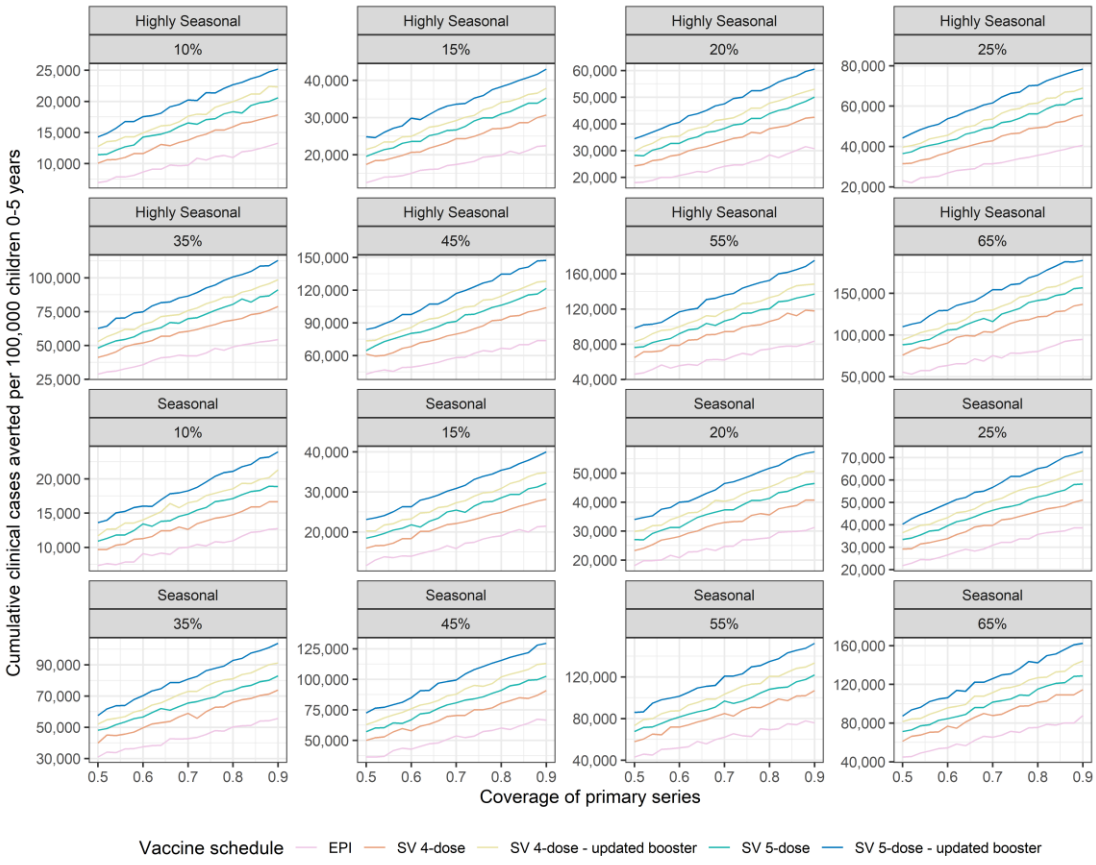

Figure 5.13 Effect of increasing vaccination coverage of the first three RTS,S doses on potential cases averted. Cumulative clinical cases averted per 100,000 children aged 0-5 years over a period of 15 years following vaccine introduction, stratified by seasonality profile and baseline $PfPR_{2-10}$. Coverage of the fourth or fifth vaccine doses remains unchanged at a 20% drop off from the coverage of the first three doses.

Decisions surrounding what RTS,S schedule to implement will also have to account for the potential achievable coverage of the different delivery schedules. When comparing the additional cases averted with a SV-RTS,S schedule compared to an AB-RTS,S schedule, the coverage of each approach was a key determinant of the schedule with the highest impact in a particular setting (Figure 5.14). The superiority of SV-RTS,S across the majority of coverage comparisons remained especially with a five-

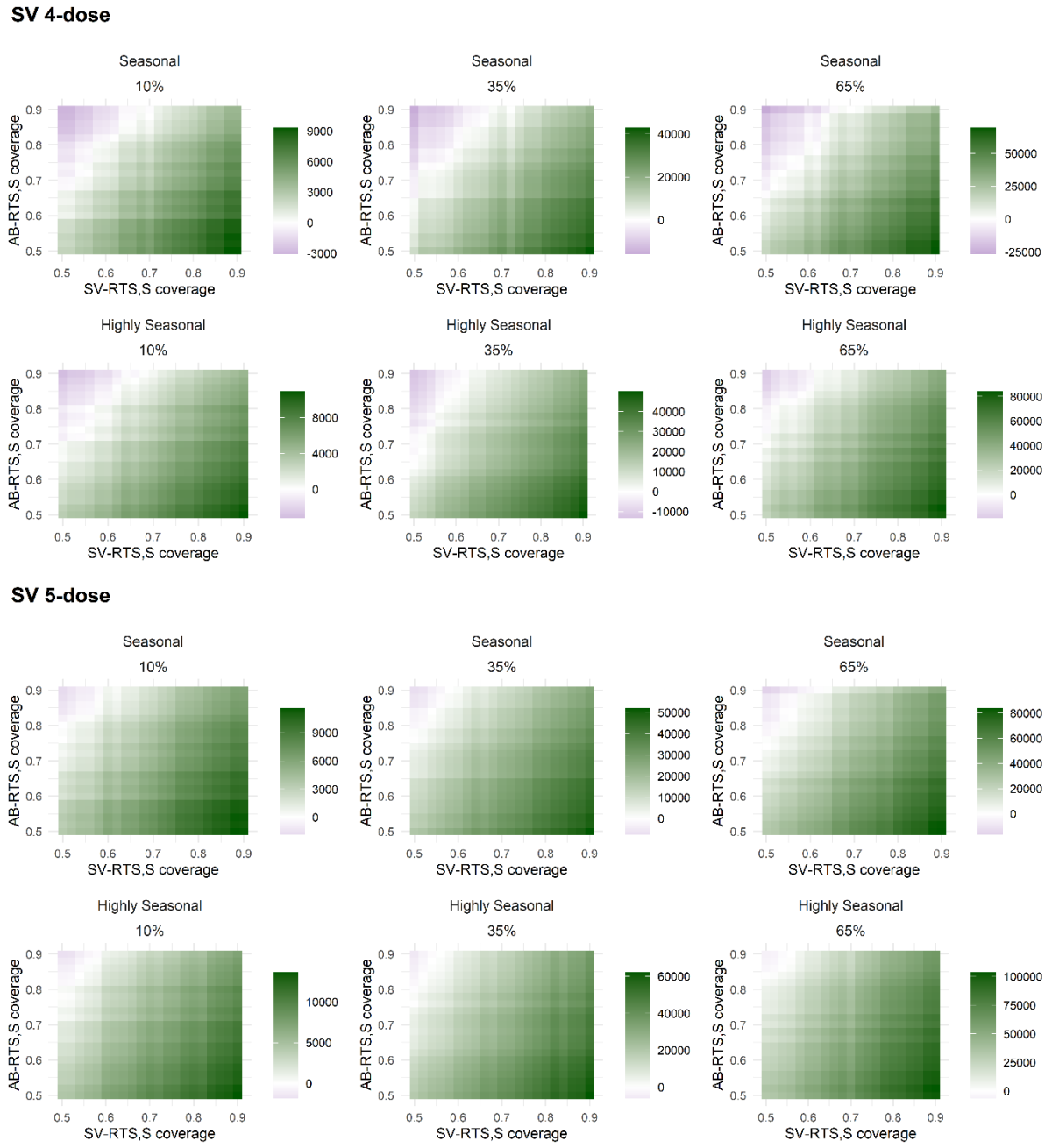

Figure 5.14. Comparability of vaccination schedules with varying levels of coverage of the first three vaccine doses. Colour scale represented the additional cases averted with a four- (top panel) of five- (bottom panel) dose SV-RTS,S schedule compared to an AB-RTS,S schedule dependent on coverage, seasonality and baseline $PfPR_{2\text{-}10}$ (three settings representative of low, medium and high transmission intensity are shown). Note that the scale varies in each subplot. Equivalent case reductions are shown in white, dominance of SV-RTS,S in green and dominance of AB-RTS,S in purple. Comparisons are made assuming the same underlying parameterizations of the efficacy model in each scenario.

dose schedule apart from at very low SV-RTS,S coverage (~<50%) and very high AB-RTS,S coverage (~>80%) (Figure 5.14 bottom panel). However, when a four-dose schedule was considered at low coverage levels (~<65%) and with high AB-RTS,S coverage (~70%) an age-based approach was predicted to avert more cases over 15 years in this target population (Figure 5.14 top panel). The comparisons between schedules shown in Figure 5.14 are made assuming the same efficacy profile model parameters (Figure 5.2A,B,D) if the seasonal-vaccination updated booster model was used (Figure 5.2C,E) SV-RTS,S outperformed AB-RTS,S across all coverage comparisons.

When combined with SMC the potential coverage of an SMC regime as well as that of the RTS,S vaccine will again be important determinants of impact. At lower SMC coverage vaccination had a higher predicted additional impact on top of SMC than when SMC coverage was high (Figure 5.15). However, even when the coverage of both interventions was high (>80%) RTS,S was predicted to provide significant additional impact on top of SMC (Figure 5.15).

Further, for countries to adopt and deploy seasonally targeted intervention packages, knowledge of the timing of the annual transmission season is vital. As an intervention, SMC impact was highly sensitive to modelled changes in delivery relative to the peak in transmission (Figure 5.16A). This can make operational deployment of SMC challenging due to annual fluctuations in the rainy season with consequent logistical or supply issues. SV-RTS,S, however, was more robust to modelled changes in delivery (Figure 5.16A), therefore when interventions were combined the potential reduction in case burden was reduced relative to SMC delivery alone (Figure 5.16B). Given that RTS,S delivery through an age-based schedule is not reliant on calendar time, this approach may mitigate some of the challenges of optimal alignment in SMC delivery (Figure 5.16C).

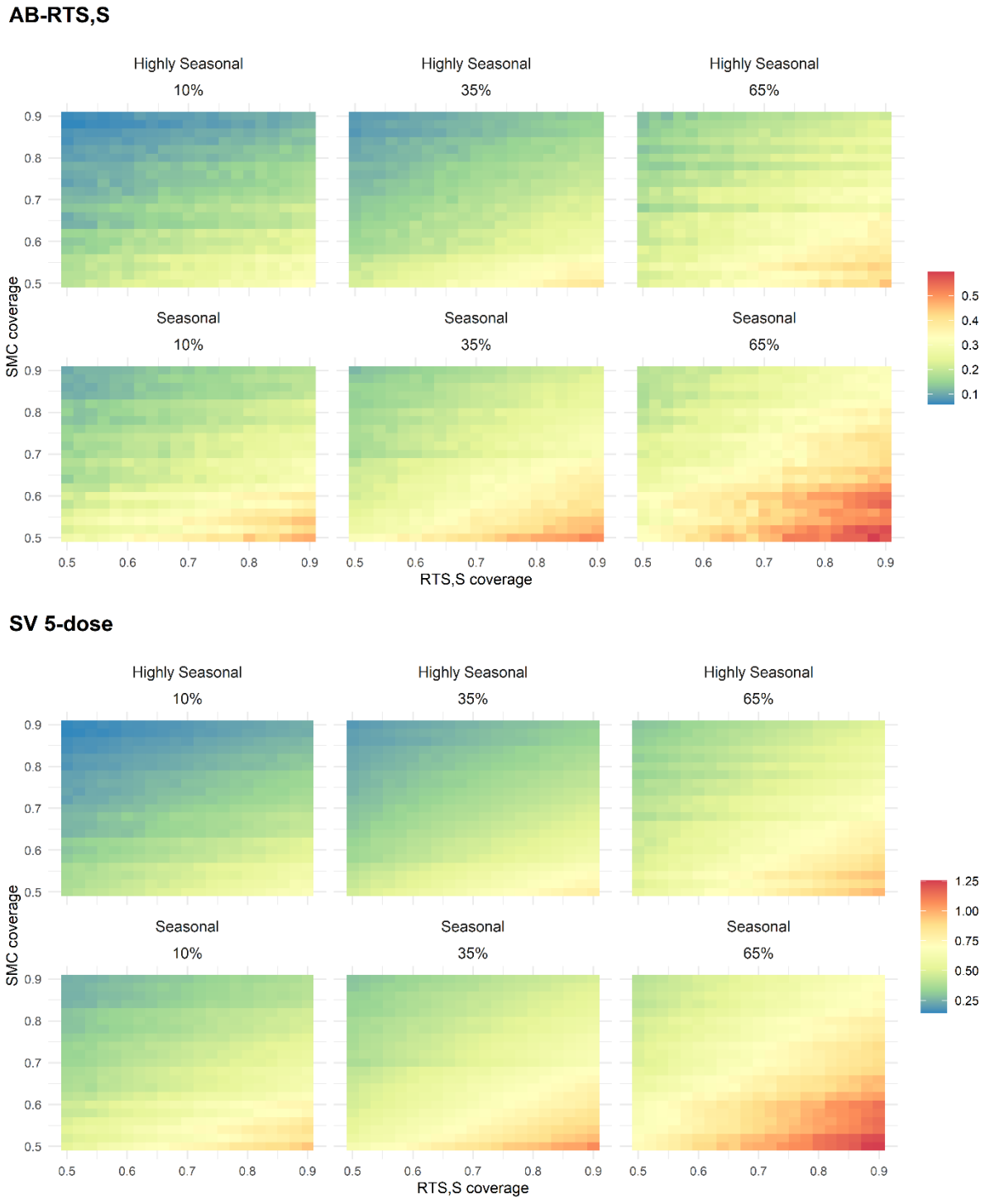

Figure 5.15 Percentage additional clinical malaria cases averted when RTS,S vaccination is combined with SMC at varying coverage levels. Colour scale represents the percentage increase in clinical malaria cases averted when RTS,S is deployed alongside SMC in model simulations compared to SMC alone, dependent on coverage, seasonality and baseline $PfPR_{2\text{-}10}$ (three settings representative of low, medium and high transmission intensity are shown). Note that the colour scale varies in each panel. AB-RTS,S and SV-RTS,S with five doses are shown here assuming the same underlying parameterizations of the efficacy model. RTS,S coverage is for the first three doses. SMC modelled as four monthly cycles.

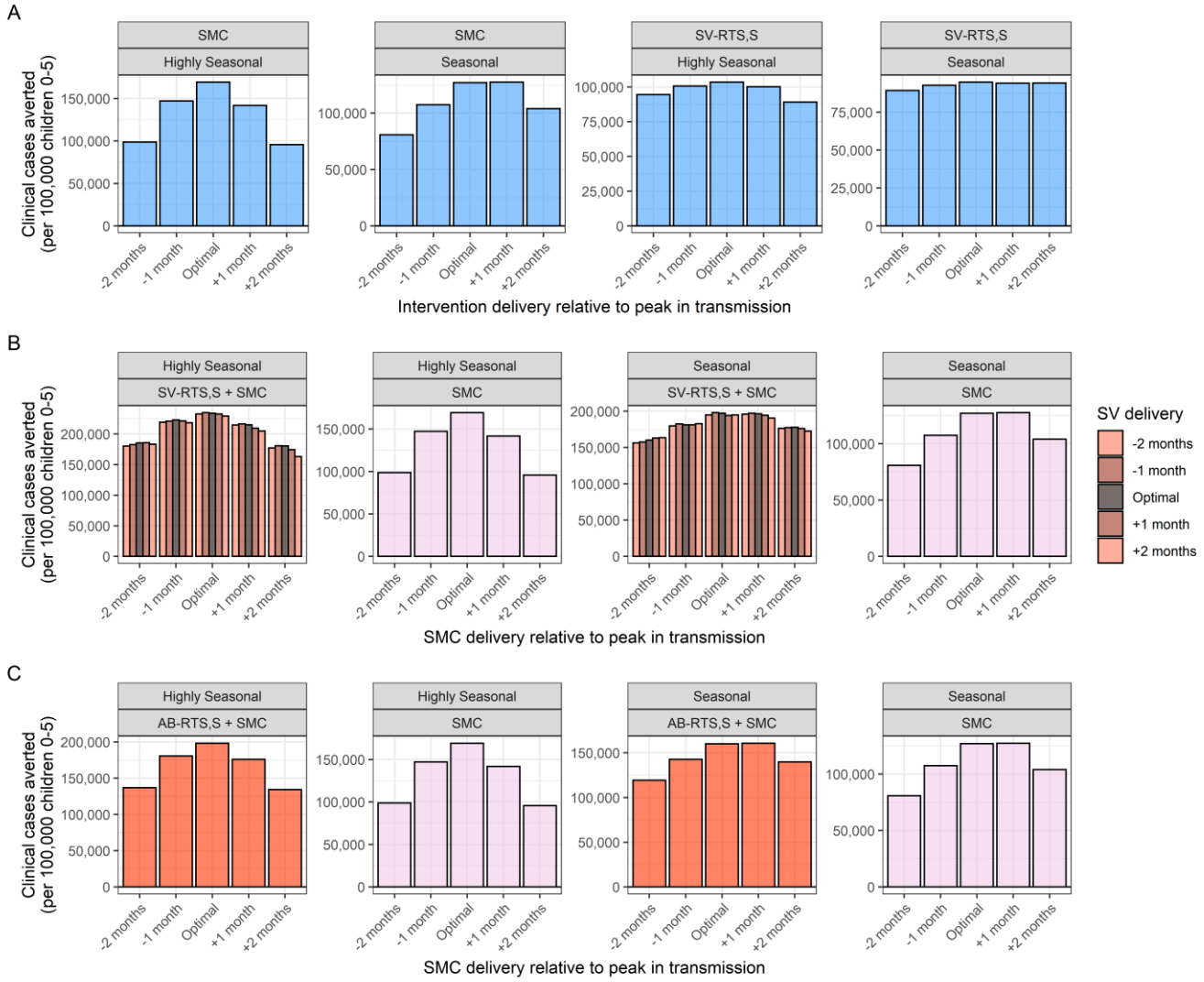

Figure 5.16 Impact of deviations in the alignment of intervention delivery relative to the malaria transmission season. Barplots show the cumulative clinical cases averted in children 0-5 over 15 years for a baseline $PfPR_{2-10}$ of 35%. **A)** Comparing SMC alone and SV-RTS,S alone. The 5-dose SV-RTS,S strategy is show here for ease of comparison. **B)** Comparing SV-RTS,S + SMC to SMC alone and **C)** Comparing AB-RTS,S + SMC to SMC alone. Vaccine coverage is fixed at 80% for the first three doses with a 20% drop off (relative to the third dose) for the fourth and fifth doses and SMC coverage of 75%.

5.4.4 Potential impact of a delayed-fractional age-based RTS,S vaccination schedule in seasonal settings

Assuming a delayed-fractional schedule, combined with the potential improvements in efficacy following the primary series that was demonstrated in human challenge trials, results show that malaria burden reductions are improved over 15 years compared to first-generation AB-RTS,S across all seasonality and transmission settings (Figure 5.17). In terms of predicted cumulative cases averted per 100,000 children aged 0–5 years over a 15 year period, the modified DF-RTS,S schedule averted between 13%–27% more clinical cases than the first-generation AB-RTS,S RTS,S/AS01, across all seasonality and transmission settings. Despite these improvements, cases and deaths averted remained lower than in all SV-RTS,S simulations.

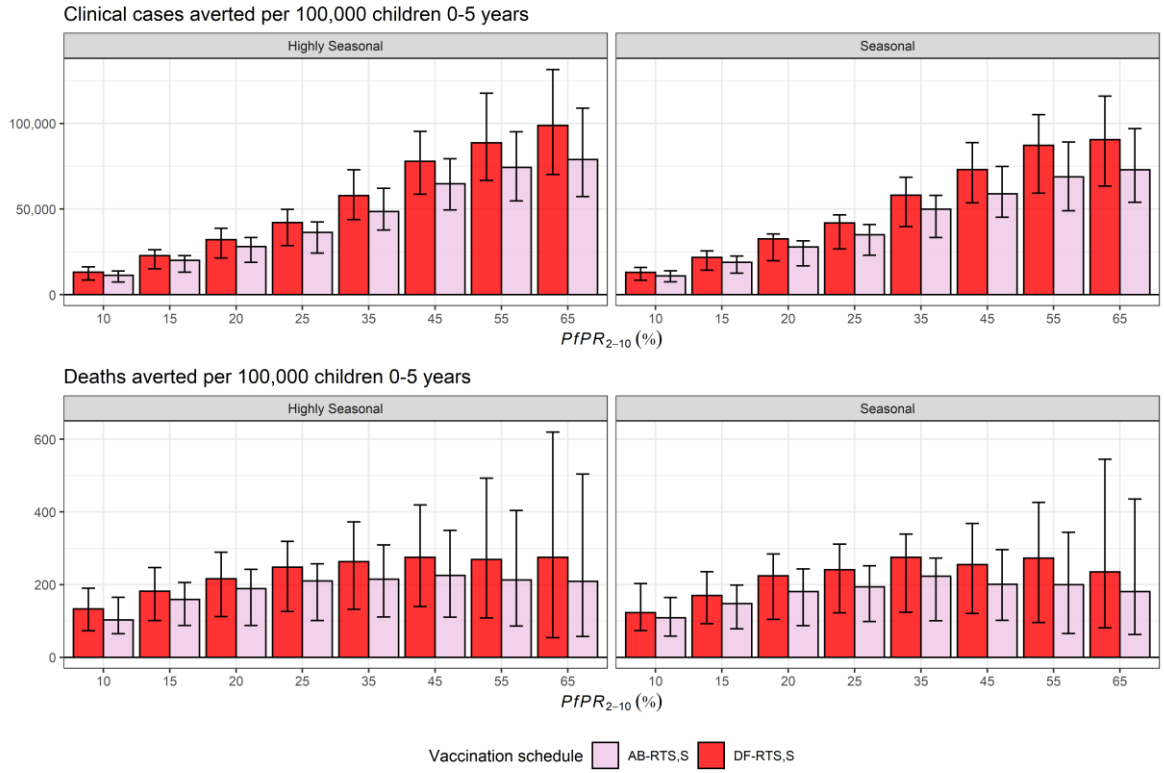

Figure 5.17 Impact of a second-generation age-based RTS,S approach following the delayed-fractional schedule of *Regules et al 2016*. A) Cumulative clinical cases averted and **B)** deaths averted over 15 years as a function of baseline $PfPR_{2-10}$ and seasonality in children aged 0-5 years old. Coverage is fixed at 80% for the first three doses with a 20% drop off (from the third dose) to the fourth dose. AB-RTS,S - is the first-generation four-dose age-based strategy, DF-RTS,S is the second-generation four dose age-based strategy. Model median values are presented in the coloured bars with error bars representing the 95% Credible Intervals.

5.5 Discussion

These modelling results demonstrate that RTS,S vaccination alone, and to a greater extent when combined with SMC, could have a significant positive impact in areas with seasonal malaria transmission when delivered at scale. Seasonally targeting RTS,S with a four- or five-dose schedule consistently resulted in greater predicted reductions than age-based delivery at equivalent coverage levels. However, the predicted difference was reduced when vaccination was combined with SMC. SMC is widely used, reaching around 33.5 million children in 2020, and is a highly effective and key malaria intervention in the Sahel region (World Health Organization, 2021g). These modelling results, along with results from the recent clinical trial, highlight the significant additional impact that RTS,S could have when delivered with SMC in these settings.

This additional impact of RTS,S when combined with SMC was driven by the protection provided by RTS,S outside the SMC target window alongside increased protection during the transmission season. The former component leads to a more prominent impact of vaccination in settings with a longer seasonal transmission season where malaria burden is more uniformly spread over 7–8 months. In these settings, even when five-monthly cycles of SMC were delivered, the additional impact of SV on top of SMC was significant averting up to 64,000 more clinical cases per 100,000 population over a 15-year time horizon than SMC alone on average. If further monthly cycles are to be considered in these settings however, the additional impact of RTS,S on top of SMC will continue to be reduced. Conversely, when comparing vaccination schedules in the absence of SMC, the incremental benefit of SV-RTS,S over AB-RTS,S was greater in highly seasonal settings due to the burden of malaria being concentrated in a shorter period. Given these results, seasonality is an important determinant of vaccination schedule impact in these settings alongside whether SMC is deployed or not.

When considering vaccination with RTS,S in the absence of SMC, by aligning peak vaccine efficacy with the period of highest malaria risk population level, reductions in malaria morbidity and mortality were maximised relative to an age-based approach. However, a child's age at first vaccination under this approach can vary from 5–17 months, meaning some children will have substantial exposure to malaria before receiving their first dose of vaccine compared to an age-based approach where age at first vaccination is fixed. This disparity in age at first vaccination meant that age-based vaccination was slightly more beneficial than seasonally targeted vaccination between 10 to 20 months of age, when children are at a higher risk of severe malaria outcomes, particularly severe malarial anaemia (Griffin et al., 2015). Therefore, a potential third vaccination strategy in seasonal settings – a hybrid strategy that uses age-based delivery for the first three doses combined with annual pre-season fourth and fifth doses – could be considered. Such an approach could preserve the young age at first vaccination

while retaining the population-level benefits of seasonally targeted fourth and fifth doses that result in greater aggregate reductions in morbidity and mortality at older ages. Further modelling work is needed to examine the potential benefit of a hybrid RTS,S vaccination strategy in seasonal settings along with research into the minimum dose spacing between third and fourth doses.

For countries considering RTS,S, the potential achievable coverage of each vaccination strategy will be an important determinant in deciding which strategy to adopt. For example, if other routine EPI vaccine coverage levels are low, but SMC or historical campaign vaccination coverage is high, SV campaigns that reach high coverage will likely have the greatest impact in that setting compared to low coverage uptake through age-based immunization and vice-versa. However, the inclusion of a fifth dose of RTS,S in the seasonal schedule may mitigate this differential, but additional boosters might result in higher rates of drop off in coverage that were not considered in this present analysis.

Further, vaccine demand may outstrip initial supply as countries decide to adopt RTS,S. In this situation it will be vital to understand where and how to prioritise RTS,S, dependent on endemicity, seasonality and current interventions. Previous modelling work has highlighted that sub-national allocation of RTS,S through age-based EPI delivery would maximise the overall public health benefit if targeted to countries with the highest incidence—particularly those in the Sahel region (Hogan, Winskill & Ghani, 2020). Given the potential for SV-RTS,S to have a greater public health impact in seasonal settings, further work is needed to understand how limited doses can best be geographically allocated given this new mode of delivery. The importance of sub-national allocation of interventions and tailoring of intervention packets to local malaria epidemiology is increasingly important with the limited resources available to malaria control. Within countries malaria seasonality patterns can vary greatly and as a result national malaria control programmes may want to consider different RTS,S vaccination strategies in different locations, dependent on observed seasonality. Further modelling work examining optimal vaccination strategies dependent on the local epidemiology and current intervention packets should be considered to help guide future RTS,S policy.

A further operational consideration highlighted by this work is the relative robustness of vaccination impact to fluctuations in the start of the transmission season compared to SMC. The relatively short duration of protection provided by each SMC monthly course means that potential case reductions are highly sensitive to optimised delivery. Along with the associated public health gains of combining RTS,S with SMC, this also enhances the robustness of SMC. Given the threat of climate change in impacting weather patterns across sub-Saharan Africa, flexible intervention packages that can adapt to fluctuations or changes in seasonality patterns are, and will be vital (Monerie, Pohl & Gaetani, 2021; Ryan, Lippi & Zermoglio, 2020; Nissan, Ukawuba & Thomson, 2021).

The inclusion of a potential DF-RTS,S vaccination schedule was found in this analysis to result in higher levels of burden reduction to first-generation AB-RTS,S vaccination, highlighting that despite the 5-month delay in receiving the third dose of RTS,S the increase in efficacy modelled under this schedule offsets any loss of protection during these 5 months. In modelled seasonal settings I predict similar levels of additional cases averted with this DF-RTS,S (13%–27% more clinical cases) as was modelled in perennial settings in *Hogan et al.* (21–25% more clinical cases) (Hogan et al., 2018). Despite these improvements in efficacy, in seasonal settings the targeting of RTS,S to the peak in the transmission season resulted in higher impact. The potential efficacy of this delayed-fraction regime however has not yet been tested in target populations in malaria endemic countries and further work on understanding the role of dose spacing and dose reductions is needed. As such, results are presented as a potential indication of what an increase in third dose efficacy might mean for population level impacts in seasonal settings.

While currently outside the remit of this work, as the associated costs of seasonally targeted delivery are not yet available, it will be important to consider the cost-effectiveness of different RTS,S vaccination strategies in seasonal transmission settings moving forwards given the limited resources available for malaria control. When comparing AB-RTS,S to SV-RTS,S the potential introductory costs of a SV campaign compared to the costs associated with adding RTS,S into an existing Expanded Programme on Immunization schedule and the costs of additional doses requited by SV-RTS,S will be important determinant of the cost-effectiveness of each schedule. Previous work has shown that the introduction of RTS,S in seasonal setting though an age-based immunization schedule tended to enter the cost-effectiveness scale up pathway later once bed-net and SMC coverage had reached higher levels (Winskill et al., 2017). The temporal targeting of SMC to the peak in transmission increased its cost-effectiveness relative to age-based RTS,S in this work. However, a further study evaluating the cost-effectiveness scale up pathway of interventions in Ghana found that introducing AB-RTS,S would be the optimal first step followed by SMC (Sauboin et al., 2019b). These different conclusions are largely a result of different constraints placed on achievable coverage levels and costing inefficiencies (Galactionova, Smith & Penny, 2021). With the associated improvements of seasonally-targeting RTS,S a further understanding its cost-effectiveness in seasonal settings alongside SMC will be essential.

5.6 Limitations

There are several limitations to the current work. Due to the results of the model validation in Chapter 4, several SV-RTS,S and SP+AQ efficacy profiles had to be considered in this evaluation. This resulted in large variation in the potential reductions in malaria morbidity and mortality presented in the results. The outputs of this work should therefore be considered in the context of understanding overall patterns and relationships between interventions given these uncertainties. And further work examining the potential efficacy alterations of RTS,S under this seasonal-vaccination schedule are needed to refine estimates further. Further these results are presented in generic transmission settings, assuming existing levels of malaria vector control and treatment coverage that are not scaled up over time. In particular, I do not consider the scale up of insecticide treated bed-nets which are currently one of our most cost-effective interventions against malaria (Winskill et al., 2017). I also do not consider the impact of increasing the age-range of SMC delivery to children up to 10 years old which recent evidence from Senegal showed to be highly effective and had an indirect effect on transmission, reducing the incidence of malaria in older individuals who did not receive SMC (Cissé et al., 2016). This is currently being explored in additional modelling work (Cairns et al., 2019). Throughout this work I did not model any vaccine-induced protection until after the third dose. It is possible that some protection would be conferred from the first two doses, but additional data would be needed to capture this impact in the model. Finally, the results presented for the delayed-fractional efficacy model are indicative of only the potential for this approach. We currently lack data from any field trials to parameterise the efficacy model.

5.7 Conclusions

Overall, this work along with the clinical trial results demonstrate that RTS,S/AS01 vaccination in seasonal transmission settings could be a valuable tool to add to existing seasonal interventions, with seasonal delivery maximising impact relative to an age-based approach. Results of the trial and modelling suggest that RTS,S should not replace SMC where it is already implemented but that it can have significant health benefits when combined with SMC or could be introduced in seasonal settings where SMC is currently not implemented or recommended for use but malaria transmission is seasonal. Decisions surrounding deployment strategies of RTS,S in seasonal settings will need to consider the local and regional variations in seasonality, current levels of other anti-malarial interventions and the potential achievable RTS,S coverage.

Chapter 6

6 Discussion

"This is a historic moment. The long-awaited malaria vaccine for children is a breakthrough for science, child health and malaria control. Using this vaccine on top of existing tools to prevent malaria could save tens of thousands of young lives each year" – WHO Director-General Dr Tedros Adhanom Ghebreyesus, October 2021.

After more than 60 years of research, the licensure of first-generation RTS,S is a huge step forward for malaria control and malaria vaccinology. Despite the partial protection that RTS,S provides, its deployment in the Malaria Vaccine Implementation Programme (MVIP) highlights the positive public health impact that RTS,S can have in malaria endemic countries. Pooled data across the 3 MVIP countries showed that hospitalization with severe malaria among children eligible for at least 3 doses of vaccine was reduced by 29%, and hospitalization with malarial parasitaemia or antigenaemia was reduced by 21% (World Health Organization, 2022). Critically the MVIP has also demonstrated that delivery of malaria vaccines is feasible and equitable (World Health Organization, 2022). While this is a significant achievement, the development of a highly efficacious malaria vaccine continues with priority strategic goals for second-generation vaccines against *P. falciparum* now calling for a vaccine with protective efficacy of at least 75% against clinical malaria to be licensed by 2030 (Malaria Vaccine Funders Group, 2013).

To achieve these goals the field needs to continue to develop and build on the initial successes of RTS,S and the promise of other potential first-generation vaccines such as R21 and PfSPZ which are now in late-stage clinical trials, while maintaining and progressing the diverse development pipeline of other second-generation approaches. The development of RTS,S, its deployment and continued evaluation has facilitated the synthesis of knowledge and data from across a wide range of different disciplines involved in research into malaria vaccines. This depth of data has enhanced the

development of mathematical modelling frameworks that combine immunological insights, into epidemiological transmission models to address public health questions (White et al., 2011b, 2013, 2014, 2015; Penny et al., 2016; Winskill et al., 2017; Galactionova, Smith & Penny, 2021). These frameworks have formed a core part of the evaluation and policy recommendations surrounding RTS,S (Penny et al., 2016; World Health Organization, 2016, 2022) and highlight how we can combine vaccinology and mathematical modelling to provide insights to help us further research into second-generation approaches.

In this thesis, I have used mathematical models as a tool to evaluate characteristics of and the potential public health impacts of novel RTS,S delivery schedules and dosing regimens. In this Chapter I summarise the findings of my thesis, highlight important data and research gaps before reviewing directions for future research.

6.1 Summary and implications of findings

In Chapter 2, using data from the delayed-fractional RTS,S human challenge study in malaria-naïve adults (Regules et al., 2016), I investigated the association between vaccine-induced anti-CSP antibody quantity and quality and protection from malaria infection. To do so, I extended a previously published individual-based mathematical model of sporozoite inoculation and merozoite release (White et al., 2013) to include dose-response curves that relate antibody IgG titre and avidity indices ('dose') to probability of sporozoite survival ('response'). Using this framework, I was able to combine data on the relative risk of infection and delays in the time to onset of detectable parasitaemia in trial volunteers into measures of vaccine efficacy against infection (defined as the reduction in the probability of infection following challenge) and vaccine efficacy per sporozoite (defined as the reduction in the liver-to-blood parasite inoculum). I found that incorporating both antibody titre and avidity measurements into the model framework provided a substantially better fit to the observed trial data than titre alone and helped to explain the observed efficacy improvement of the delayed-fractional regime better than if titre alone was used to predict efficacy. No thresholds of protection for either immune marker were identified in this work but the model predicted that vaccine efficacy against infection in excess of 70% could be achieved if avidity indices greater than 70 are induced, regardless of the number of antibodies induced. For individuals with a high antibody titre response that also showed high avidity (both metrics in the top tercile of observed values in the trial) delayed-fractional vaccination provided near sterile protection upon first challenge (98.2% [95% CrI 91.6–99.7%]). Further, I found that delayed-fractional vaccination resulted in a 99.7% (95% CrI 98.7 %–99.8%) reduction in the number of parasites entering the blood.

Considerable focus has been on the quantity of the vaccine-induced immune response as a marker for pre-erythrocytic efficacy. However, these results have shown that the quality of vaccine induced responses is also an important consideration when evaluating associations between immune markers and protection from infection. Given the need for continued development of a highly efficacious malaria vaccine and the challenges of testing new vaccine formulations in large field trials, the establishment of predictive immune correlates will be invaluable. These results provide an early insight into the use of avidity as a surrogate marker of the quality of the vaccine-induced antibody response to form part of future malaria vaccine evaluation frameworks and highlights the need for avidity measurements to be performed alongside antibody titre measurements in ongoing field trials.

In Chapter 3, using data from previously published clinical trials of seasonal malaria chemoprevention (SMC) I fitted and validated the functional parametric form of the protective efficacy profile of Sulfadoxine-Pyrimethamine plus Amodiaquine (SP+AQ), the drug combination currently recommended for SMC. Currently all interventions in the Imperial College London transmission model have been fitted and validated against clinical trial data and their associated uncertainty in effect has been quantified. The previous model of SP+AQ efficacy in the model structure lacked this same level of validation and had no quantified uncertainty in effect. Therefore, to be able to make realistic model projections of a seasonal RTS,S vaccination approach that could be delivered concurrently with SMC I first characterised the protective efficacy of SMC to be used in the model structure. Using a Bayesian survival analysis framework and adjusting for a participant's age and use of an insecticide treated bed-net, the model was able to replicate the observed malaria incidence in both arms of the trial cohort. The findings showed that efficacy was best characterised by a Weibull cumulative distribution function and that a high level of protection was maintained for around 20 days before declining to zero protection by day 60. The estimated duration of protection against clinical malaria at a level of 50% or over from the best fitting model was 35 days (95% CrI 28 days–44 days). A higher degree of uncertainty was observed in the tail of the fitted distribution as a result of the reduction in population at risk at these later time points. Critically, results of the validation exercise in this Chapter provide further evidence that this functional form of SP+AQ efficacy is well characterised. This validation was an important additional step given that trial sites in these studies had varying levels of malaria transmission intensity which will have affected the time to infection, dosing rules (weight or age-based), detection methods, seasonality, vector control and other individual-level variations.

The results of this chapter further our understanding of the rate of decay in SP+AQ efficacy over time highlighting the continued need to ensure four-week gaps between cycles. Furthermore, current guidelines for implementing SMC are considered restrictive in terms of the number of monthly cycles that can be deployed and the locations that match the seasonality criteria (World Health Organization,

2012b; Word Health Organization, 2019). Ongoing discussions have focused on ways in which SMC policy could be less restrictive (Word Health Organization, 2019). The possibility of SMC being delivered in new geographies, in areas with longer transmission seasons, to older age groups, and with increases in the number of cycles delivered, are being researched as potential options to provide essential control interventions to those communities not currently reached. Findings from my work indicate that if SMC could be administered over a longer period of five, six or even seven months this could optimise SMC, maximising reductions in malaria case incidence in countries currently delivering SMC. The results further suggest that the expansion of SMC to geographical areas where the period of highest malaria risk is greater than four months can incur greater benefits in averting malaria cases. While the focus of this chapter is on chemoprophylaxis rather than vaccination, the results of this work are critical for the work in Chapters 4 and 5.

In Chapter 4 I used the newly fitted SP+AQ efficacy model along with a model of first-generation RTS,S efficacy to replicate the Phase 3b seasonal vaccination clinical trial - the first completed large-scale field trial of a seasonal RTS,S delivery approach - in the Imperial College London malaria transmission model. In replicating the trial in the transmission model framework, I aimed to understand if the current parameterisations of intervention models were able to satisfactorily capture the results of the trial. I found that while model results were consistent with the trial data in terms of estimating the correct relationships between the interventions, the existing RTS,S vaccine model that was previously fitted to antibody titre measurements and clinical disease endpoints from the RTS,S Phase 3 clinical trial (White et al., 2015) underestimated the impact of seasonal vaccination. While this could be driven by heterogeneity that the model is not able to capture, model results were aligned with the trial results by making biologically-motivated changes to the underlying intervention models. The first change was to increase the peak efficacy of the fourth and fifth RTS,S doses so that efficacy returned to the same levels as after the first three doses. Despite this change, the impact of the combined seasonal-vaccination and SMC arm in the model framework was still underestimated. Through sampling over the posterior distributions of efficacy model parameters I found that by selecting parameters that significantly increased the duration of SP+AQ protection, alongside a more modest increase in the duration of RTS,S protection I was able to capture the observed trial results. This suggested a potential synergistic interaction between pre-erythrocytic vaccination and chemoprevention when combined in this schedule. The required reparameterizations of intervention models provides early evidence for potential underlying immunological changes in the relationship between antibody titre and efficacy along with potential synergistic relationships between pre-erythrocytic vaccines and anti-malarial drugs which have not previously been studied.

Finally in Chapter 5, I combined the work of all previous Chapters to investigate the long-term public health impacts of the seasonal RTS,S vaccination schedules described in Chapter 4 compared to age-based delivery of RTS,S in seasonal settings in the presence and absence of SMC. I found that seasonally targeting RTS,S resulted in greater absolute reductions in malaria cases and deaths compared to an age-based strategy, averting between 32%–100% more clinical cases dependent on seasonality and transmission intensity. I predict that adding seasonally targeted RTS,S to SMC would reduce clinical incidence by up to an additional 36%–56% in children younger than five compared with SMC alone. I found that the duration of the transmission season was a key determinant of intervention impact, with the advantage of adding RTS,S to SMC predicted to be smaller in areas with shorter transmission seasons. Further I found that a model of RTS,S efficacy that emulates the potential peak efficacy of the delayed-fractional approach could potentially avert more cases than those averted by the original RTS,S dosing schedule despite the delayed age at third vaccination, but that impact remained lower than a seasonally targeted approach.

The results of this work have direct implications for global malaria policy and were presented to the Malaria Policy Advisory Committee in October 2021 (World Health Organization, 2021b). This work was therefore part of the evidence package that led to the recommendation by the WHO for countries to consider providing RTS,S seasonally, with a five-dose strategy, in areas with highly seasonal malaria or with perennial malaria transmission with seasonal peaks (World Health Organization, 2022). Further, the results of this work highlight several operationally important considerations for countries when deciding to adopt RTS,S including: patterns of seasonality and transmission intensity, coverage of other malaria interventions, and potential achievable coverage levels of RTS,S delivery schedules.

6.2 Limitations and Future directions

Mathematical models have a long history in epidemiological research, and although no model can claim to be 'right', most can offer insights into different aspects of the transmission of and impacts of interventions on malaria. The results presented in this thesis must therefore be viewed in the context of the assumptions and limitations of the models used and the data available to parameterise them. In doing so I also highlight potential research gaps and directions for future research.

In Chapter 2, a mechanistic model of malaria infection was combined with data from a human challenge study of the delayed-fractional RTS,S vaccination approach. Primarily the dataset that was available from this study was small with only 46 volunteers, 10 of whom developed parasitaemia. This limits the precision of the estimated biological parameters and leads to wide uncertainty intervals on all estimates in this chapter. Given this small sample size, informative priors were needed to ensure convergence during model fitting. Further, the model assumed that each sporozoite that successfully

developed within the liver would release on average 30,000 merozoites into the blood stream, an estimate derived from recent work by *Coffeng et al.* (Coffeng et al., 2017). This parameter could not be fitted to the data in this thesis, due to the lack of datapoints and the high levels of correlation between this parameter and the parameter describing the mean number of sporozoites. Future work with larger datasets could extend the fitting to be able to further characterise the variation in this parameter. In addition, the model could be improved in several ways and applied to further datasets from human challenge studies for both RTS,S and other pre-erythrocytic vaccines. In the past few years, the literature surrounding the delayed-fractional approach has developed, suggesting that looking only at the NANP repeat region (as was identified as the leading correlate of RTS,S induced protection (White et al., 2013, 2015; Olotu, Fegan & Bejon, 2010)) may overlook antibody responses to other regions that are also important for protection from infection. Recently antibody responses to the C-terminal region of CSP have been identified as potentially important additional correlates of RTS,S induced protection (Dobaño et al., 2019; Das et al., 2021; Neafsey et al., 2015). Given these results the model described in Chapter 2 could be further extended to include dose-response curves of C-terminal antibody titres and avidity to understand the relative contribution of C-terminal and NANP repeat antibody quantity and quality to protective efficacy. In addition, separate models could be fit to both delayed-fractional and standard dosing of RTS,S to characterise any further potential changes to correlates of vaccine protection between the different approaches if larger datasets were available. Finally, additional studies are needed to see whether these findings extrapolate to children and infants living in malaria endemic regions.

Despite the newly fitted SP+AQ efficacy profile in Chapter 3 showing good alignment with the results of clinical trials identified in the validation section, there are several limitations to this work and the model structure used to estimate SP+AQ efficacy. Firstly, a relatively small dataset was available from a single trial, limiting statistical power in drawing conclusions. Furthermore, the control cohort, while enrolled under the same inclusion criteria and followed up in a similar manner as the treated cohort, was not randomised and did not receive any placebo treatment. While the potential confounding factors of age and bed-net use were accounted for in the model structure, some residual bias or confounding as a result of this non-randomised control group may remain. To address this, further model extensions that include a fitted gamma frailty term to account for unobserved heterogeneity either at the individual or study village level could be considered (Balan & Putter, 2020). Currently in the model, each individual in the trial is assumed to experience the same weekly constant baseline force of infection, not accounting for any heterogeneity in exposure to infectious mosquitos, which has previously been shown to potential bias estimates of vaccine efficacy (White et al., 2010). Including a gamma frailty term would also account for this heterogeneity. Further, the dataset was

only available from the period following the third and final delivery of SMC. It is possible that individuals could have residual levels of SP+AQ concentrations from the two preceding months, which might slow parasite growth for example, that I was unable to account for. This could potentially lead to overestimates of the efficacy profile in this work. Therefore, a potential extension to this work would be to contact authors of the studies identified in the model validation to collect a larger and more diverse dataset in order to then fit a joint model of SP+AQ efficacy over time to all of these trials. Further this model framework could be applied to future studies that intend to examine the impact of potential drug-resistance markers in a population and their impact on the duration of SP+AQ protection as this will be a critical area of malaria surveillance and research moving forwards to ensure drugs remain highly effective in target populations.

In Chapter 4, the reparameterizations to the intervention efficacy models are driven by the discordance between the model and trial results and the biologically-motivated hypotheses for the reasons between these differences. These hypotheses of sustained efficacy of the fourth and fifth doses and the potential for a synergistic relationship between vaccination and chemoprevention need to be further examined in future research. Current results suggest that despite some reductions in anti-CSP antibody titre following fourth and fifth doses of RTS,S, efficacy itself did not decline significantly (Chandramohan et al., 2021; Sagara et al., 2021). While the full panel of serological assays and data are not yet available from this study, when they do become available, future studies could adopt a similar approach to *White et al.* (2015), potentially extending the model of antibody kinetics and vaccine efficacy to also incorporate novel additional immunological makers such as antibody avidity or antibody responses to the C-term as determinants of vaccine efficacy over time. Additionally, the potential for synergistic relationships between pre-erythrocytic vaccines and anti-malarial chemoprevention needs to be investigated further. Recent work has highlighted that pre-erythrocytic vaccines can achieve higher efficacy when sporozoite loads are reduced via transmission blocking vaccines (Sherrard-Smith et al., 2018). RTS,S has been shown in Chapter 2 to potentially reduce the liver-to-blood inoculum by >95%, which could potentially result in increased efficacy of SP+AQ at lower drug concentrations. A within-host modelling framework that explicitly captures parasite densities across pre-erythrocytic and blood-stages and accounts for the impact of RTS,S and SP+AQ on both the prevalence and density of infection could be used to investigate such an interaction.

Finally in Chapter 5, the long-term effectiveness of RTS,S in seasonal settings both in the presence and absence of SMC was considered using the Imperial College London model of malaria transmission. There are several key knowledge and data gaps that contribute to the uncertainty of model estimates of intervention impact in this work. The first, is that the underlying uncertainty in interventions models

of seasonal RTS,S vaccination, delayed-fractional vaccination and SMC when combined with seasonal RTS,S drives significant uncertainty in the modelled outputs. As mentioned further research into the characterisation of intervention models are needed to resolve this. Secondly, I was not able to perform a cost-effectiveness analysis of the seasonal vaccination approach due to the current lack of cost data surrounding this delivery platform. This will be vital moving forwards for countries and funders to make informed choices about RTS,S delivery strategies, particularly as the recurrent and introductory costs of a seasonal vaccination schedule might be significantly higher than those associated with adding RTS,S into existing Expanded Program on Immunization schedules (Baral et al., 2021). Further to this, GSK has yet to release the cost per dose of RTS,S which as previous modelling has shown will be an important contributory factor to the overall cost-effectiveness of different RTS,S delivery approaches (Penny et al., 2016; Galactionova, Smith & Penny, 2021). Further, this work highlighted the potential for a third RTS,S schedule to be considered in seasonal settings, that of a hybrid age-based primary series with additional pre-season boosters. Modelling work can be used to assess the impact of such a schedule; however, no such schedule has yet been delivered and decisions on minimal dose-spacing rules will need to be established to be able to characterise impact. The long-term modelling in this chapter was performed assuming archetypal endemicity and seasonality settings with no constraints on vaccine dose supply and no consideration of the scale-up of other interventions. However, demand for RTS,S is expected to outstrip initial supply (World Health Organization, 2021e), therefore mathematical models parameterised at sub-national levels across sub-Saharan Africa can be used to identify where vaccine introduction should be prioritised, and under what immunization schedule to maximise public health impact given a range of different supply constraints and other intervention packets currently in use at a sub-national level (Hogan, Winskill & Ghani, 2020). Finally, as was shown with the initial evaluation of the long-term impacts of RTS,S (Penny et al., 2016) and in evaluation mass-drug administration programmes (Brady et al., 2017) it is beneficial to combine the predictions of different mathematical models to assess whether the same recommendations are made. This allows for differences in model assumptions and parameterisations of interventions to be brought within a single framework. Although the uncertainty in the underlying transmission model was accounted for in this thesis, future policy-relevant modelling of malaria vaccines should aim for consensus modelling approaches to improve our understanding of impacts in light of different model assumptions. Given the positive policy recommendation for the adoption of seasonally targeted RTS,S vaccination in areas with highly seasonal malaria or with perennial malaria transmission with seasonal peaks, further operational research will be needed specifically related to the feasibility and scalability of seasonal delivery of vaccine doses. Further evaluation will also be required to determine how best to deliver the combination of SMC and seasonal malaria vaccination

in these areas, and if there is the potential for concurrent or paired delivery systems. Further data on the immunogenicity and effectiveness of seasonal annual doses will also be vital especially from different seasonal settings and countries as the role out begins.

6.3 Conclusions

Malaria may, one day, be controlled effectively using highly effective multi-stage vaccines or through the advent of other novel control interventions. However, malaria control cannot wait for a silver bullet intervention. Reversing the recent stagnation in burden reductions is a global public health priority and achieving effective malaria control is likely only to be achieved through tailoring of different combinations of interventions dependent on the local epidemiology and operational constraints of a country. The value of RTS,S when delivered through country EPI schedules on top of existing tools has been demonstrated in the recent MVIP evaluation. New RTS,S dosing and delivery approaches that aim to improve on the efficacy of initial RTS,S schedules are being developed. In this thesis, I have used mathematical models of malaria infection and population level transmission to highlight the potential improvements and benefits of these novel approaches. The results of this thesis have consequently contributed to the WHO recommendation that seasonally-targeted vaccination strategies should also be considered for widescale use in seasonal settings. Going forwards, important decisions on local deployment strategies in the face of constrained dose supply and in the face of other next-generation vaccines, such as R21, will need to be considered by policy makers. Mathematical models can be used to help support such decisions.

7 References

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Chapter 8

8 Appendices

8.1 Formal model description of the Imperial College London transmission model

Individuals begin life susceptible to infection (S) (see Figure 8.1 for model structure). At birth individuals are assumed to possess a level of immunity that is maternally inherited and this immunity decays exponentially over the first 6 months of life. throughout their life individuals (i) are exposed to infectious bites from mosquitos, with the hazard of infection to each individual governed by the force of infection acting on each individual (Λ_i). Λ_i is dependent on their level of pre-erythrocytic immunity and several vector determined parameters (mosquito population size, biting rates, level of infectivity). Once infected, individuals experience a latent period of infection of 12 days (d_E), and then either develop symptomatic clinical disease (D) or asymptomatic infection (A). This outcome is determined by their probability of acquiring clinical disease (ϕ_i), which is dependent on their level of clinical immunity. If individuals develop clinical disease, they receive appropriate treatment with a fixed probability (f_T) whereby they enter the infection state (T) otherwise they do not seek treatment, with probability $(1 - f_T)$, and move to the untreated disease state (D). Treated individuals recover from infection at a rate r_T. They then enter a period of prophylactic protection as drug dependent protection from re-infection wanes over time and then return to the fully susceptible state (S). Individuals who did not receive treatment recover to the asymptomatic state (A) at rate r_D. From this asymptomatic state, individuals move to the sub-patent infection state (U) with rate r_A, as parasite density is controlled due to immune responses. Finally they clear natural infection and move back to the susceptible state (S) at rate r_U. Super-infection is included in the model (re-infection) and is possible for all individuals in states D, A and U. If an individual is super-infected, they will move to state T if they receive treatment, if no treatment is received, they will either develop clinical disease (D) or asymptomatic infection (A) in the same process as for primary infection.

The population size in simulations remains constant through the replacement of deaths (from natural causes) with a new-born individual with the same individual biting rate due to heterogeneity in biting patterns. These deaths are modelled using national life tables, with individuals removed from the population at age-specific rates to match the required age distribution (United Nations, 2019).

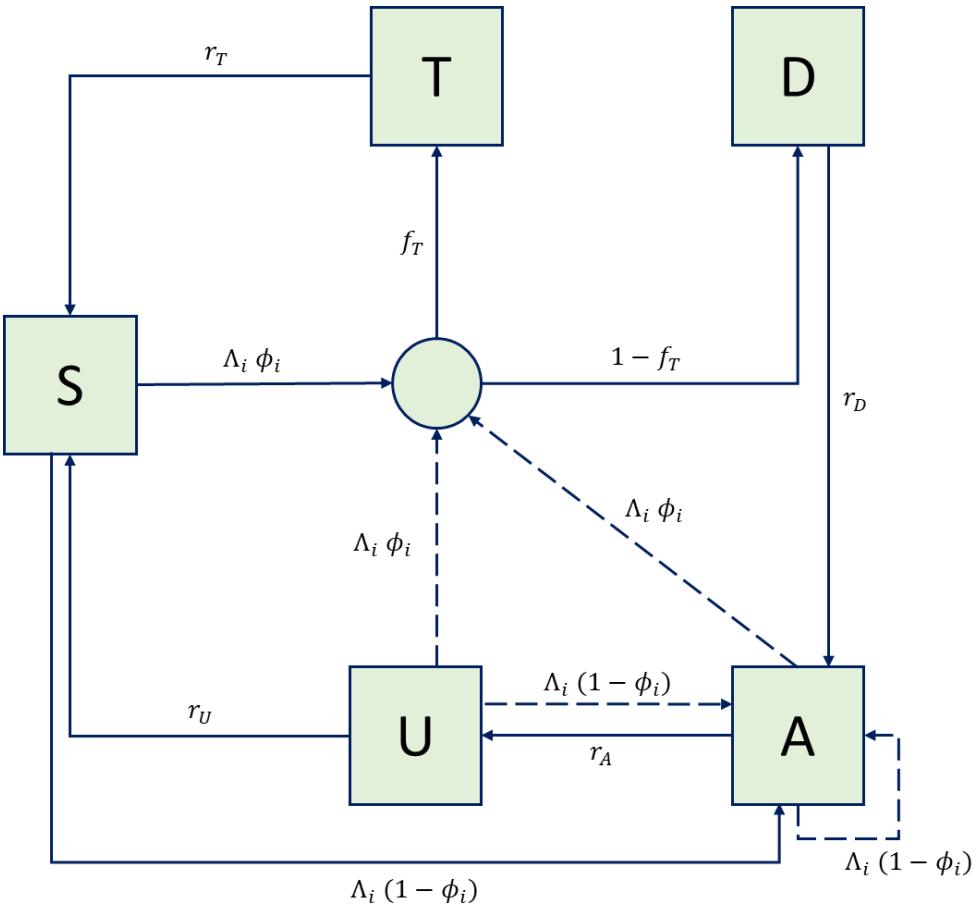

Figure 8.1 Human Transmission Model. Flow diagram for the progression between human compartments of the transmission model. States are shown in boxes and transitions with arrows, dashed arrows representing superinfection, associated hazard rates are given with transitions. The circle represents the treatment node. S, susceptible; T, treated clinical disease; D, untreated clinical disease; P, prophylaxis; A, asymptomatic patent infection; U, asymptomatic sub-patent infection. All parameters and rates are described and referenced within Table 8.1.

Table 8.1. Infection state transition rates between human compartments

Process	Transition	Rate
Infection	S → I	$\Lambda_i(t-d_E)$
Progression of untreated disease to asymptomatic infection	D → A	$r_D = \frac{1}{d_D}$
Progression of asymptomatic to subpatent infection	A → U	$r_A = \frac{1}{d_A}$
Progression of subpatent infection to susceptible	U → S	$r_U = \frac{1}{d_U}$
Progression of treated disease to susceptible*	T → S	$r_T = \frac{1}{d_T}$
Super-infection from untreated clinical disease, asymptomatic or subpatent infection	D →I A→I U→I	$\Lambda_i(t-d_E)$

* Treated individuals experience a period of drug-dependent partial protection from reinfection.

8.1.1 Heterogeneity in biting rates

Each individual in the model is assumed to have a unique biting rate. This is the product of their relative biting rate ζ_i and their relative age-dependent biting rate ψ_i which for a given age (a) is:

$$\psi_i(a) = 1 - \rho exp\left(-\frac{a}{a_0}\right) \quad (8.1)$$

ere ρ and a_0 are parameters that determine the relationship between age (i.e. body size) and biting rate. ω is a normalising constant for the biting rate with age:

$$\omega = \int_0^\infty \psi(a)g(a)da \quad (8.2)$$

where $g(a)$ is the cross-sectional human population age-distribution. The relative biting rate is drawn from a Log-normal distribution with a mean of 1 and it is assumed that this heterogeneity persists throughout their lifetime:

$$log(\zeta_i) \sim N\left(\frac{-\sigma^2}{2}, \sigma^2\right) \quad (8.3)$$

The EIR $\varepsilon_i(a,t)$ and force of infection $\Lambda_i(a,t)$ experienced by individual i with age a at time t is thus given by:

$$\varepsilon_i(a,t) = \varepsilon_0(t)\zeta_i\psi_i(a) \quad (8.4)$$

$$\Lambda_i(a,t) = b_i(t)\varepsilon_i(a,t) \quad (8.5)$$

where $\varepsilon_0(t)$ is the mean EIR experienced by adults at time t and $b_i(t)$ is the probability that an infectious bite leads to a patent infection. This probability is determined by the level of pre-

erythrocytic immunity (see below) and is subject to a lag of d_E days to account for the latent period of sporozoite infection.

8.1.2 Immunity

Several levels of immunity are captured within the model each responding to a distinct stage of infection. These are:

1. Maternal immunity I_{CM} , I_{VM} – leads to a reduction in the probability of clinical or severe disease given an infection;
2. Acquired immunity to infection (pre-erythrocytic immunity) I_B – reduction in the probability of infection given an infectious bite;
3. Blood stage immunity I_{VA} , I_{CA} , I_D – reduction in the probability of developing clinical or severe disease and ultimately the detectability of asymptomatic infection and infectiousness to mosquitoes;
4. Detection immunity – blood stage immunity that reduces both the probability of detection and reduces infectiousness to mosquitoes.

The acquisition and loss of natural immunity is modelled dynamically and is dependent on both age and exposure. Newborns acquire a level of maternally derived immunity to clinical disease (I_{VM}) and severe disease (I_{VM}) at birth (through placental transfer of antibodies). The level of immunity at birth is set to a proportion P_M of the acquired immunity in a randomly chosen population of 15-35 year-olds with the same biting heterogeneity level. Maternal immunity decays exponentially from birth: $r_M = \frac{1}{d_M}$

Pre-erythrocytic immunity develops once individuals have reached older ages and is boosted by one following each exposure to an infectious bite provided it is after u_B days since their last exposure. Pre-erythrocytic immunity decays exponentially between exposures with rate: $r_B = 1/d_B$. Blood stage immunity is assumed to act by controlling parasite densities in the blood, thereby impacting the probability of an individual developing severe disease, clinical disease, and the detectability of asymptomatic infection. Acquired immunity to each of these outcomes is tracked separately and is developed in the above order through the associated parameterisations below. Each is boosted by one following each patent infection provided it is at least u_V, u_C, or u_D days respectively since the last exposure. Again, each of these decays exponentially between exposures with rate $r_{VA} = \frac{1}{d_{VA}}$, $r_C = \frac{1}{d_{CA}}$ and $r_{ID} = \frac{1}{d_{ID}}$ respectively.

Immunity levels to infection, severe and clinical disease and detectability are subsequently converted to individual time-dependent probabilities using Hill functions. Therefore, the probability that an individual i will develop infection at time t following an infectious bite is given by:

$$b_i(t) = b_0\left(b_1 + \frac{1-b_1}{1+\left(\frac{I_B(i,t)}{I_{B0}}\right)^{\kappa_B}}\right) \tag{8.6}$$

where b_0 is the probability of infection with no immunity, $b_0 b_1$ is the minimum probability, I_{B0} and κ_B are the shape and scale parameters respectively and $I_B(i,t)$ is the level of pre-erythrocytic immunity of individual i at time t.

The probability that individual i develops clinical disease at time t conditional on being infected is defined as:

$$\phi_i(\mathrm{t}) = \phi_0\left(\phi_1 + \frac{1-\phi_1}{1+\left(\frac{I_{CA}(\mathrm{i,t}) + I_{CM}(i,t)}{I_{C0}}\right)^{\kappa_C}}\right) \tag{8.7}$$

where ϕ_0 is the probability of disease with no immunity, $\phi_0\phi_1$ is the minimum probability, I_{C0} and κ_C are scale and shape parameters respectively, $I_{CA}(i,t)$ is the level of acquired immunity to clinical disease and $I_{CM}(i,t)$ is the level of maternally acquired immunity to clinical disease of individual i at time t.

The probability that individual i develops severe disease at time t and age a conditional on being infected is defined as:

$$\theta_i(a,t) = \theta_0\left(\theta_1 + \frac{1-\theta_1}{1+f_V(i,a)\left(\frac{(I_{VA}(i,t) + I_{VM}(i,t)}{I_{V0}}\right)^{\kappa_V}}\right) \tag{8.8}$$

where θ_0 is the probability of disease with no immunity, $\theta_0\theta_1$ is the minimum probability, I_{V0} and κ_V are scale and shape parameters respectively, $I_{VA}(i,t)$ is the level of acquired immunity to severe disease, $I_{VM}(i,t)$ is the level of maternally acquired immunity to severe disease of individual i at time t and

$$f_V(i,a) = 1 - \frac{(1-f_{V0})}{\left(1+\left(\frac{a}{a_V}\right)^{\gamma_V}\right)} \tag{8.9}$$

is an age-dependent (physiological) modifier of the risk of severe disease, where f_{V0}, a_V and γ_V are parameters.

The detectability of an asymptomatic infection by microscopy in individual i of age a at time t is given by:

$$q_i(a,t) = d_1 + \frac{(1 - d_{min}}{\left(\left(\frac{1 + I_D(i,t)}{I_{D0}}\right)^{\kappa_D} f_D(i,a)\right)} \tag{8.10}$$

where d_{min} is the minimum probability of detection, I_{D0} and κ_D are scale and shape parameters respectively, $I_D(i,t)$ is the level of acquired immunity to the detectability of infection of individual i at time t and

$$f_D(i,a) = 1 - \frac{(1 - f_{D0})}{\left(1 + \left(\frac{a}{a_D}\right)^{\gamma_D}\right)} \tag{8.11}$$

is an age-dependent (physiological) modifier of the detectability of infection where f_{D0}, a_D and γ_D are parameters.

8.1.3 Onwards infectivity

The reduction in parasite density that reduces the probability of detection is also assumed to decrease the probability of onwards transmission to mosquitos (each infection state is assumed to be onwardly infectious to mosquitoes who bite an individual, with highest infectivity associated with diseased states). Onwards infectiousness is c_D and c_U in states D and U respectively, and c_T following treatment. In state A infectiousness is modified by q_i, the detectability of individual i, and is given by the function $c_U + (c_D + c_U)\, q_i^{\gamma i}$.

8.1.4 Severe disease and mortality

Severe disease incidence and malaria associated mortality are derived in the model from modelled estimates of clinical incidence. Following *Griffin et al* (2016), the incidence of severe malaria requiring hospitalisation in the age range a_L to a_U at time t is given by:

$$\lambda_H\big(t,(a_L,a_U)\big) = \frac{\sum_{i:\, a_L < a_i(t) < a_U} \big((1 - f_T) + f_T f_T\big)\Lambda_i(t)\, \theta_i(t)}{\#\{i\, :\, a_L < \ a_i(t)\ < a_U\}} \tag{8.12}$$

where $\Lambda_i(t)$ is the force of infection experienced by individual i at time t and $\theta_i(t)$ is the probability that individual i develops severe disease at time t conditional upon being infected. Malaria related mortality is assumed to be proportionally related to the incidence of severe disease and is defined as:

$$\mu = \big(t, (a_L, a_U)\big) = v\lambda_H\big(t, (a_L, a_U)\big) \tag{8.13}$$

where v is a scaling factor, estimated to be 0.215 (Griffin et al., 2016). Individuals receiving treatment are assumed to experience a reduction, f_{VT}, in the probability of progression from clinical to severe disease and hence death.

8.1.5 Vector model structure

The vector model captures infection in the mosquito population. It is based on the deterministic model previously described in *White et al*. (2011) but is implemented it its equivalent compartmental stochastic structure for adult female mosquitos (the transmitters of parasites). Adult female mosquitos lay eggs at rate β, upon hatching from these eggs, larvae progress through early and late larval stages (E *and* L compartments) before developing to the pupal stage (P_L) before maturing into adult mosquito (M). The duration spent in each larval stage is denoted by d_E, d_L, d_{P_L}. The larval stages are regulated by density dependent mortality (μ_E, μ_L, μ_{P_L}) with a time varying carrying capacity, K, that represents the ability of the environment to sustain breeding sites through different periods of the year. The density of larvae relative to the carrying capacity is regulated by parameter γ. The environmental carrying capacity determines the mosquito density and hence the baseline transmission intensity in the absence of interventions. Adult mosquitos are stratified according to their infection status with *P. falciparum,* adults begin life susceptible (S_M), once infected mosquitos enter an exposed but not infectious state (E_M) of a fixed length (τ_{EM}) before they become infectious to humans (I_M) following the extrinsic incubation period. Mosquitos are assumed to remain infectious until they die at rate μ_M. This mosquito death rate is defined as: $\mu_M = -f_R \log(p_1 p_2)$, where p_1 is the probability of a mosquito surviving one feeding cycle, p_2 the probability of surviving one resting cycles and f_R is the feeding rate. The probability that a mosquito survives the extrinsic incubation period of malaria is thus given by: $P_M = e^{-\mu_M \tau_{EM}}$.

The differential equations describing the larval and adult population models are as follows:

$$\frac{dE}{dt} = \beta M - \mu_E\left(1 + \frac{E+L}{K}\right)E - \frac{E}{d_E} \tag{8.14}$$

$$\frac{dL}{dt} = \frac{E}{d_{EL}} - \mu_L\left(1 + \gamma\,\frac{E+L}{K}\right)L - \frac{L}{d_L} \tag{8.15}$$

$$\frac{dP_L}{dt} = \frac{L}{d_L} - \mu_P P_L - \frac{P_L}{d_{P_L}} \tag{8.16}$$

$$\frac{dS_M}{dt} = \frac{P_L}{2d_{PL}} - \mu_M S_M \tag{8.17}$$

$$\frac{\partial E_M}{\partial t} = \Lambda_M S_M - \Lambda_M(t-\tau_{EM})S_M(t-\tau_{EM})P_M - \mu_M E_M \quad (8.18)$$

$$\frac{dI_M}{dt} = \Lambda_M(t-\tau_{EM})S_M(t-\tau_{EM})P_M - \mu_M I_M \quad (8.19)$$

It is assumed that 50% of the emergent adult mosquitoes from hatching are female and all enter the susceptible state (S_M). The rate at which adult female mosquitoes become infected is a function of the infectiousness of the human population including an appropriate time-lag (t_l) to account for the period between humans becoming infected and becoming infectious. The force of infection experienced by mosquitoes (Λ_M) is a function of infectious compartments in the human population and the relative infectivity of each state integrated over all human age groups and heterogeneity in exposures and is given by:

$$\Lambda_M(t) = \frac{\alpha}{\omega}\int_0^\infty \int_0^\infty \zeta\psi(a)(c_D D(\zeta,a,t-t_l) + c_T T(\zeta,a,t-t_l) + c_A A(\zeta,a,t-t_l) + c_U U(\zeta,a,t-t_l)dad\zeta \quad (8.20)$$

where α is the biting rate on humans:

$$\alpha = \frac{Q_0}{\delta} \quad (8.21)$$

Q_0 quantifies the level of anthropophagy and δ is the mean time between feeds. The parameter ω represents a normalising constant for the biting rate over all ages:

$$\omega = \int_0^{\infty\int} \psi(a)\, g(a)\, da \quad (8.22)$$

where $g(a)$ is the human age distribution. There is a fixed delay τ_M before female mosquitoes become infectious to humans (I_M) and they are assumed to remain infectious after this.

8.1.6 Vector bionomics

The model captures a range of different vector species compositions (*An.gambiae s.s, An. arabiensis* and *An.funestus*) within an area based on estimates from the Malaria Atlas Project (Sinka et al., 2016). The characterising bionomics parameters for each species are shown in Table 8.2.

Table 8.2 Vector Bionomics Parameters (Griffin et al., 2016)

Bionomics trait	*An.gambiae s.s*	*An.arabiensis*	An.funestus
Anthropophagy	0.92	0.71	0.94
Endophily	0.81	0.42	0.81
% bites indoors	0.97	0.96	0.98
% bites indoors and in bed	0.89	0.90	0.90
Daily mortality of adults with no interventions μ_M	0.132	0.132	0.112

8.1.7 Seasonality

Seasonality is incorporated in the model by allowing a time-varying carrying capacity of the environment to support mosquito larvae, at time *t*, characterised by the functional form:

$$K(t) = K_0 \frac{R(t)}{\bar{R}} \tag{8.23}$$

Where K_0 is the carrying capacity, $\bar{R}$ the mean rainfall over the year and $R(t)$, the time-varying seasonal curve:

$$R(t) = g_0 + \sum_{j=1}^{3} g_j \, cos(\, 2\pi tj) + h_j \, sin(\, 2\pi tj)\, m \tag{8.24}$$

derived from the first three frequencies of a Fourier transformation to historic rainfall patterns from the Climate Hazards Group InfraRed Precipitation with Station (CHIRPS) data (Funk et al., 2015; CHIRPS, 1999; Garske, Ferguson & Ghani, 2013).

8.1.8 Model Parameter Values

All baseline model parameter estimates are given in Table 8.3 below: these are obtained from previous publications on the transmission model and associated model fitting exercises (Griffin et al., 2010; Griffin, Ferguson & Ghani, 2014; Griffin et al., 2015, 2016; White et al., 2011a).

Table 8.3 Model parameters

Parameter	Symbol	Estimate
Human infection duration (days)		
Latent period	d_E	12
Patent infection	d_A	195
Clinical disease (treated)	d_T	5
Clinical disease (untreated)	d_D	5
Sub-patent infection	d_U	110
Treatment Parameters		
Probability of seeking treatment if clinically diseased	f_T	Variable
Age and heterogeneity		
Age-dependent biting parameter	ρ	0.85
Age-dependent biting parameter	a_0	8 years
Variance of the log heterogeneity in biting rates	σ^2	1.67
Immunity reducing probability of infection		
Maximum probability due to no immunity	b_0	0.59
Maximum relative reduction due to immunity	b_1	0.5
Inverse of decay rate	d_B	10 years
Scale parameter	I_{B0}	43.88
Shape parameter	κ_B	2.16
Duration in which immunity is not boosted	u_B	7 days

New-born immunity relative to mother's	P_{CM}	0.77
Immunity reducing probability of clinical disease		
Maximum probability due to no immunity	ϕ_0	0.79
Maximum relative reduction due to immunity	ϕ_1	0.000737
Inverse of decay rate	d_{CA}	30 years
Scale parameter	I_{C0}	18.02
Shape parameter	κ_C	2.37
Duration in which immunity is not boosted	u_C	6 days
Inverse of decay rate of maternal immunity	d_M	68 days
Immunity reducing probability of detection		
Minimum probability due to maximum immunity	d_1	0.16
Inverse of decay rate	d_{ID}	10 years
Scale parameter	I_{D0}	1.58
Shape parameter	κ_D	0.48
Duration in which immunity is not boosted	u_D	9.5 days
Scale parameter relating age to immunity	a_D	22 years
Timescale with which immunity changes with age	f_{D0}	0.007055
Shape parameter relating age to immunity	γ_D	4.82
Immunity reducing probability of severe disease and mortality		
Maximum probability due to no immunity	θ_0	0.0749886
Maximum relative reduction due to immunity	θ_1	0.0001191
Scale parameter	I_{V0}	1.10
Shape parameter	κ_V	2.00
Inverse of decay rate	d_{VA}	30 years
Duration in which immunity is not boosted	u_V	11 days
Inverse of decay rate of maternal immunity	d_{VM}	77 days
New-born immunity relative to mother's	P_{VM}	0.20
Reduced probability of death due to treatment	f_{VT}	0.5
Age-dependent severe disease risk modifier parameter	f_{V0}	0.141
Age-dependent severe disease risk modifier parameter	a_V	2493.41
Age-dependent severe disease risk modifier parameter	γ_V	2.91
Mortality scaling factor from severe disease	ν	0.065
Infectiousness to mosquitoes		
Lag from parasites to infectious gametocytes	d_g	12 days
Untreated disease	c_D	0.068
Treated disease	c_T	0.022
Sub-patent infection	c_U	0.00062
Parameter for infectiousness of state *A*	γ_1	1.82
Adult mosquito population model		
Daily mortality of adults with no interventions	μ_M	Varies by species – see Table 2.4 above
Mean time between feeds	δ	3 days
Extrinsic incubation period	τ_{EM}	10 days
Larval model		
Average number of eggs laid per female mosquito per day	β	21 /day
Early instar larval developmental period	d_E	6.64 days

Late instar developmental period	d_L	3.72 days
Pupal developmental period	d_P	0.643 days
Mortality rate of early-stage larvae (density dependent)	μ_E	0.0338/day
Mortality rate of late-stage larvae (density dependent)	μ_L	0.0348/day
Mortality rate of pupae (density independent)	μ_P	0.249/day
Effect of density dependence on late instars relative to early instars	γ	13.25

8.1.9 Intervention Models

The model incorporates a range of malaria control interventions. Here I describe the models pertaining to interventions that are not a key focus of this thesis. I discuss the other interventions that are a focus of this thesis (vaccination and chemoprophylaxis) in more detail in Chapters 3-5.

8.1.9.1 Treatment

Appropriate case management is implemented within the model. Treatment acts to return an individual from a clinically infected state to the susceptible class while also providing them with a drug-dependent period of prophylaxis resulting in partial protection from infection. We assume that artemisinin combination therapies (ACTs) have a 95% probability of successfully clearing infection and non-ACTs a 75% probability of clearing an infection. For ACTs protection from infection was characterised using a previously published pharmacokinetic-pharmacodynamic (PKPD) model, fitted to clinical trial data from six different sites in sub-Saharan Africa (Okell et al., 2014). The protection from infection at time *u* after effective treatment is denoted by $P_T(u)$ and the probability of re-infection is multiplied by $1 - P_T(u)$ relative to the period with no prophylaxis. The overall degree of protection can be quantified by the area under the curve:

$$A_T = \int_0^\infty P_T(u)\ du \tag{8.25}$$

For first line treatment (Artemether-Lumefantrine) A_T varies from 7 to 16 days depending on age.

8.1.9.2 Long lasting insecticide treated bed nets and Indoor residual spraying

The two main forms of vector control methods: long lasting insecticide treated bed-nets (LLINs) and indoor residual spraying (IRS) are modelled according to *Griffin et al.* 2010 (Griffin et al., 2010; Le Menach et al., 2007), with the addition of one modification that accounts for the possibility of a mosquito being killed by IRS prior to feeding (Griffin et al., 2016). LLINs and IRS act by repelling female mosquitos from feeding on a human host or killing the mosquito during its feeding and resting cycle. When LLINs and IRS are present in the model there are 6 different outcomes of a mosquito attempting to feed, each modelled probabilistically:

1. It bites a non-human host

2. It is killed by the LLIN before biting
3. It is killed by IRS before biting
4. It is killed by IRS after biting
5. It successfully feeds and survives
6. It is repelled without feeding either through the actions of the LLIN or IRS, once repelled a mosquito will attempt to find alternative blood meal sources.

The probability that a female mosquito seeking a blood meal successfully feeds is both species dependent (due to species specific bionomics and behaviours) and dependent on the anti-vectorial interventions in the human population. In the model it is assumed that only humans reside inside a house and all livestock are kept outside the house and therefore all mosquitoes that enter a house will attempt to bite humans.

Assume that person i is protected by a given LLIN/IRS efficacy. Let the probability that a mosquito of a given species bites host i during a single attempt be y_i; the probability that a mosquito bites a host and survives the feeding attempt be w_i, and the probability that it is repelled without feeding be z_i. These probabilities exclude natural vector mortality, so that for an individual with no protection, $y_i = w_i = 1$ and $z_i = 0$.

During a single feeding attempt, on either a human or animal, the probability that a female mosquito successfully feeds is:

$$W = (1 - Q_0) + Q_0 \sum_i \pi_i w_i \tag{8.26}$$

or is repelled without feeding with probability:

$$Z = Q_0 \sum_i \pi_i z_i \tag{8.27}$$

where Q_0 is the proportion of bites taken on humans in the absence of interventions and π_i is the proportion of bites that person i receives in the absence of any interventions.

The length of time a mosquito spends looking for a blood meal and resting between feeds are δ_1 and δ_2 respectively. The mosquito feeding ratef_R is given by $f_R = \frac{1}{\delta_1+\delta_2}$. Parameter δ_2 is assumed to be unaffected by the interventions, whilst δ_1 is increased to $\delta_1 = \frac{\delta_{10}}{1-Z}$ where δ_{10} is the value with no interventions.

In the absence of interventions, the probability that a mosquito survives these periods of feeding and resting are p_1 and p_2 respectively, with no interventions:

$$p_{10} = \exp(-\mu_0 \delta_{10}) \tag{8.28}$$

$$p_2 = \exp(-\mu_0 \delta_2) \tag{8.29}$$

where μ_0 is the natural death rate. And in the presence of interventions:

$$p_1 = \frac{p_{10}\, W}{1 - Z\, p_{10}} \tag{8.30}$$

Multiplying $p_1 p_2$ gives the probability of surviving a single feeding cycle. This allows us to find the mosquito death rate (μ_M) as:

$$p_1 p_2 = \exp\left(-\frac{\mu}{f_R}\right) \tag{8.31}$$

$$\mu = -f_R \log(p_1 p_2) \tag{8.32}$$

As μ changes so to does the probability that a mosquito survives through the extrinsic incubation period (p_M).

The probability that a single feeding cycle then ends with a successful bite on human i, q_i, is given by:

$$q_i = p_{10}(Q_0 \pi_i w_i + Z\, q_i) \tag{8.33}$$

$$q_i = \frac{p_{10} Q_0 \pi_i w_i}{1 - Z\, p_{10}} \tag{8.34}$$

The probability that a feeding cycle ends with a bite on a non-human host, q_A, is:

$$q_A = p_{10}(1 - Q_{10} + Z\, q_A) \tag{8.35}$$

$$q_A = \frac{p_{10}(1 - Q_0)}{1 - Z\, p_{10}} \tag{8.36}$$

This means the proportion of successful bites on humans (Q) is given by:

$$Q = 1 - \frac{q_A}{q_A + \sum_i q_i} = 1 - \frac{(1 - Q_0)}{(1 - Q_0) + Q_0 \sum_i \pi_i w_i} = 1 - \frac{(1 - Q_0)}{W} \tag{8.37}$$

and the human biting rate by:

$$\alpha = Q\, f_R \tag{8.38}$$

The rate at which person i is bitten by a given species is:

$$\lambda_i = \frac{\alpha\, \pi_i w_i}{\sum_i \pi_i w_i} \tag{8.39}$$

In instances where IRS is used, some mosquitoes may successfully bite a human and then die after this feed while resting on a treated wall inside the house. In this instance when calculating the force of

infection on humans, the biting rate on each individual person need to be modified by a factor $\frac{y_i}{w_i}$ giving:

$$\tilde{\lambda}_i = \frac{\alpha\, \pi_i y_i}{\sum_i \pi_i w_i} \tag{8.40}$$

Thus, the EIR experienced by a human i due to a single mosquito species is $\tilde{\lambda}_i I_M$ and the total EIR a single human experiences is given by the sum of each present vector species EIRs.

The impact of LLIN and IRS are dependent on the proportion of bites a human receives while under the protection of each intervention (e.g. sleeping under the bed-net and being inside a sprayed house) (Griffin et al., 2016, 2010). Therefore the degree of protection from LLINs and IRS will depend human host movement and sleeping patterns, the behaviour of mosquito vectors and the intrinsic efficacy of the intervention.

Let therefore, $\lambda_I(t)$ be the rate at which a person who is indoors at time t is bitten, and $\lambda_O(t)$ be the outdoors rate. Knowing the proportion of human hosts who are indoors $p_I(t)$ or in bed $p_B(t)$ at time t enables the calculation of the proportion of bites taken while a human host is indoors (Φ_I) and in bed (Φ_B) as:

$$\Phi_I = \frac{\sum_t p_I(t)\lambda_I(t)}{\sum_t \Big(\big(1 - p_I(t)\big)\lambda_O(t) + p_I(t)\lambda_I(t)\Big)} \tag{8.41}$$

$$\Phi_B = \frac{\sum_t p_B(t)\lambda_I(t)}{\sum_t \Big(\big(1 - p_I(t)\big)\lambda_O(t) + p_I(t)\lambda_I(t)\Big)} \tag{8.42}$$

Pulling this probabilistic model together, when a mosquito enters a house one of three outcomes can happen: it is repelled, feeds successfully or dies. The probabilities of each outcome in the presence of LLINs, IRS or both combined are given in

Table **8.4** and the parameter values used in the model are summarised in Table 8.5.

Table 8.4 Vector control probabilistic model. Outcome probabilities in the presence of LLIN or IRS alone and in combination.

Outcome	Probability
Any biting (y_i)	
IRS only	$1-\Phi_I+\Phi_I(1-r_I)(1-r_{IW}-d_{IW})$
LLIN only	$1-\Phi_B+\Phi_B(1-r_N)(1-r_{NW}-d_{NW})$
IRS + LLIN	$1-\Phi_I+(\Phi_I-\Phi_B)(1-r_I)(1-r_{IW}-d_{IW})$ $\Phi_B(1-r_I)(1-r_{IW}-d_{IW})(1-r_N)(1-r_{NW}-d_{NW})$
Bites and survives (w_i)	
IRS only	$1-\Phi_I+\Phi_I(1-r_I)(1-r_{IW}-d_{IW})(1-d_{IF})$
LLIN only	$1-\Phi_B+\Phi_B(1-r_N)(1-r_{NW}-d_{NW})$
IRS + LLIN	$1-\Phi_I+(\Phi_I-\Phi_B)(1-r_I)(1-r_{IW}-d_{IW})(1-d_{IF})$ $\Phi_B(1-r_I)(1-r_{IW}-d_{IW})(1-d_{IF})(1-r_N)(1-r_{NW}-d_{NW})$
Repelled without feeding (z_i)	
IRS only	$\Phi_I(r_I+(1-r_I)r_{IW})$
LLIN only	$\Phi_B(r_N+(1-r_N)r_{NW})$
IRS + LLIN	$(\Phi_I-\Phi_B)(r_I+(1-r_I)r_{IW})$ $+\Phi_B\big(r_I+(1$ $-r_I)(r_N+(1-r_N)(r_{IW}+(1-r_{IW}-d_{IW})r_{NW})\big)$

Φ_I, Φ_B time dependent probability of feeding indoors and on someone in bed respectively

r_N, r_I probability of being repelled before entering the house due to LLINs and IRS respectively

r_{NW}, r_{IW} the probability of being repelled by LLIN after entering the house or IRS before feeding respectively

d_{NW}, d_{IW} the probability of being killed by LLIN after entering the house or IRS before feeding respectively

d_{IF} probability of being killed after feeding after entry to a house due to IRS (this is a species-specific parameter)

Table 8.5 Vector control parameters (Griffin et al., 2016, 2010)

Intervention	Probability	Symbol	Value
LLINs	Repelled before entering the house	r_N	0.113
	Repelled by the bednet	r_{NW}	0.295
	Killed by the bednet	d_{NW}	0.533
IRS	Repelled before entering the house	r_I	0.687
	Repelled by the IRS	r_{IW}	0
	Killed before feeding	d_{IW}	0.295
	Killed after feeding (An. gambiae ss and funestus)	d_{IF}	0.813
	Killed after feeding (An. arabiensis)	d_{IF}	0.422

8.2 Binary infection model

The binary infection model was fitted using the same MCMC techniques as the main sporozoite infection model. Due to the reduction in the number of parameters being fitted we could increase the number of iterations to 800,000 without sacrificing computational time, 160,000 of which were discarded as burn-in. All updates were attempted with a multivariate-Normal proposal distribution. The covariance of the multivariate-Normal proposal distributions for Metropolis-Hastings updates were adaptively tuned using the estimated posterior distributions during iterations 500 – 10,000. This ensured the MCMC process adapted to the target distribution to keep the search effective at all times due to correlation between the parameters to be estimated. Further the magnitude of the proposed step size was calibrated using a Robbins-Munro algorithm to ensure an acceptance rate of approximately 23% as per the main text. Prior distributions were kept the same as in the main text (Table 8.6), and model comparisons were made using Deviance Information Criterion.

For the same combination of dose-response curves explored in the sporozoite infction model, the results of model fitting using Bayesian methods are presented in **Error! Reference source not found.**. W ith the best fitting model describing the dose-response between titre and avidity with Hill-function curves. The efficacy against infection estimated by the best fitting binary infection model was *VE* = 77.9% (95% CrI, 75.9% - 79.8%). In the binary model of infection the dose-response curves relate directly to the proportion protected from infection and not sporozoite survival and therefore can only be used to estimate vaccine efficacy against infection and not per-sporozoite.

Table 8.6 Parameter estimates for binary infection models with different combinations of dose-response curves and model comparison with Deviance-Information Criterion (DIC). Priors and Posteriors are presented as median and 95% Credible intervals in brackets. U denotes a uniform distribution, N normal and G gamma. Posterior estimates are shown for models with different combinations of dose-response curves and are ordered left to right based on model fitting comparisons with Deviance Information Criterion. Hill denotes a Hill-function dose response curve and Exp and exponential dose-response curve, read as "titre dose-response curve – avidity index dose-response curve".

Parameter	**Description**	**Prior**	**Posterior**				
			Hill-Hill	Exp-Hill	Hill-Exp	Exp-Exp	Interaction
β_t	Anti-NANP antibody titre needed for 50% reduction in infection probability	16,666 (10,000-70,000)	4,864 (620-39,578)	135,417 (97,899-149,379)	848 (511-3,269)	78,759 (55,386-124,711)	64,185 (30,822-136,969)
α_t	Shape parameter for antibody dose-response	U(0,30)	0.1 (0-0.2)	-	0.2 (0.1-0.3)	-	-
β_{ai}	Anti-NANP antibody titre needed for 50% reduction in infection probability	U(0,100)	30.1 (3.5-91.1)	2.9 (1.1-8.9)	82.6 (57.9-99.2)	29.7 (27.1-33)	29.2 (26.3-33.2)
α_{ai}	Shape parameter for antibody dose-response	U(0,30)	0.2 (0-0.6)	0.4 (0.3-0.6)	-	-	-
γ	Shape parameter for interation dose-response curve	N(0,10)	-	-	-	-	-0.3 (-0.9-1.5)
ΔDIC	Difference in deviance informative criterion		0.00	9.73	14.98	91.25	165.54

8.3 Cochrane RoB2 tool results

The following tables detail the framework of questions and answers from the RoB2 (Sterne et al., 2019) for each study identified in the literature review (see "Ref or Label"cell in the table for the study reference. The final table is a summary table highlighting the results from the RoB2 assessment across studies.

Unique ID	smc1	**Study ID**	1	**Assessor**	HT
Ref or Label	Bojang et al 2010	**Aim**	assignment to intervention (the 'intention-to-treat' effect)		
Experimental	SP+AQ	**Comparator**	non-randomised arm	**Source**	Journal article(s)
Outcome	Clinical malaria any parasitaemia	**Results**	0.93	**Weight**	1

Domain	Signalling question	Response	Comments
Bias arising from the randomization process	1.1 Was the allocation sequence random?	Y	
	1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?	Y	
	1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?	Y	Control cohort was non-randomised in a neighbouring area and characteristic differences were not listed
	Risk of bias judgement	**Some concerns**	Control cohort was non-randomised in a neighbouring area and characteristic differences were not listed
Bias due to deviations from intended interventions	2.1.Were participants aware of their assigned intervention during the trial?	N	Children were not aware of the drug regime given. Study nurses not involved with the evaluation of safety or efficacy measurements and who did not communicate any information on group allocation to the team in charge of the evaliation knew the study drugs allocated to each child at delivery.
	2.2.Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	Y	

	2.3. If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the experimental context?	PN	
	2.4 If Y/PY to 2.3: Were these deviations likely to have affected the outcome?	NA	
	2.5. If Y/PY/NI to 2.4: Were these deviations from intended intervention balanced between groups?	NA	
	2.6 Was an appropriate analysis used to estimate the effect of assignment to intervention?	Y	ITT
	2.7 If N/PN/NI to 2.6: Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomized?	NA	
	Risk of bias judgement	**Low**	Children were not aware of the drug regime given. Study nurses not involved with the evaluation of safety or efficacy measurements and who did not communicate any information on group allocation to the team in charge of the evaliation knew the study drugs allocated to each child at delivery. ITT
Bias due to missing outcome data	3.1 Were data for this outcome available for all, or nearly all, participants randomized?	Y	Loss to follow up was similar among the treatment groups and was not associated with any of the baseline characteristics
	3.2 If N/PN/NI to 3.1: Is there evidence that result was not biased by missing outcome data?	NA	
	3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?	NA	
	3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?	NA	

	Risk of bias judgement	**Low**	Loss to follow up was similar among the treatment groups and was not associated with any of the baseline characteristics
Bias in measurement of the outcome	4.1 Was the method of measuring the outcome inappropriate?	Y	Passive surveillance for malaria was maintained throughout the transmission season for 16 weeks (September to December). Parents/guardians of children in the trial and controls were encouraged to take their child to the health centre identified as being closest to their home at any time that their child became unwell. Project staff were based at each of these health facilities to identify children in the trial and to ensure that they were seen, properly investigated and treated promptly. A dipstick for diagnosis of malaria (CORE Diagnostics, Birmingham, UK) was used if fever (axillary temperature of ≥37.5°C) or a history of fever within the previous 48 hours was present. In such cases, a thick blood smear was also collected for subsequent confirmation of the diagnosis.
	4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	PY	No information was give but control group potentially attended different health clinics to those of the treatment arm.
	4.3 Were outcome assessors aware of the intervention received by study participants?	NA	
	4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	NA	
	4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?	NA	
	Risk of bias judgement	**High**	Passive surveillance for malaria was maintained throughout the transmission season for 16 weeks (September to December). Parents/guardians of children in the trial and controls were encouraged to take their child to the health centre identified as being closest to their home at any time that their child became unwell. Project staff were based at each of these health facilities to identify children in the trial and to ensure that they were seen, properly investigated and treated promptly. A dipstick for diagnosis of malaria (CORE Diagnostics, Birmingham, UK) was used if fever (axillary temperature of ≥37.5°C) or

			a history of fever within the previous 48 hours was present. In such cases, a thick blood smear was also collected for subsequent confirmation of the diagnosis. No information was give but control group potentially attended different health clinics to those of the treatment arm.
Bias in selection of the reported result	5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?	Y	
	5.2 ... multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?	N	Results were reported for malaria any parasitaemia and malaria parasitaemia ≥5000/µl
	5.3 ... multiple eligible analyses of the data?	N	
	Risk of bias judgement	**Low**	Results were reported for malaria any parasitaemia and malaria parasitaemia ≥5000/µl
Overall bias	**Risk of bias judgement**	**High**	

Unique ID	smc2	**Study ID**	2	**Assessor**	HT
Ref or Label	Dicko et al 2011	**Aim**	assignment to intervention (the 'intention-to-treat' effect)		
Experimental	SP+AQ	**Comparator**	placebo	**Source**	Journal article(s)
Outcome	clinical malaria	**Results**	0.83	**Weight**	1

Domain	Signalling question	Response	Comments
Bias arising from the randomization process	1.1 Was the allocation sequence random?	Y	
	1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?	Y	
	1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?	N	
	Risk of bias judgement	**Low**	
Bias due to deviations from intended interventions	2.1.Were participants aware of their assigned intervention during the trial?	N	
	2.2.Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	PN	
	2.3. If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the experimental context?	NA	
	2.4 If Y/PY to 2.3: Were these deviations likely to have affected the outcome?	NA	
	2.5. If Y/PY/NI to 2.4: Were these deviations from intended intervention balanced between groups?	NA	

	2.6 Was an appropriate analysis used to estimate the effect of assignment to intervention?	Y	
	2.7 If N/PN/NI to 2.6: Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomized?	NA	
	Risk of bias judgement	**Low**	
Bias due to missing outcome data	3.1 Were data for this outcome available for all, or nearly all, participants randomized?	Y	
	3.2 If N/PN/NI to 3.1: Is there evidence that result was not biased by missing outcome data?	NA	
	3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?	NA	
	3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?	NA	
	Risk of bias judgement	**Low**	
Bias in measurement of the outcome	4.1 Was the method of measuring the outcome inappropriate?	N	
	4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	PN	
	4.3 Were outcome assessors aware of the intervention received by study participants?	N	
	4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	NA	
	4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?	NA	
	Risk of bias judgement	**Low**	

Bias in selection of the reported result	5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?	Y	
	5.2 ... multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?	N	
	5.3 ... multiple eligible analyses of the data?	N	
	Risk of bias judgement	**Low**	
Overall bias	**Risk of bias judgement**	**Low**	

Unique ID	smc3	**Study ID**	3	**Assessor**	HT
Ref or Label	Konate et al 2011	**Aim**	assignment to intervention (the 'intention-to-treat' effect)		
Experimental	SP+AQ	**Comparator**	placebo	**Source**	Journal article(s)
Outcome	clinical malaria	**Results**	0.71	**Weight**	1

Domain	Signalling question	Response	Comments
Bias arising from the randomization process	1.1 Was the allocation sequence random?	Y	
	1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?	Y	
	1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?	N	
	Risk of bias judgement	**Low**	
Bias due to deviations from intended interventions	2.1.Were participants aware of their assigned intervention during the trial?	N	
	2.2.Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	PN	
	2.3. If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the experimental context?	NA	
	2.4 If Y/PY to 2.3: Were these deviations likely to have affected the outcome?	NA	
	2.5. If Y/PY/NI to 2.4: Were these deviations from intended intervention balanced between groups?	NA	

	2.6 Was an appropriate analysis used to estimate the effect of assignment to intervention?	Y	
	2.7 If N/PN/NI to 2.6: Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomized?	NA	
	Risk of bias judgement	**Low**	
Bias due to missing outcome data	3.1 Were data for this outcome available for all, or nearly all, participants randomized?	Y	
	3.2 If N/PN/NI to 3.1: Is there evidence that result was not biased by missing outcome data?	NA	
	3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?	NA	
	3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?	NA	
	Risk of bias judgement	**Low**	
Bias in measurement of the outcome	4.1 Was the method of measuring the outcome inappropriate?	N	
	4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	PN	
	4.3 Were outcome assessors aware of the intervention received by study participants?	PN	
	4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	NA	
	4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?	NA	
	Risk of bias judgement	**Low**	

Bias in selection of the reported result	5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?	Y	
	5.2 ... multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?	N	
	5.3 ... multiple eligible analyses of the data?	N	
	Risk of bias judgement	**Low**	
Overall bias	**Risk of bias judgement**	**Low**	

Unique ID	smc4	**Study ID**	4	**Assessor**	HT
Ref or Label	Sesay et al 2011	**Aim**	assignment to intervention (the 'intention-to-treat' effect)		
Experimental	SP+AQ	**Comparator**	placebo	**Source**	Journal article(s)
Outcome	clinical malaria	**Results**	0.66	**Weight**	1

Domain	Signalling question	Response	Comments
Bias arising from the randomization process	1.1 Was the allocation sequence random?	Y	
	1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?	Y	
	1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?	N	
	Risk of bias judgement	**Low**	
Bias due to deviations from intended interventions	2.1.Were participants aware of their assigned intervention during the trial?	N	
	2.2.Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	N	
	2.3. If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the experimental context?	NA	
	2.4 If Y/PY to 2.3: Were these deviations likely to have affected the outcome?	NA	
	2.5. If Y/PY/NI to 2.4: Were these deviations from intended intervention balanced between groups?	NA	

	2.6 Was an appropriate analysis used to estimate the effect of assignment to intervention?	Y	
	2.7 If N/PN/NI to 2.6: Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomized?	NA	
	Risk of bias judgement	**Low**	
Bias due to missing outcome data	3.1 Were data for this outcome available for all, or nearly all, participants randomized?	Y	
	3.2 If N/PN/NI to 3.1: Is there evidence that result was not biased by missing outcome data?	NA	
	3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?	NA	
	3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?	NA	
	Risk of bias judgement	**Low**	
Bias in measurement of the outcome	4.1 Was the method of measuring the outcome inappropriate?	N	
	4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	PN	
	4.3 Were outcome assessors aware of the intervention received by study participants?	N	
	4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	NA	
	4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?	NA	
	Risk of bias judgement	**Low**	

Bias in selection of the reported result	5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?	Y	
	5.2 ... multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?	N	
	5.3 ... multiple eligible analyses of the data?	N	
	Risk of bias judgement	**Low**	
Overall bias	**Risk of bias judgement**	**Some concerns**	

Unique ID	smc5	**Study ID**	5	**Assessor**	HT
Ref or Label	Zongo et al 2015	**Aim**	assignment to intervention (the 'intention-to-treat' effect)		
Experimental	SP+AQ	**Comparator**	non-randomised arm	**Source**	Journal article(s)
Outcome	clinical malaria	**Results**	0.8	**Weight**	1

Domain	Signalling question	Response	Comments
Bias arising from the randomization process	1.1 Was the allocation sequence random?	Y	
	1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?	Y	
	1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?	PY	The control arm was not randomised control so there were differences between the groups that needed to be accounted for.
	Risk of bias judgement	**Some concerns**	
Bias due to deviations from intended interventions	2.1.Were participants aware of their assigned intervention during the trial?	PN	
	2.2.Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	Y	
	2.3. If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the experimental context?	N	
	2.4 If Y/PY to 2.3: Were these deviations likely to have affected the outcome?	NA	
	2.5. If Y/PY/NI to 2.4: Were these deviations from intended intervention balanced between groups?	NA	

	2.6 Was an appropriate analysis used to estimate the effect of assignment to intervention?	Y	
	2.7 If N/PN/NI to 2.6: Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomized?	NA	
	Risk of bias judgement	**Low**	
Bias due to missing outcome data	3.1 Were data for this outcome available for all, or nearly all, participants randomized?	Y	
	3.2 If N/PN/NI to 3.1: Is there evidence that result was not biased by missing outcome data?	NA	
	3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?	NA	
	3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?	NA	
	Risk of bias judgement	**Low**	
Bias in measurement of the outcome	4.1 Was the method of measuring the outcome inappropriate?	N	
	4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	PN	These children were followed up in a manner similar to that used for the other study children. However were not placebo so difficult to characterise differences in treatment seeking behaviour.
	4.3 Were outcome assessors aware of the intervention received by study participants?	PN	Open trial, as blinding was not feasible due to difference in the appearance of the study drugs but steps were taken to ensure concealed randomisation and staff who performed laboratory analyses were not aware of the child's treatment group. Unsure if the study nurses/physians were aware of the treatment group.
	4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	NA	
	4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?	NA	

	Risk of bias judgement	**Some concerns**	
Bias in selection of the reported result	5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?	Y	
	5.2 ... multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?	N	
	5.3 ... multiple eligible analyses of the data?	N	
	Risk of bias judgement	**Low**	
Overall bias	**Risk of bias judgement**	**Some concerns**	

Unique ID	smc6	**Study ID**	6	**Assessor**	HT
Ref or Label	Tine et al 2011	**Aim**	assignment to intervention (the 'intention-to-treat' effect)		
Experimental	SP+AQ	**Comparator**	cluster randomised	**Source**	
Outcome	clinical malaria	**Results**	0.79	**Weight**	1

Domain	Signalling question	Response	Comments
Bias arising from the randomization process	1.1 Was the allocation sequence random?	Y	The randomization unit was the CHW in order to avoid contamination. Each CHW is covering one village. The CHWs were randomized using a random number generator from Excel software.
	1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?	NI	
	1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?	N	
	Risk of bias judgement	**Some concerns**	
Bias due to deviations from intended interventions	2.1.Were participants aware of their assigned intervention during the trial?	PY	Potential for contamination between villages and clusters making participants aware? Unsure if the trial was placebo controlled so participants might be aware as drug regimes would have been delivered or not. CHW delivering the interventions also aware they were delivering IPTc. In the four villages with combined HMM and IPTc, all doses of AQ and SP were administered by CHWs under direct observation. IPTc drug delivery was organized at the level of health huts. At scheduled days for IPTc administration, parents were asked to bring their child at the health huts for IPTc delivery.
	2.2.Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	PY	

			In case a child was not seen at time of administration, the CHW was advised to visit that child at home and give the treatment. To facilitate SP and AQ administration, treatment doses were tabulated on a document and distributed to each CHW to serve as job aid.
	2.3. If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the experimental context?	PN	
	2.4 If Y/PY to 2.3: Were these deviations likely to have affected the outcome?	NA	
	2.5. If Y/PY/NI to 2.4: Were these deviations from intended intervention balanced between groups?	NA	
	2.6 Was an appropriate analysis used to estimate the effect of assignment to intervention?	Y	
	2.7 If N/PN/NI to 2.6: Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomized?	NA	
	Risk of bias judgement	**Some concerns**	
Bias due to missing outcome data	3.1 Were data for this outcome available for all, or nearly all, participants randomized?	Y	
	3.2 If N/PN/NI to 3.1: Is there evidence that result was not biased by missing outcome data?	NA	
	3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?	NA	
	3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?	NA	
	Risk of bias judgement	**Low**	
Bias in measurement of the outcome	4.1 Was the method of measuring the outcome inappropriate?	N	

	4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	PN	
	4.3 Were outcome assessors aware of the intervention received by study participants?	Y	CHW would have been aware that IPTc was delivered to the children in their village.
	4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	N	RDTs were used and temperature so no judegement was required by CHW.
	4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?	NA	
	Risk of bias judgement	**Some concerns**	
Bias in selection of the reported result	5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?	Y	
	5.2 ... multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?	N	
	5.3 ... multiple eligible analyses of the data?	N	
	Risk of bias judgement	**Low**	
Overall bias	**Risk of bias judgement**	**Some concerns**	

Unique ID	smc7	**Study ID**	7	**Assessor**	HT
Ref or Label	Nidaye et al 2019	**Aim**	assignment to intervention (the 'intention-to-treat' effect)		
Experimental	SP+AQ	**Comparator**	Cluster randomised	**Source**	
Outcome	clinical malaria	**Results**	0.83	**Weight**	1

Domain	Signalling question	Response	Comments
Bias arising from the randomization process	1.1 Was the allocation sequence random?	Y	Cluster randomisation was chosen to allow the overall impact of SMC, including any indirect effect on transmission, to be captured, and to allow assessment of the impact of adding SMC on the overall workload of the health workers. Randomisation in each stratum was constrained to ensure balanced allocation with respect to population size, distance to the nearest health post or hospital, and the malaria incidence in the village the year before the trial, and was performed using Stata version 11 (StataCorp, College Station, Texas) by a statistician at the London School of Hygiene & Tropical Medicine.
	1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?	Y	
	1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?	N	
	Risk of bias judgement	**Low**	
Bias due to deviations from intended interventions	2.1.Were participants aware of their assigned intervention during the trial?	Y	cluster randomised so those who recieved SMC knew they were recieving SMC in that village there was also radio and other announcements about SMC delivery and the CHM knew they were also delivering SMC in their village. Those that weren't recieving would also be aware of this.
	2.2.Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	Y	
	2.3. If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the experimental context?	N	
	2.4 If Y/PY to 2.3: Were these deviations likely to have affected the outcome?	NA	

	2.5. If Y/PY/NI to 2.4: Were these deviations from intended intervention balanced between groups?	NA	
	2.6 Was an appropriate analysis used to estimate the effect of assignment to intervention?	Y	ITT
	2.7 If N/PN/NI to 2.6: Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomized?	NA	
	Risk of bias judgement	**Low**	
Bias due to missing outcome data	3.1 Were data for this outcome available for all, or nearly all, participants randomized?	Y	
	3.2 If N/PN/NI to 3.1: Is there evidence that result was not biased by missing outcome data?	NA	
	3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?	NA	
	3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?	NA	
	Risk of bias judgement	**Low**	
Bias in measurement of the outcome	4.1 Was the method of measuring the outcome inappropriate?	N	
	4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	PN	
	4.3 Were outcome assessors aware of the intervention received by study participants?	Y	
	4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	N	
	4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?	NA	

	Risk of bias judgement	**Some concerns**	
Bias in selection of the reported result	5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?	Y	
	5.2 ... multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?	N	
	5.3 ... multiple eligible analyses of the data?	N	
	Risk of bias judgement	**Low**	
Overall bias	**Risk of bias judgement**	**Low**	

Unique ID	smc8	**Study ID**	8	**Assessor**	HT
Ref or Label	Tagbor et al 2016	**Aim**	assignment to intervention (the 'intention-to-treat' effect)		
Experimental	SP+AQ	**Comparator**	placebo	**Source**	
Outcome	clinical malaria	**Results**	0.39	**Weight**	1

Domain	Signalling question	Response	Comments
Bias arising from the randomization process	1.1 Was the allocation sequence random?	Y	ID numbers comprising a four digit code and a check digit were randomly allocated to one of three intervention groups in a 1:1:1 ratio in permuted blocks of 12 using Stata version 13 (StataCorp, College Station, TX, USA). Treatment allocation was held in opaque sealed envelopes labelled only with the study ID number on the front. Upon enrolment to the study, the next envelope in the sequence was opened by the study team member to determine the treatment allocation of the child to be used throughout the study. This was carried out separately from the screening process; individuals screening children for eligibility were unaware of subsequent assignment.
	1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?	Y	
	1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?	PY	Despite the large number of children randomised, there were some imbalances between treatment groups at baseline.
	Risk of bias judgement	**Some concerns**	
Bias due to deviations from intended interventions	2.1.Were participants aware of their assigned intervention during the trial?	N	To facilitate community-based treatment of malaria with the assigned regimen (artemether-lumefantrine or dihydroartemisinin-piperaquine), and to ensure that children received the correct regimen if they attended at health centres in the study area, ID cards were colour-coded according to intervention group and labelled with the regimen to be used for case management. The study was therefore open-label with respect to the regimen used for case management but blinded with respect to whether seasonal malaria chemoprevention was active or placebo (members of the research team from KNUST/CGHR and LSHTM were aware of the
	2.2.Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	N	

			allocation, but those who administered the SMC, and mothers/children were blinded).
	2.3. If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the experimental context?	NA	
	2.4 If Y/PY to 2.3: Were these deviations likely to have affected the outcome?	NA	
	2.5. If Y/PY/NI to 2.4: Were these deviations from intended intervention balanced between groups?	NA	
	2.6 Was an appropriate analysis used to estimate the effect of assignment to intervention?	Y	
	2.7 If N/PN/NI to 2.6: Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomized?	NA	
	Risk of bias judgement	**Low**	
Bias due to missing outcome data	3.1 Were data for this outcome available for all, or nearly all, participants randomized?	Y	
	3.2 If N/PN/NI to 3.1: Is there evidence that result was not biased by missing outcome data?	NA	
	3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?	NA	
	3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?	NA	
	Risk of bias judgement	**Low**	
Bias in measurement of the outcome	4.1 Was the method of measuring the outcome inappropriate?	N	
	4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	N	

	4.3 Were outcome assessors aware of the intervention received by study participants?	N	blinded with respect to whether seasonal malaria chemoprevention was active or placebo
	4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	NA	
	4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?	NA	
	Risk of bias judgement	**Low**	
Bias in selection of the reported result	5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?	Y	
	5.2 ... multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?	N	
	5.3 ... multiple eligible analyses of the data?	N	
	Risk of bias judgement	**Low**	
Overall bias	**Risk of bias judgement**	**Some concerns**	

Ref or Label	Study ID	Experimental	Comparator	Outcome	Weight	D1	D2	D3	D4	D5	Overall
Bojang et al 2010	1	SP+AQ	non-randomised arm	Clinical malaria any parasitaemia	1	!	+	+	-	+	-
Dicko et al 2011	2	SP+AQ	placebo	clinical malaria	1	+	+	+	+	+	+
Konate et al 2011	3	SP+AQ	placebo	clinical malaria	1	+	+	+	+	+	+
Sesay et al 2011	4	SP+AQ	placebo	clinical malaria	1	+	+	+	+	+	!
Zongo et al 2015	5	SP+AQ	non-randomised arm	clinical malaria	1	!	+	+	!	+	!
Tine et al 2011	6	SP+AQ	cluster randomised	clinical malaria	1	!	!	+	!	+	!
Ndiaye et al 2019	7	SP+AQ	luster randomised	clinical malaria	1	+	+	+	!	+	+
Tagbor et al 2016	8	SP+AQ	placebo	clinical malaria	1	!	+	+	+	+	!

+ Low risk

! Some concerns

- High risk

D1 Randomisation process

D2 Deviations from the intended interventions

D3 Missing outcome data

D4 Measurement of the outcome

D5 Selection of the reported result

The “traffic light” plots are of the domain-level judgements for each individual result and an overall summary of the risk of bias in each study.

8.4 Additional research outputs and publications

Relevant journal articles

- **Thompson HA**, Hogan AB, Walker PG, White MT, Cunnington AJ, Ockenhouse CF, Ghani AC. *Modelling the roles of antibody titre and avidity in protection from Plasmodium falciparum malaria infection following RTS, S/AS01 vaccination*. Vaccine. 2020
- **Thompson HA**, Hogan AB, Walker PG, Winskill P, Zongo I, Sagara I, Tinto H, Ouedraogo JB, Dicko A, Chandramohan D, Greenwood B, Cairns M, Ghani AC. *Mathematical modelling of a seasonal use-case for the RTS,S/AS01 malaria vaccine.* The Lancet global health (in review). 2022

Evidence submitted to the Malaria Policy Advisory Group October 2021

http://terrance.who.int/mediacentre/data/malaria/documents/mpag-october2021-session5-rtss-malaria-vaccine.pdf?sfvrsn=9507a63b_10

Oral presentations

- "Modelling the roles of antibody titre and avidity in protection from Plasmodium falciparum malaria infection following RTS, S/AS01 vaccination" American Society of Tropical Medicine and Hygiene 2018
- "Modelling the roles of antibody titre and avidity in protection from Plasmodium falciparum malaria infection following RTS, S/AS01 vaccination" School of Public Health PhD Symposium 2018

Other publications during the PhD

- **Thompson HA,** Mousa A, Dighe A, Fu H, Arnedo-Pena A, Barrett P, Bellido-Blasco J, Bi Q, Caputi A, Chaw L, De Maria L. *Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) setting-specific transmission rates: a systematic review and meta-analysis.* Clinical Infectious Diseases. 2021
- **Thompson HA**, Imai N, Dighe A, Ainslie KE, Baguelin M, Bhatia S, Bhatt S, Boonyasiri A, Boyd O, Brazeau NF, Cattarino L. *SARS-CoV-2 infection prevalence on repatriation flights from Wuhan City, China.* Journal of travel medicine. 2020
- Verity R, Okell LC, Dorigatti I, Winskill P, Whittaker C, Imai N, Cuomo-Dannenburg G, **Thompson HA**, Walker PG, Fu H, Dighe A. *Estimates of the severity of coronavirus disease 2019: a model-based analysis*. The Lancet infectious diseases. 2020

- **Thompson HA,** Mboup A, Cisse B, Nayagam S, Watson OJ, Whittaker C, Walker PG, Ghani AC, Mboup S. *The projected impact of mitigation and suppression strategies on the COVID-19 epidemic in Senegal: A modelling study*. medRxiv. 2020.
- Walker PG, Whittaker C, Watson OJ, Baguelin M, Winskill P, Hamlet A, Djafaara BA, Cucunubá Z, Olivera Mesa D, Green W, **Thompson, H** et al. *The impact of COVID-19 and strategies for mitigation and suppression in low-and middle-income countries*. Science. 2020.

These are a selection of the main publications I worked on during my time with the Imperial College London COVID-19 response team during the pandemic 2020-2021. In addition to these papers listed here I was a co-author on further published articles and reports that can be found here: https://www.imperial.ac.uk/people/hayley.thompson12/publications.html

I also spent three months between Feb-June 2020 working for the World Health Organisation in the Health Emergencies Programme as an epidemiologist during the early stages of the COVID-19 pandemic. I Provided technical epidemiological analysis support to understand the evolving global spread of COVID-19. Explored and quantified key epidemiological parameters in real-time using large global datasets in R. Automated key data cleaning and analysis protocols in R and produced data visualisations using ggplot2 in R. Developed a dashboard in Rshiny for internal and external users to examine global trends. Led training sessions and workshops on how to interpret epidemiological analysis presented in this dashboard. Contributed to standard operating procedures. And I presented multiple analyses to WHO leadership which fed into meetings with member states, daily press briefings and guidance documents

Public engagement

- Interviewed on BBC Radio 4 “More or Less” to discuss the work in: *Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) setting-specific transmission rates: a systematic review and meta-analysis*
- Volunteer at Imperial College Lates and Festival 2019-2020 leading an interactive activity called *“Pandemic Potential”*