Models for measuring and predicting malaria vaccine efficacy

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Abstract

In the past decade several candidate malaria vaccines have undergone clinical trials in artificial challenge studies and studies of natural infection under field conditions. GlaxoSmithKline's RTS,S vaccine against *Plasmodium falciparum* infection has taken the lead, with Phase III trials in African children demonstrating 55.8% (97.5% CI, 51.3% - 59.8%) efficacy against clinical malaria and 34.8% (95% CI, 16.2% – 49.2%) efficacy against severe malaria. Mathematical models can contribute to multiple stages of malaria vaccine development, from measuring efficacy in clinical trials, understanding the relationship between naturally acquired and vaccine-induced immunity, identifying correlates of protection, and predicting the likely impact of vaccination programs in the field.

When measuring vaccine efficacy in field trials under natural exposure to malaria, there are many factors which can bias estimates of efficacy. We demonstrate how heterogeneity in exposure can cause efficacy to be underestimated and heterogeneity in vaccine response can cause efficacy to be overestimated.

Most infection-blocking vaccines rely on boosting some element of the pre-erythrocytic immune response, however the relationship between the naturally acquired pre-erythrocytic responses and protection from infection remains poorly understood. By analysing studies from a systematic of the published literature, I demonstrate that although many studies report a statistically significant relationship between cellular pre-erythrocytic immune responses and protection from infection, many studies do not have sufficient statistical power to evaluate the effects of the pre-erythrocytic immune response.

Mathematical models are developed for investigating the relationship between pre-erythrocytic antibodies and protection from infection, and fitted to data from a longitudinal study of malaria infection in Kenyan adults. The relationship between antibodies to the antigens circumsporozoite protein (CSP) and thrombospondin-related adhesion protein (TRAP) and protection from infection is characterised using dose-response curves.

Using data from an artificial challenge trial of the RTS,S malaria vaccine, I demonstrate that vaccine-induced protection from infection depends on both anti-CSP antibodies and CSP-specific T cells. I estimate that RTS,S causes a 97.7% (95% CI, 96.3% – 98/7%) reduction in the number of parasites entering the blood from the liver.

The immune effector mechanisms determining the duration of vaccine-induced protection from infection are likely to be similar to those involved in naturally acquired immunity. Models of antibody kinetics were fitted to data from longitudinal studies of the antibody response to *P. falciparum* infection in Ghanaian and Gambian children, and the parameters determining the duration of antibody response are estimated.

Upon licensure, a successful malaria vaccine is likely to be administered to young African children. A model of malaria transmission, extensively fitted to clinical data, is used to investigate the impact of vaccination in different transmission settings; the interaction between vaccines and other interventions such as insecticide treated nets; and the interaction between vaccination and naturally acquired immunity. Finally, the potential cost-effectiveness of vaccination is explored.

Declaration of Originality

I declare that this work is my own, completed under the supervision of Prof. Azra Ghani and Dr. Jamie Griffin. Much of the work involved in this thesis has been of a collaborative nature. All individuals who have shared data, made suggestions for analysis, and commented on manuscripts have been acknowledged.

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ACD	active case detection
ACT	artemisinin combination therapy
ADI	active detection of infection
AIC	Akaike information criterion
AMA-1	apical membrane antigen 1
AS	adjuvant system
ASC	antigen secreting cells
BSV	blood-stage vaccine
CMI	cell-mediated immunity
CSP	circumsporozoite protein
DALY	disability adjusted life years
DDT	dichloro-diphenyl-trichloroethane
EBA-175	erythrocyte binding antigen 175
EIR	entomological inoculation rate
ELISA	enzyme linked immunosorbent assay
EPI	expanded program on immunisation
GMEP	Global Malaria Elimination Program
GSK	GlaxoSmithKline
ibppy	infectious bites per person year
ICER	incremental cost-effectiveness ratio
IFN-γ	interferon gamma
Ig	immunoglobulin
IL-2	interleukin 2
IPTc	intermittent preventive treatment of children
IPTi	intermittent preventive treatment of infants
IPTp	intermittent preventive treatment of pregnant women
IRS	indoor residual spraying
ITN	insecticide treated nets

LSA-1	liver-stage antigen 1
LSA-3	liver-stage antigen 3
LLIN	long-lasting insecticide treated nets
MBC	memory B cells
MCMC	Markov Chain Monte Carlo
MDA	mass drug administration
MSAT	mass screen and treat
MSP-1	merozoite surface protein 1
MSP-2	merozoite surface protein 2
NANP	Asparagine-Alanine-Asparagine-Proline
PBMC	peripheral blood mononuclear cells
PCD	passive case detection
PCR	polymerase chain reaction
PE	pre-erythrocytic
PEV	pre-erythrocytic vaccine
*Pf*EMP1	*Plasmodium falciparum* erythrocyte membrane protein 1
*Pf*PR	*Plasmodium falciparum* parasite rate
PH	proportional hazards
PYAR	person years at risk
RDT	rapid diagnostic test
SMC	seasonal malaria chemoprophylaxis
Swiss TPH	Swiss Tropical and Public Health Institute
TBV	transmission-blocking vaccine
TNF-α	tumour necrosis factor alpha
TRAP	thrombospondin related adhesive protein
YLD	years lost due to disability
YLL	years of life lost
WHO	World Health Organisation
WRAIR	Walter Reed Army Institute of Research

Acknowledgements

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Chapter 1. Introduction

1.1. Background

In 1967 Nussenzweig *et al* [1] reported that mice immunized with radiation-attenuated *Plasmodium berghei* sporozoites developed sterile immunity against further infection, a finding subsequently repeated by Vanderberg *et al* [2]. Buoyed by these early successes, the challenge of developing an effective vaccine for malaria in humans appeared to lie in translating these findings from animal models to appropriate technology for the immunisation of humans, as had been done for other human pathogens. Studies followed demonstrating that following exposure to the bites of large numbers of infectious irradiated mosquitoes, sterile immunity to infection could be induced in humans challenged with either *Plasmodium falciparum* [3-5] or *Plasmodium vivax* [6]. An effective malaria vaccine appeared to be just around the corner. However progressing from immunity induced by the bites of infectious mosquitoes to immunity induced by vaccination proved to be an unexpectedly challenging hurdle. A similar pattern of optimism followed by failure was repeated in the 1990's with the development of the SPf66 malaria vaccine where initial indications of efficacy in South American trials [7] were followed by disappointing results in Africa and Asia [8, 9].

The successes and failures in the malaria vaccine development effort were mirrored by those in the wider malaria control effort. In the 1950's the World Health Organisation (WHO) launched the Global Malaria Elimination Project (GMEP) with the laudable aim of eliminating malaria from many of the world's endemic countries [10]. Based on indoor residual spraying with the recently discovered insecticide DDT and effective treatment with chloroquine, the program experienced early successes eliminating malaria from several countries including Spain and Italy [11]. Unfortunately these early successes were followed by failure as resistance to both DDT and chloroquine emerged. Notably, the GMEP made no serious attempt to tackle malaria in sub-Saharan Africa where transmission was at its most intense. The early optimism for widespread malaria elimination was replaced by a resigned acceptance that malaria was here to stay. Following this disappointment the problem of malaria elimination was reduced to waiting for a vaccine [12], arguably to the detriment of innovative research into other tools for malaria control.

In the past decade there has been renewed enthusiasm for widespread control of malaria, and even elimination in some settings, built upon advances in malaria control tools, in particular insecticide treated nets [13] and effective treatment with artemisinin combination therapies (ACTs) [14]. Against this background of increased research into more traditional malaria control tools targeting the vector with insecticides and the parasite with drugs, there has finally been substantial progress towards an effective vaccine, with several promising candidates under development [15-17]. The most advanced candidate is GlaxoSmithKline's RTS,S vaccine which has demonstrated 55% efficacy against clinical malaria and 47% efficacy against severe malaria [15]. Phase III trials are

scheduled for completion in 2014 after which the vaccine is likely to become licensed and available as a tool for protecting young African children from malaria.

1.2. Epidemiology of malaria

1.2.1. Burden of malaria

Malaria, caused by the *Plasmodium* parasite, inflicts an enormous burden of morbidity and mortality on the world's population, the bulk of which is borne by young children and pregnant women living in *P. falciparum* endemic regions of sub-Saharan Africa (Figure 1.1). The annual number of deaths attributable to malaria was estimated to be 655,000 in 2010 [18], although this number has been in steady decline for the past decade [19]. *P. falciparum* is just one of the five species of parasite responsible for malaria in humans, the others being *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesei*. Often neglected in the past, *P. vivax* is increasingly being recognised as a major public health problem with an estimated 132 – 391 million cases per year [20]. Approximately 2.6 billion people live in *P. vivax* endemic areas, predominantly in South and East Asia and parts of South America [21]. *P. vivax* is rare in most parts of Africa due to very high prevalence of the Duffy-negative phenotype which confers resistance to *P. vivax* infection [22]. *P. malariae* and *P. ovale* are usually found at low prevalence throughout Africa, Asia and South America [23, 24] and give rise to mild illness, although the full range of clinical outcomes remain poorly understood [24]. *P. knowlesei* was thought to only infect old world monkeys but has recently been diagnosed in humans in Malaysian Borneo [25] and is increasingly being recognised as potentially life threatening [26].

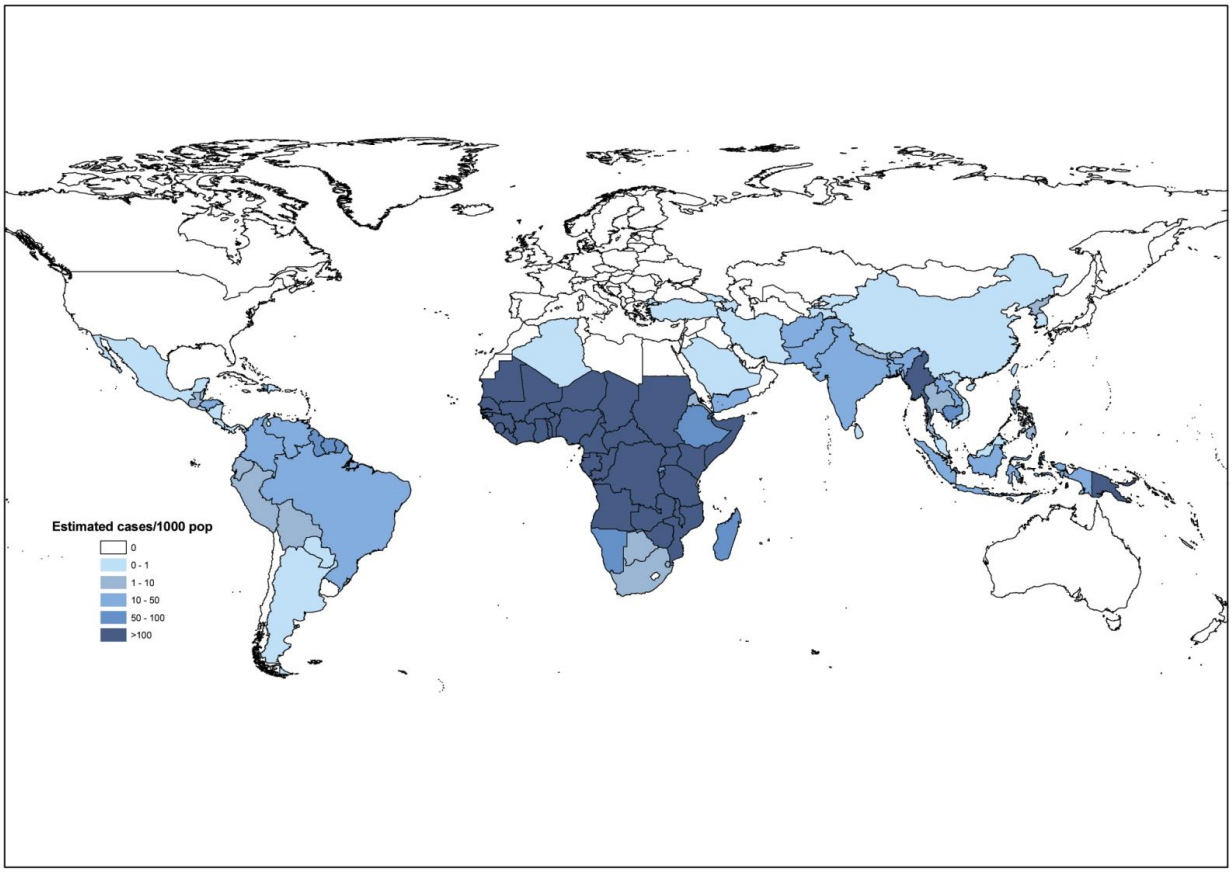

Figure 1.1: Estimated number of malaria cases per 1,000 population in 99 endemic countries in 2009. Figure reproduced from Cibulskis *et al* [19].

1.2.2. The lifecycle of the *Plasmodium falciparum* parasite

The lifecycle of the *Plasmodium falciparum* parasite begins when an infectious *Anopheles* mosquito takes a blood meal from a human host (Figure 1.2). As the mosquito feeds, sporozoites (the infectious stage of the parasite) are inoculated into the skin through the mosquito's proboscis. Sporozoites migrate through the skin until they reach a blood vessel and travel to the liver in the blood circulation. Once in the liver a sporozoite will invade a hepatocyte and transform into a trophozoite [27]. The trophozoite undergoes schizogonic development and differentiates into approximately 20,000 merozoites [28] over a period of approximately 6.5 days [29]. The combined skin and liver stage of the parasite lifecycle is termed the pre-erythrocytic stage. Hepatic merozoites enter the blood from the liver to begin the erythrocytic stage of the lifecycle. Merozoites rapidly invade red blood cells, typically on a time scale of less than 10 minutes, where they replicate and differentiate into 8 – 32 merozoites over a period of two days after which the infected blood cell ruptures releasing the next generation of merozoites into the blood stream [30]. The rupturing of infected red blood cells every two days results in the periodic cycle of fever characteristic of clinical malaria. A proportion of merozoites will differentiate into male or female gametocytes – the sexual stage of the parasite. Gametocytes circulate in the blood stream until they are ingested by another mosquito taking a blood meal.

The parasites' multiplication in the mosquito is known as the sporogonic cycle. In the mosquito's midgut, male microgametes penetrate female macrogametes generating zygotes. The zygotes in turn become motile ookinetes which invade the midgut wall where they develop into oocysts. The oocysts grow, rupture and release sporozoites which migrate to the mosquito's salivary glands [30]. When the mosquito next takes a blood meal from a human, sporozoites are inoculated as the mosquito salivates, thus completing the parasites' lifecycle.

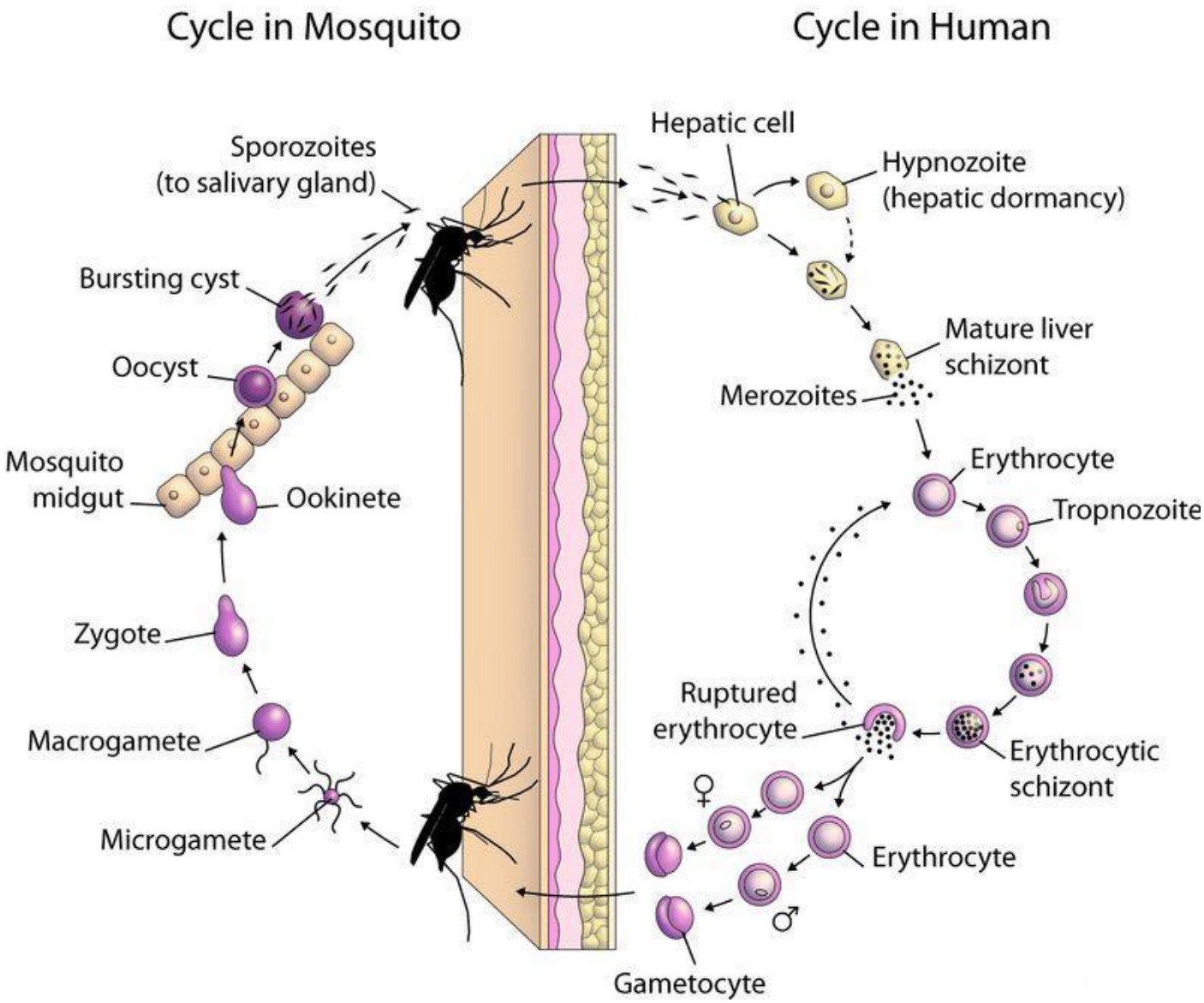

Figure 1.2: The lifecycle of the malaria parasite in humans and mosquitoes. Figure reproduced from Johns Hopkins Open Courseware: "Life cycle of the malaria parasite" from Epidemiology of Infectious Diseases. © Johns Hopkins Bloomberg School of Public Health. Creative Commons BY-NC-SA.

1.2.3. Malaria transmission

Ever since Ronald Ross and Giovanni Grassi first demonstrated that malaria was transmitted from mosquitoes to humans [31], malariologists have been measuring the transmission of malaria and the effects of malaria on

exposed populations. Malaria transmission can be quantified using measurements from epidemiological studies. The entomological inoculation rate (EIR) measures the average number of infectious bites per person per year (ibppy). The EIR is usually estimated from the number of mosquitoes taking a blood meal on a human adult over a day and the proportion of mosquitoes testing positive for sporozoites. Not all infectious mosquito bites progress to blood-stage malaria, either due to chance [32] or due to an infection-blocking immune response [33]. The number of infectious bites that progress to blood stage malaria is termed the force of infection. The proportion of a population infected with malaria is measured by the parasite prevalence or parasite rate (*Pf*PR for *P. falciparum*) – defined to be the proportion of a cross-sectional cohort testing positive for parasites. The parasite prevalence will depend on the method used for parasite detection, with parasite prevalences measured by PCR generally being higher than those measured by slide microscopy [34]. Malaria infection can either be asymptomatic with no detectable clinical symptoms, or progress to an episode of clinical malaria – usually defined as fever plus parasite density above a given threshold. The average number of episodes of clinical malaria per person per year is termed the clinical incidence. A proportion of episodes of clinical malaria will progress to severe malaria. Severe malaria can be measured on a population level as the number of cases per person year at risk, but in practice is often measured via the number of cases presenting to hospital [35]. There are several manifestations of severe malaria, including but not limited to, severe anemia, cerebral malaria and respiratory distress. Variation in the diagnosis of severe malaria can lead to variation in estimates of severe malaria incidence [36, 37]. The relationship between these epidemiological measures is shown in Figure 1.3.

A number of malariological quantities derived from entomological data on vector behaviour are also of importance for malaria transmission. The stability index gives a measure of an environments capacity to sustain malaria transmission and is defined to be the expected number of human bites taken by a vector over its lifetime [38]. The vectorial capacity estimates the number of infectious bites on humans that arise from all the mosquitoes that are infected by a single person on a single day [39]. The human blood index measures the proportion of mosquito blood meals that are taken from humans. The basic reproductive number (R_0) is defined as the expected number of human cases that would arise from a typical malaria case in an otherwise uninfected population with no immunity and no control [40].

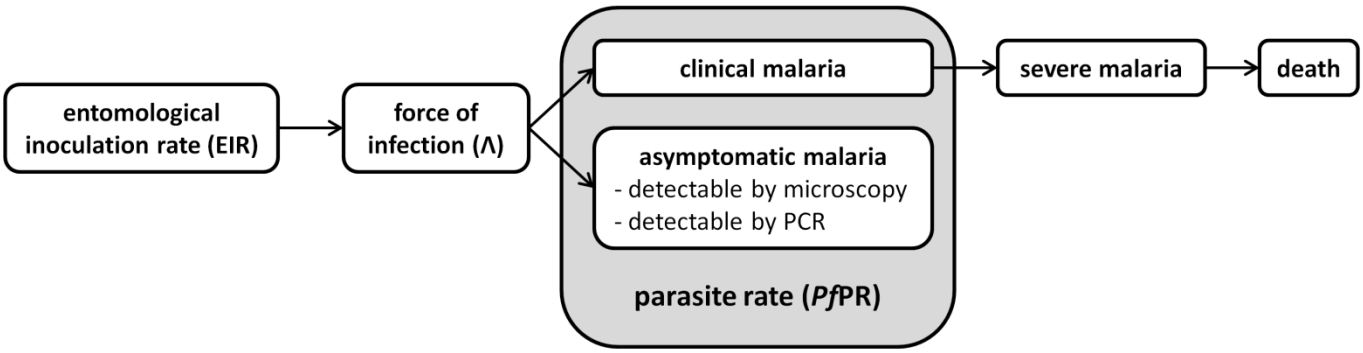

Figure 1.3: Schematic diagram of the relationship between epidemiological measurements of malaria transmission and clinical outcomes.

In addition to its average intensity, malaria transmission can also be characterised by its variation. Heterogeneity in malaria transmission exists on all spatial scales, from large scale variation between geographic regions to micro-heterogeneity within a single household. Large scale variation in transmission is primarily due to differences in climate [41], vector species distribution [42] and economic development. On a community or village level, heterogeneity in transmission can arise due to proximity to larval breeding sites [43] or variation in the quality of housing [44]. Mosquito biting is heterogeneous even between members of the same household due to different sleeping patterns or olfactory cues [45, 46].

Malaria transmission also shows substantial temporal variation, primarily driven by seasonal changes in mosquito densities, which are in turn regulated by climatic factors [47]. Most malaria endemic regions experience some degree of seasonality dependent on their rainfall patterns [48], with transmission at its highest during the rainy season and at its lowest during the dry season.

1.2.4. The *Anopheles* mosquito

Although there are thousands of species of mosquito belonging to several genera, only one genus, *Anopheles*, is capable of transmitting malaria to humans. The genus *Anopheles* contains around 430 known species, but only 40 of these are competent malaria vectors [49]. The most important vectors in sub-Saharan Africa are *An. gambiae sensu stricto*, *An. arabiensis* and *An. funestus.* The *Anopheles* mosquito begins its life upon emergence from a pupa in a larval breeding site. Both male and female mosquitoes feed on sugar from nearby plants and mate in swarms. Female mosquitoes must take a blood meal from an animal host to facilitate egg production – hence only female mosquitoes are responsible for malaria transmission. After feeding, a mosquito must rest while the blood meal is digested and eggs develop in the abdomen. After a period of 2 – 3 days [50], the mosquito will search for a suitable breeding site and oviposit 50 – 200 eggs [51]. The duration between consecutive blood meals is termed the gonotrophic cycle, and is a key quantity for malaria control as it determines the expected number of bites a mosquito will make in her lifetime. Oviposited eggs develop into larvae which feed on bacteria, yeasts, protozoa and particulate organic matter in the water. Growth takes place via a series of moults through four morphologically distinct larval instars. Fourth instar larvae moult to become non-feeding pupae which develop into adult mosquitoes. The duration of the larval period depends mainly on temperature and, in tropical areas, lasts 7-15 days [52].

1.3. Immunity to malaria

1.3.1. Naturally acquired immunity to malaria

Children and adults living in malaria-endemic areas acquire substantial protection from malaria, but rarely, if ever, achieve sterile immunity. The rate of acquisition of immunity is both age [53] and exposure [54] dependent, leading to non-linear patterns of immunity across a population. Effective immunity to malaria is acquired in a sequential manner, with rapid development of immunity to severe malaria, followed by immunity to episodes of clinical malaria, and finally the acquisition of immune tolerance to blood-stage parasites [55].

Young children are particularly vulnerable to episodes of severe malaria after maternally acquired immunity has waned and before the acquisition of effective immunity [56]. The acquisition of immunity to severe malaria is believed to be rapid, requiring only one or two infections [57], however there is substantial variation in the rates of acquisition of immunity to different forms of severe malaria, with cerebral malaria manifesting in older children [35]. Immunity to episodes of clinical malaria is acquired at a slower rate than immunity to severe malaria with a peak in clinical incidence occurring in children between 1 – 10 years of age depending on the intensity of transmission (Figure 1.4).

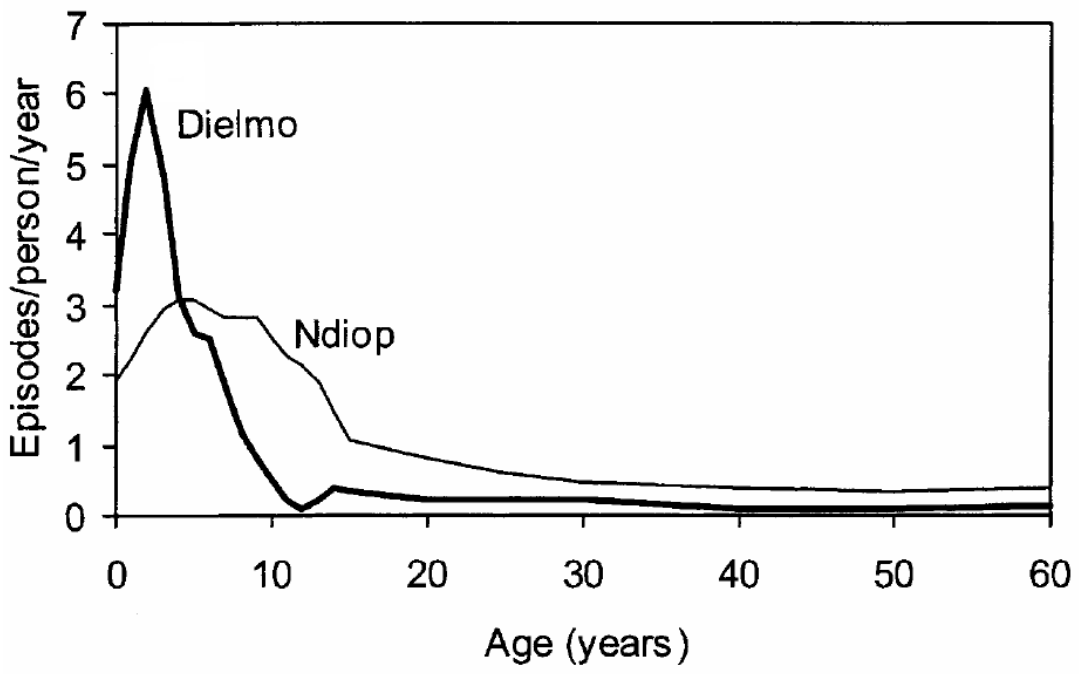

Figure 1.4: Annual clinical incidence of malaria in the Senegalese villages of Dielmo (EIR = 200 ibppy) and Ndiop (EIR = 20 ibppy). The patterns demonstrate that clinical incidence peaks earlier in high transmission settings. Figure reproduced from Smith *et al* [58] based on data from Trape and Rogier [59].

Patterns of immunity to asymptomatic parasite infection are observable in studies of age-stratified parasite prevalence [60-62] with the highest prevalence rates typically being in younger children before the acquisition of effective immunity (Figure 1.5). These patterns can be explained by two types of immune response: a blood-stage immune response controlling parasite densities, a pre-erythrocytic immune response that prevents infections, or

a combination of the two. The relative contribution of these two types of immune response to protection from parasites has been difficult to estimate from epidemiological data [63, 64].

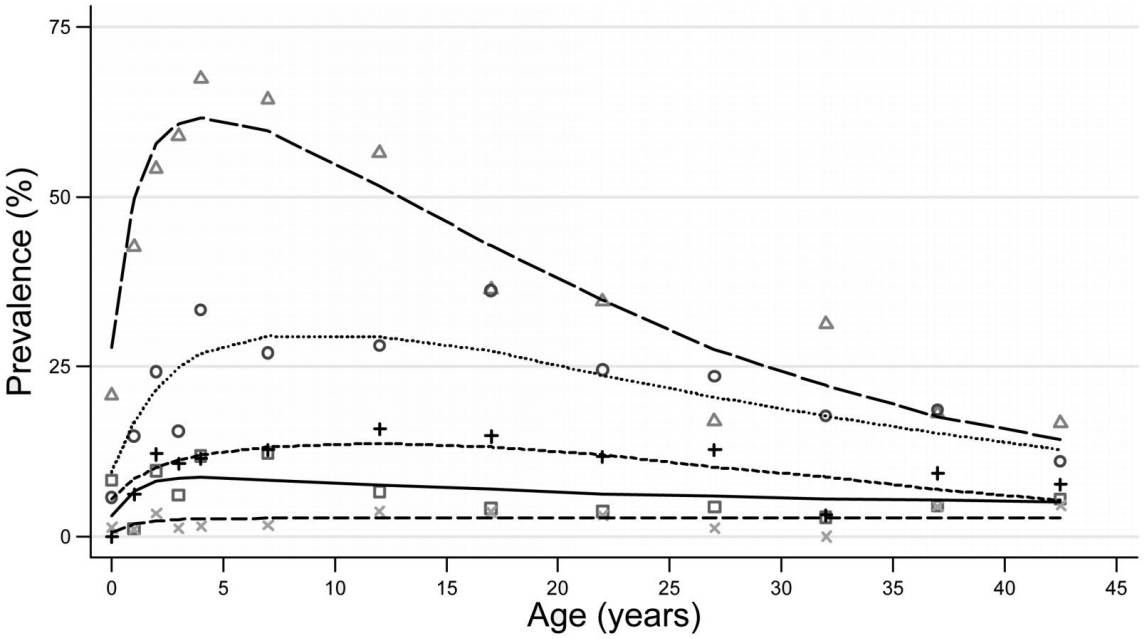

Figure 1.5: Prevalence of *P. falciparum* parasites by age stratified by altitude band and region. Triangles, Tanga < 600m; circles, Tanga 600 – 1200m; squares, Kilimanjaro 600 – 1200m; plus signs, Tanga > 1200m; crosses, Kilimanjaro > 1200m. Figure reproduced from Drakeley *et al* [60].

1.3.2. Immunology of malaria

Upon entering a host, the malaria parasite must first invade and replicate within hepatocytes in the liver, and then erythrocytes in the blood, before finally differentiating into gametocytes that circulate in the blood. The parasites face a succession of challenges during this process, as they must overcome physical barriers and evade both non-specific innate responses and adaptive immune responses. Upon exposure to blood-stage parasites the non-specific innate immune response is first to be activated, triggering a release of cytokines which inhibit parasite development, and allow effective priming of the adaptive antibody and cell-mediated immune responses [65].

After initial exposure to malaria parasites, the adaptive immune response is primed. Protection against malaria can be induced by the following mechanisms: antibodies opsonise and immobilise sporozoites and block invasion into liver cells [66]; interferon-γ (IFN-γ) and $CD8^+$ T cells inhibit parasite development in hepatocytes [16]; antibodies block invasion of merozoites into erythrocytes; and IFN-γ and $CD4^+$ T cells activate macrophages which phagocytose infected red blood cells and free merozoites.

Underlying the adaptive immune response is the phenomenon of pattern recognition of parasite antigens by lymphocytes: either B cells responsible for humoral immunity or T cells responsible for cell-mediated immunity. In a simplified model of the human immune system, antigen presenting cells internalise parasite antigen and present it to T cells and B cells [67]. T cells proliferate into antigen-specific $CD4^+$ or $CD8^+$ T cells which provide cell-

mediated immunity in the liver and blood. When antigen-specific memory B cells (MBC) recognise a presented antigen they will undergo proliferation generating an expanded memory B cell compartment [68] (Figure 1.6). Some MBCs will terminally differentiate into either short-lived or long-lived plasma B cells which reside in survival niches in the spleen or bone marrow [69]. Plasma B cells, also referred to as antibody secreting cells, are responsible for generating the immunoglobulin (Ig) antibodies which are the basis of the humoral immune response. There are five classes of immunoglobulin antibody: IgG, IgA, IgD, IgE and IgM. The first time an individual is exposed to a particular antigen, the majority of antibody produced is IgM. Upon re-exposure to the same antigen, a rapid secondary response can be mounted in which large amounts of antibody of other classes are produced, most notably IgG.

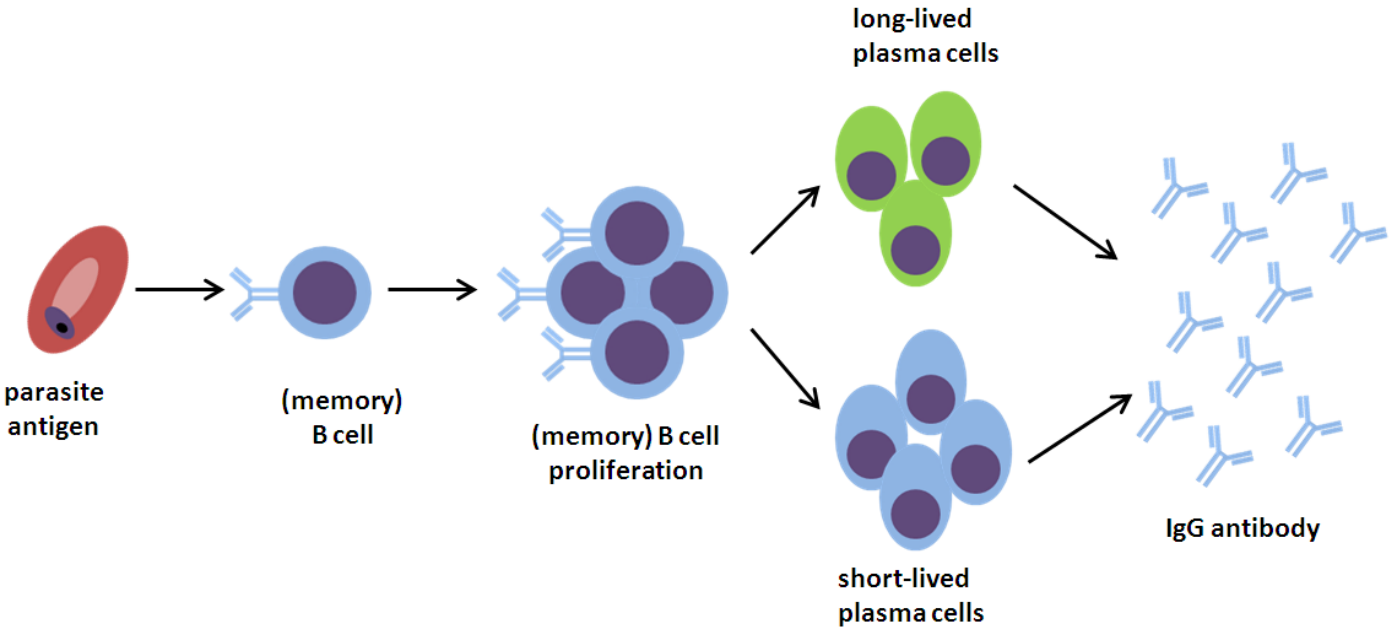

Figure 1.6: Schematic model of the immunological processes underlying the generation of IgG antibody. Upon recognition of parasite antigen, memory B cells proliferate and terminally differentiate into short-lived or long-lived plasma cells. Plasma cells continuously secrete the antibodies required for maintaining humoral immunity.

1.3.3. Antibodies to *P. falciparum* antigens

Sequencing of the *P. falciparum* genome has identified approximately 5,400 proteins [70], although which of these proteins elicit the production of protective antibodies remains unclear [71]. There are a small number of well studied antigens of the pre-erythrocytic, erythrocytic and sexual stages of the parasite.

At the pre-erythrocytic stage, the circumsporozoite protein (CSP) covers the entire surface of the sporozoite and is found on the plasma membrane of liver-stage parasites [72]. Antibodies to CSP immobilize sporozoites and inhibit parasite invasion of hepatocytes [73]. Thrombospondin-related adhesive protein (TRAP) is found primarily near the tip of the sporozoite (the microneme) [74]. Antibodies to TRAP inhibit sporozoite gliding motility and hepatocyte invasion [75]. Liver-stage antigen 1 (LSA-1) and liver-stage antigen 3 (LSA-3) are expressed once the

sporozoite invades a liver hepatocyte. As LSA-1 and LSA-3 are expressed only in the hepatocyte which antibodies are unable to access, they are not expected to provide protection from infection, although they are likely targets of cell-mediated immunity [76].

Several proteins have been identified that are expressed on the surface of merozoites, or during merozoite invasion of erythrocytes, the most well studied being merozoite surface protein 1 (MSP-1), merozoite surface protein 2 (MSP-2), apical membrane antigen 1 (AMA-1), and erythrocyte binding antigen 175 (EBA-175). Antibodies to these blood-stage antigens have been shown to inhibit parasite replication, and correlate with protection from clinical malaria [77].

Members of the *P. falciparum* erythrocyte membrane protein 1 (*Pf*EMP1) family of proteins are particularly important as they allow binding of infected red blood cells to the vascular endothelium [78]. This sequestration enables parasites to remove themselves from the blood circulation and to avoid spleen-dependent immune mechanisms. Sequestration is associated with the pathogenesis of severe malaria and pregnancy associated malaria [79]. *Pf*EMP1 proteins are encoded by approximately 60 members of the *var* gene family, only one of which is expressed at a time. Sequential switching between *var* genes allows the *P. falciparum* parasite to avoid recognition by the immune system while sequestered [80].

1.4. Malaria control and elimination

The past decade has seen dramatic declines in cases of *P. falciparum* malaria across a range of settings throughout the world [19]. These declines have been associated with increased distribution of long-lasting insecticide-treated nets and a switch from failing drug regimens to artemisinin-based combination therapies as first-line treatment [81, 82]. Although changing trends in the epidemiology of malaria are often attributed to expanded coverage of malaria control interventions [83, 84], often discussed but rarely researched explanations for the decline in malaria are increased economic development and urbanisation [85].

Following a call from the Bill and Melinda Gates Foundation in 2007 there has been a renewed sense of optimism in the malaria community regarding prospects for malaria eradication [86]. The Gates Foundation, endorsed by WHO [87], has called for the global eradication of the malaria parasite by expanding coverage of existing interventions and investing in the development of new interventions such as vaccines. Although the steps taken towards the different targets of malaria control, elimination or eradication are often identical, there are some key differences between these aims [87]:

- **Malaria control** is reducing the disease burden to a level at which it is no longer a public health problem.
- **Malaria elimination** is interrupting local mosquito-borne malaria transmission in a defined geographical area, i.e. zero incidence of locally contracted cases.

- **Malaria eradication** is the permanent reduction to zero of the worldwide incidence of malaria infection caused by a specific agent; i.e. applies to a particular malaria parasite species.

Whether or not eradication is possible in the foreseeable future is a contentious issue but local elimination should be possible in many parts of the world [88].

1.4.1. Malaria control interventions

There is a wide range of existing malaria control interventions that target the parasite and vector at several stages of their respective lifecycles. Anti-malarial drugs can be used to treat clinical episodes of malaria and clear patients of parasitemia by targeting blood-stage parasites. In the past chloroquine and sulphadoxine-pyrimethamine (SP) were widely used as first-line treatment for malaria, however the emergence of drug resistance has rendered these drugs ineffective in many regions [89]. The current first-line treatment in almost all malaria endemic countries is artemisinin combination therapy (ACT) – an artesunate drug combined with a longer lasting partner drug. ACTs have the additional benefit of killing gametocytes, thus reducing onward malaria transmission from humans to mosquitoes [90]. Improvements in treatment have also been accompanied by improved diagnostic techniques, in particular rapid diagnostic tests (RDTs) which allow malaria parasites to be quickly tested for [91]. RDTs can prevent over-diagnosis of malaria leading to cost savings on treatment, improved health outcomes for patients without malaria, and can reduce the risk of emergence of drug resistance.

In addition to treating malaria, anti-malarial drugs can be used as preventive interventions. There are several interventions based on the use of anti-malarial drugs as prevention:

- **Intermittent preventive therapy in infants (IPTi)** protects infants in the vulnerable first months of life by administering several courses of drugs, usually SP [92].
- **Intermittent preventive therapy in pregnant women (IPTp)** protects pregnant women from their second trimester onwards by administering SP – due to safety considerations the only drug for such use [93].
- **Seasonal malaria chemoprophylaxis (SMC)** – previously referred to as IPT in children – protects school age children in regions with high seasonality of transmission by administering drugs throughout the rainy season [94].
- **Mass drug administration (MDA)** involves treatment of an entire population with drugs to reduce parasite rates and malaria transmission [95].
- **Mass screen and treat (MSAT)** involves screening a population for parasites using diagnostic tools such as RDTs and only treating those identified as parasite positive [95].

Arguably, the most effective interventions for preventing malaria are those that target the vector, either by killing the mosquito outright or by preventing contact between mosquitoes and human. The most important vector control interventions are:

- **Insecticide treated bed nets (ITNs)** protect the user by creating a physical barrier around their sleeping space with a net [13]. Treating the net with a pyrethroid insecticide provides additional protection by killing any mosquitoes that contact the net.
- **Long lasting insecticidal nets (LLIN)** are a technological improvement on ITNs. Whereas ITNs need to be regularly re-treated with insecticide, in LLINs the insecticide is impregnated in the fibre of the net. If maintained properly, an LLIN can remain efficacious for 3-5 years [96].
- **Indoor residual spraying (IRS)** involves spraying the inside walls of houses in a community with residual insecticide [97]. Community level protection is conferred by reducing the size of the mosquito population and repelling mosquitoes from sprayed houses. There are several classes of insecticide suitable for IRS including pyrethroids, carbamates and DDT.
- **Larviciding** involves the application of insecticides to larval breeding sites to kill the aquatic stages of the mosquito [98].

There are several other novel vector control tools under development including genetically modified mosquitoes [99], sterile insect techniques [100], attractive toxic sugar bait [101], auto-dissemination of insecticides by adult mosquitoes [102], improved housing structure [44], and insecticide treating of cattle to target outdoor biting mosquitoes [103].

1.5. Malaria vaccines

Perhaps the most important class of novel interventions under development are vaccines. Malaria vaccines can be split into three categories depending on the stage of the *Plasmodium* parasite targeted (Figure 1.7).

- **Pre-erythrocytic vaccines (PEV)** prevent infection with malaria by targeting the pre-erythrocytic stages of the parasite, namely sporozoites inoculated into the skin by blood-feeding mosquitoes and the intra-cellular forms of the parasite in liver hepatocytes.
- **Blood-stage vaccines (BSV)** do not prevent malaria infection but reduce the probability of clinical or severe episodes of malaria by reducing blood-stage parasite densities.
- **Transmission-blocking vaccines (TBV)** aim to prevent transmission of the *Plasmodium* parasite from human to mosquito, either by targeting the sexual stages of the parasite in humans or by interrupting the process of parasite development in mosquitoes who have taken a blood meal from a vaccinated human. TBVs do not confer direct protection to the vaccinated individual, but instead protect the wider community by reducing transmission intensity.

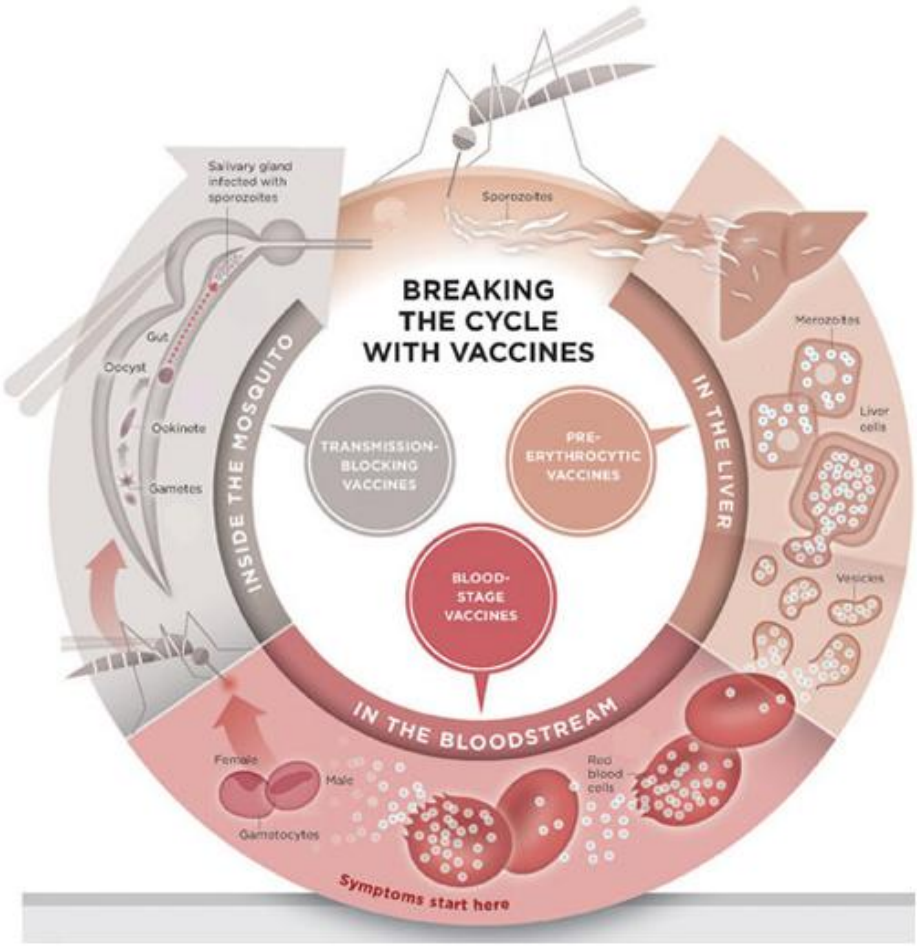

Figure 1.7: Lifecycle of the *P. falciparum* parasite with targets for pre-erythrocytic, blood-stage and transmission-blocking vaccines highlighted. Figure reproduced from PATH Malaria Vaccine Initiative (http://www.malariavaccine.org/malvac-lifecycle.php).

In each of the three categories, there are several candidate vaccines currently undergoing clinical trials. All vaccines currently undergoing clinical trials, and those which have been discontinued are described in detail in the WHO's rainbow tables [104]. The most promising candidates in each of the categories are summarised in Table 1.1.

Table 1.1: A selection of malaria vaccines currently being tested in clinical trials based on data from the WHO's Rainbow Table [104].

Vaccine	Developer	Antigen	Trial phase	Countries tested	Registration	Reference
Pre-erythrocytic vaccines						
RTS,S/AS01E	GlaxoSmithKline	CSP	Phase III	several sub-Saharan countries	NCT00866619	Agnandji [15]
Ad35 vectored CSP+ RTS,S/AS01	GlaxoSmithKline	CSP	Phase I/IIa	Belgium, USA	NCT01366534	Shott [105]
ChAd63/MVA ME-TAP	University of Oxford	TRAP + multiple epitopes	Phase I/IIa	UK, Gambia	NCT00890760	Reyes-Sandoval[106]
PfSPZ	Sanaria, Inc.	Whole sporozoite	Phase I/IIa	USA	NCT01001650	Epstein [16]
Blood-stage vaccines						
FMP2.1/AS02	GlaxoSmithKline	AMA1	Phase II	Mali	NCT00460525	Thera [107]
AMA1-C1/ Alhydrogel	NIAID	AMA1	Phase II	Mali	NCT00341250	Sagara [108]
AdCh63/MVA MSP1	University of Oxford	MSP1	Phase Ia	UK	NCT01003314	Sheehy [109]
Transmission-blocking vaccines						
Pfs25	NIAID	Pfs25	Phase I	USA	NCT00295581	Wu [110]

1.5.1. Clinical trials for testing malaria vaccines

Candidate malaria vaccines must be tested for safety, immunogenicity and efficacy in clinical trials. In addition, clinical trials are used to provide data to regulatory authorities, assess how vaccines can be integrated into existing health systems, and to contribute to research and development into vaccine technology. Clinical trials can also be used to test vaccines on groups of special interest such as young children and pregnant women. A full set of clinical trials for a candidate malaria vaccine can take up to 15 years, involve up to 50,000 volunteers, and cost $500 million or more [111, 112].

The following is a summary of the typical clinical trial phases for a candidate malaria vaccine according to guidelines from PATH Malaria Vaccine Initiative [113].

- **Research and preclinical development** involves identifying relevant antigens, testing in animal models, combining with adjuvants, developing manufacturing processes, and conducting preclinical evaluation.
- **Phase I clinical trials** assess the safety profile of a candidate vaccine and its ability to induce a relevant immune response. Trials usually involve less than 100 volunteers and last for up to a year. For malaria, trials conducted in non-endemic countries on malaria-naïve volunteers are classified as Phase Ia trials, and trials in endemic countries on malaria-exposed volunteers are classified as Phase Ib trials. If the vaccine is found to be safe it can proceed to Phase II trials.
- **Phase II clinical trials** monitor safety and potential side effects, immunogenicity, and produce estimates of efficacy against infection, clinical malaria or severe malaria. Phase II trials can involve up to a few

thousand volunteers and can last for several years. Phase IIa trials are usually undertaken in non-endemic countries on malaria-naïve volunteers who are vaccinated and then artificially challenged with infection via exposure to infectious mosquitoes. Volunteers are monitored for signs of parasites and treated with anti-malarial drugs if necessary. Phase IIb trials are undertaken in malaria-endemic countries under conditions of natural exposure to malaria. If the vaccine demonstrates suitable safety and efficacy, it can proceed to Phase III trials.

- **Phase III clinical trials** monitor safety and potential side effects, and evaluate efficacy on a large scale across a range of transmission settings. Phase III trials can include thousands of volunteers and last for up to five years. If Phase III trials demonstrate safety and sufficient efficacy, the manufacturer can apply for a license to bring the vaccine to market, and submit a plan for long-term, post-licensure safety monitoring.
- **Phase IV clinical trials** involve monitoring the safety and effectiveness of a vaccine post-licensure. They do not involve comparisons against a control group. Additional monitoring ensures that any rare or delayed serious adverse events are identified. Phase IV trials can also evaluate the duration of protection, and effectiveness when the vaccine is delivered through different health systems.

1.5.2. Measuring vaccine efficacy

Malaria is a disease with a complex life history. As such, vaccination can affect infection rates, episodes of clinical and severe malaria, and malaria associated deaths. This diversity of outcomes makes designing field trials for estimating vaccine efficacy against multiple endpoints a challenging problem. Summarised below are the endpoints against which pre-erythrocytic or blood-stage vaccines can be evaluated.

- **Efficacy against infection** estimates the reduction in the force of infection due to vaccination. Only pre-erythrocytic vaccines are expected to reduce the incidence of infection in the absence of community-wide effects. Measuring infection-blocking requires clearing volunteers of parasites with an anti-malarial and following them with active case detection (ACD) for infection by testing for parasites every week or fortnight.
- **Efficacy against clinical malaria** estimates the reduction in the incidence of clinical malaria due to either pre-erythrocytic or blood-stage vaccines. Measuring efficacy against clinical malaria requires monitoring volunteers for episodes of clinical malaria with ACD of malaria episodes or passive case detection (PCD) where symptomatic episodes of malaria are reported by the trial participants. Efficacy against clinical malaria will depend on the definition of an episode of clinical malaria: usually taken to be fever plus parasite density above a given cut-off, e.g. > 2500 parasites/µL. The choice of parasite density cut-off will affect the efficacy estimate: too high a cut-off and sensitivity will be low, too low a cut-off and specificity will be lost as fever due to other causes may be classified as due to malaria [114].
- **Efficacy against severe malaria** measures the vaccine-induced reduction in the incidence of severe malaria. Efficacy estimates will depend on the clinical definition of an episode of severe malaria, usually

defined according to WHO guidelines [37]. Clinical trials for estimating efficacy against severe malaria need to be large to ensure sufficient statistical power to detect an effect as the incidence of severe malaria can often be low.

- **Efficacy against malaria-associated death** estimates the reduction in deaths attributable to malaria due to vaccination. As deaths due to malaria can often be quite rare, (particularly in clinical trials where access to good quality healthcare is high) most clinical trials are underpowered to evaluate vaccine efficacy against death. In addition to efficacy against malaria-attributable deaths, the efficacy against all cause mortality can be estimated [115].

Due to the epidemiology of malaria transmission, almost all volunteers in trials in endemic settings are likely to develop infection and many will experience multiple episodes of clinical malaria. For malaria, vaccine efficacy can thus be measured either against the first episode of malaria, or against multiple episodes. Furthermore, calculation of vaccine efficacy can be either risk-based or rate-based [116]. Risk-based efficacy is based on the difference in malaria associated events between the vaccine and control groups a fixed time after vaccination. It can be defined as

$$\text{risk-based efficacy} = 1 - \frac{\text{events in vaccine group}}{\text{events in control group}}$$

If the force of infection is high such that the majority of volunteers experience an event, then risk-based measures of efficacy can be uninformative. Furthermore, risk-based measurements do not properly account for delayed times to event due to vaccination. An alternative is to use rate-based measures of efficacy which account for person years at risk (PYAR): the time between vaccination and first event or censoring at the end of the trial. Rate-based efficacy can be defined as

$$\text{rate-based efficacy} = 1 - \frac{\text{events in vaccine group}/\text{PYAR}}{\text{events in control group}/\text{PYAR}}$$

Estimates of rate-based vaccine efficacy based on time to first event can be calculated from clinical trial data using Cox Proportional Hazards (Cox PH) analysis which allow estimates to be adjusted for covariates such as LLIN use, gender, trial site and age [117]. Vaccine efficacy against multiple events can be calculated using regression models adjusted for covariates. Poisson regression models have been used for analysis of data from Phase II clinical trials of RTS,S [118], but there has been a switch to negative binomial regression models for the analysis of Phase III trial data to capture the over-dispersion in the incidence of malaria [117].

Malaria vaccines are not characterised by their efficacy alone, the variation in efficacy between individuals and the duration of efficacy must also be considered. The variation in efficacy of a partially efficacious vaccine can be described by two simple models: leaky and all-or-nothing [119]. A leaky vaccine with 50% efficacy will provide 50% protection to all vaccinees, whereas an all-or-nothing vaccine with 50% efficacy will provide 100% protection

to 50% of vaccinees and no protection to the other 50%. In reality the protection profile of a vaccine is likely to be heterogeneous, providing a different level of protection to all vaccinees [120].

The efficacy induced by existing vaccines to several pathogens has been observed to wane over time [121], with malaria vaccines likely to be no different. Two models for the waning of vaccine efficacy have been proposed: the selection and deterioration models [122]. In the selection model, waning arises from heterogeneity in the duration of protection, i.e. a vaccinated person is protected for a period of time dependent on the vaccine half-life and then loses all protection. In the deterioration model, waning arises from the gradual decline in an individual's efficacy. The rate of waning of vaccine efficacy under both models can be estimated from extended follow up of clinical trials by fitting an interaction between time and vaccination status in Cox regression models.

1.5.3. The RTS,S malaria vaccine

During the development of the hepatitis B vaccine, it was reported that viral surface proteins (HBsAg, also known as S antigen) could be made to spontaneously assemble into viral particles devoid of DNA [123]. Scientists working at GlaxoSmithKline (GSK) in collaboration with the Walter Reed Army Institute of Research (WRAIR) were able to fuse the Asparagine-Alanine-Asparagine-Proline (NANP) repeat of the *P. falciparum* CSP antigen to the N-terminus of HbsAg, and produce virus like particles in yeast cells (*Saccharomyces cerevisiae*) [124]. High anti-CSP antibody titres were induced in mice immunized with these particles. In order to induce a cell-mediated immune response, a second generation particle expressing the NANP repeats of the CSP protein plus T cell epitopes from the C-terminal of the CSP protein was created. To stabilise these viral particles, they were co-expressed in yeast cells with wild-type HBsAg antigen. The result was RTS,S comprised of 25% RTS (B cell repeats (R) + T cell epitopes (T) + HBsAg (S) antigen) and 75% wild-type HBsAg (S) antigen [125].

Modern vaccine formulations are considered very pure, presenting only a small number of carefully selected antigens to the immune system, in contrast to older vaccines such as BCG for immunisation against tuberculosis which expose the vaccinee to thousands of antigens and cause the immune system to react aggressively. When a vaccine is administered with an adjuvant, the adjuvant safely triggers danger signals that mobilise a strong immune response to the vaccine antigens [126]. GSK tested a number of different adjuvant systems (AS) with RTS,S, the most successful formulations being AS02 and AS01. AS02 is an adjuvant system that contains an oil-in-water emulsion and the immunostimulants monophosphoryl lipid A (MPL) and QS21 [127]. AS01 is a related liposome-based adjuvant system that also contains MPL and QS21.

RTS,S has been tested extensively in both laboratory and field trials [128]. In laboratory studies conducted at WRAIR, malaria-naïve volunteers were vaccinated with RTS,S and then challenged with the bites of five *P. falciparum* infectious mosquitoes [129]. The resulting efficacy against infection was 32% (95% CI, 18% - 48%) and 50% (95% CI, 33% - 67%) for RTS,S/AS02 and RTS,S/AS01, respectively. RTS,S has undergone extensive testing in Phase II field trials at multiple sites and in varying age groups (Table 1.2). Across these trials, the efficacy against

clinical malaria has been estimated to be approximately 30% and 50% for RTS,S/AS02 and RTS,S/AS01, respectively. However, there is significant heterogeneity in vaccine efficacy due to differences in age, transmission intensity and choice of adjuvant (P. Bejon, M. White *et al* – in preparation). In addition efficacy is likely to wane over time, although individual Phase II trials are underpowered to estimate rates of waning.

Phase III clinical trials of RTS,S/AS01 began in 2009 in children from 11 trial sites in 7 sub-Saharan countries. In total, 15,460 children were enrolled in two age categories, 6 to 12 weeks of age and 5 to 17 months of age [15]. Children were randomly assigned to three study groups in a 1:1:1 ratio, with one group receiving a control vaccine, a second receiving 3 doses of RTS,S/AS01 at 1-month intervals, and a third receiving RTS,S/AS01 plus a booster dose 18 months after the third dose. First results from a cohort of 6,000 children in the 5 to 17 month category indicate an efficacy of 55.8% (97.5% CI, 50.6% - 60.4%) against clinical malaria (Figure 1.8), and 47.3% (95% CI, 22.4% - 64.2%) against severe malaria. In the combined age categories, followed for an average duration of 16 months after the third dose of vaccine, the efficacy against severe malaria was 34.8% (95% CI, 16.2% - 49.2%). Data from Phase III trials so far has not allowed a statistically significant estimate of efficacy against malaria-attributable deaths.

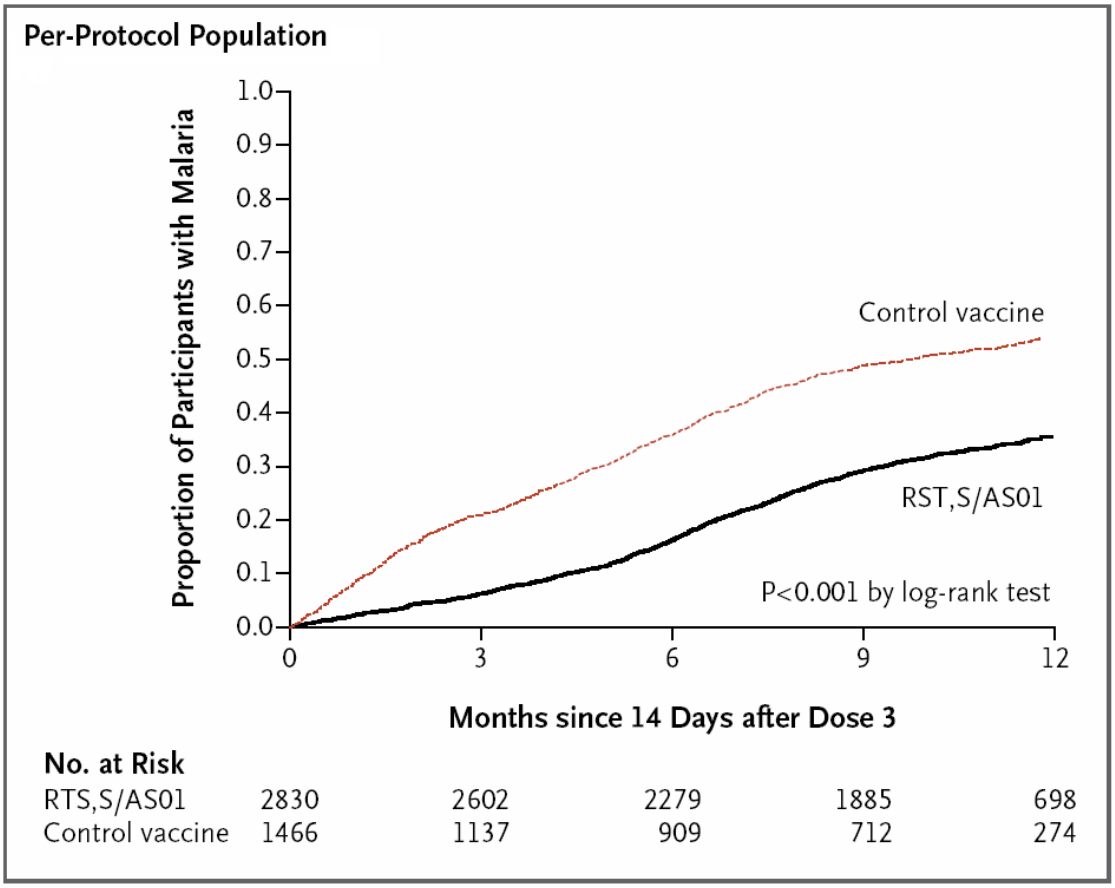

Figure 1.8: Cumulative incidence of first or only episode of clinical malaria in a cohort of children vaccinated with RTS,S/AS01 and a control cohort. Children are from the according-to-protocol cohort and are followed for 12 months. Figure reproduced from Agnandji *et al* [15].

1.5.4. Vaccination campaigns

Should RTS,S/AS01 demonstrate sufficient safety and efficacy upon completion of Phase III clinical trials in 2014, it will be submitted to regulatory authorities for approval to market. Pending regulatory approval, RTS,S/AS01 may be available as a tool for controlling malaria from 2015. At present there are three notable mechanisms through which RTS,S/AS01 could potentially be administered:

- **Expanded Program on Immunization (EPI).** The EPI is a WHO program with the aim of administering a standardized schedule of vaccines to children throughout the world. When administered through the EPI, RTS,S/AS01 has been demonstrated to be safe, efficacious, and not cause interference with other vaccines in the EPI schedule [130].
- **EPI plus booster dose.** RTS,S/AS01 could be administered through EPI campaigns with an additional booster dose 18 months later. A potential drawback of campaigns with booster doses is that in most sub-Saharan African countries, there is no existing framework such as the EPI for providing booster doses.
- **Mass vaccination campaigns** involve vaccinating as many people as possible in a community with the aim of reducing incidence of malaria and potentially causing a reduction in transmission. At present, RTS,S has not undergone the necessary clinical trials required for regulatory approval in adults. In order for mass vaccination campaigns to become a reality, further Phase III clinical trials in adults would be required, involving thousands of volunteers and lasting 3 – 5 years. In addition, special trials for at risk groups such as pregnant women and HIV positive individuals would be required.

Table 1.2: Results of RTS,S vaccine efficacy from Phase II field trials. Updated from Casares *et al* [128]. Efficacies are for according-to-protocol analysis and are adjusted by confounding factors where necessary, e.g. bed net, sickle cell trait, area, distance from health centre. Efficacies are based on the total person-years-at-risk.

Trial site	Age	Adjuvant	Immunization time (dose of RTS,S/volume of adjuvant)	Surveillance period post immunization	Adjusted vaccine efficacy from according-to-protocol analysis				Reference
					Infection (subject/total subjects)	First clinical episode (subjects/total subjects)	All clinical episodes (episodes/total subjects)	Severe malaria (subjects/total subjects)	
Gambia	18–45 years	AS02	0/1/5 months (50 µg/0.5 mL)	0–15 weeks	34% (81/131 vs. 80/119)		31% (44/131 vs. 50/119)		Bojang [131]
				0–9 weeks	71%				
				9–15 weeks	0%				
			Booster dose at 19 months	0–9 weeks	47% (19/73 vs. 29/86)				
Kenya	18–35 years	AS02A	0/1/2 months (50 µg/0.5 mL)	0–14 weeks	35% (28/79 vs. 37/75)				Polhemus [132]
		AS01B		0–14 weeks	11% (28/74 vs. 37/75)				
Mozambique (Manhica)	1–4 years	AS02A	0/1/2 months (25 µg/0.5 mL)	0–6 months		29.9% (123/745 vs. 159/745)	27.4% (153/745 vs. 190/745)	57/7% (11/745 vs. 26/745)	Alonso [133]
Cohort 1				6–18.5 months		28.9% (110/723 vs. 140/719	28.8% (157/723 vs. 193/719)	38.8% (8/723 vs. 13/719)	Alonso [134]
				0–18.5 months		35.3% (186/745 vs. 251/745)	29.8% (310/745 vs. 384/745)	48.6% (19/745 vs. 37/745)	
				0–43 months		30.5% (307/745 vs. 370/745)	25.6% (658/745 vs. 774/745)	38.3% (29/745 vs. 47/745)	Sacarlal [135]
Mozambique (Ilha Josina) Cohort2	1–4 years			0–4 months	51.1% (111/189 vs. 147/178)	61% (21/189 vs. 40/178)			Alonso [134]
				0–6 months	45% (157/189 vs. 166/178)	35.4% (46/189 vs. 56/178)	30% (52/189 vs. 68/178)		
				8.5–18.5 months		9% (60/181 vs. 59/171)			Guinovart [136]
Mozambique (Ilha Josina & Taninga)	8–10 weeks	AS02D	10/14/18 weeks of age (25 µg/0.5 mL)	0–3 months	65.9% (22/93 vs. 46/92)	65.8% (9/93 vs. 22/92)		NS (6/209 vs. 6/208)	Aponte [137]
Tanzania	8–10 weeks	AS02D	8/12/16 weeks of age (25 µg/0.5 mL)	0–6 months	65.2% (8/146 vs. 20/151)	43.2% (7/146 vs. 11/151)			Abdulla [138]

Table 1.2 (contd.): Results of RTS,S vaccine efficacy from Phase II field trials.

Trial site	Age	Adjuvant	Immunization time (dose of RTS,S/volume of adjuvant)	Surveillance period post immunization	Adjusted vaccine efficacy from according-to-protocol analysis				Reference
					Infection (subject/total subjects)	First clinical episode (subjects/total subjects)	All clinical episodes (episodes/total subjects)	Severe malaria (subjects/total subjects)	
Kenya & Tanzania	5–17 months	AS01E	0/1/2 months (50 µg/0.5 mL)	0–8 months		53% (32/402 vs. 66/407)	56% (38/402 vs. 86/407)		Bejon [118]
				0–12 months		39% (82/415 vs. 125/420)	42% (123/415 vs. 209/420)		Olotu [139]
				0–15 months		46% (58/209 vs. 85/206)	51% (108/209 vs. 175/206)		
Ghana, Tanzania & Gabon	6–10 weeks	AS01E	0/1/2 months (25 µg/0.5 mL)	0–6 months (after dose 3)		67% (9/159 vs. 23/155)			Asante [130]
				0–12 months (after dose 3)		62% (21/159 vs. 46/156)			
			0/1/7 months (25 µg/0.5 mL)	0–6 months (after dose 2)		15% (15/154 vs. 17/159)			
				0–12 months (after dose 3)		64% (23/154 vs. 48/153)			
			0/1/2 & 0/1/7 months	0–17 months (after dose 2 & 3)		53% (30/159 vs. 52/156)	59% (43/159 vs. 102/156)		

1.6. Mathematical models of malaria transmission

Mathematical models can provide insight into the dynamics of malaria transmission, inform the use of malaria-control interventions, and aid in the evaluation of malaria control programs [140]. Models are particularly useful for extrapolating findings from one transmission setting to other settings. For example, the results of a trial of LLINs in one setting can be used to estimate the impact of LLINs in other settings [141]. Models are becoming increasingly useful tools for informing policy on malaria control. For example, mathematical models formed a key part of the body of evidence to support the wide-scale use of intermittent preventive therapy in children in regions of seasonal malaria transmission [142].

The first model of malaria transmission was developed by Ronald Ross during a trip to control malaria in Mauritius in 1907 [143]. Ross' model was further developed by George MacDonald during the Global Malaria Elimination Program and used to guide malaria control interventions [144]. The resulting Ross-MacDonald model provided key analytical insights into why spraying with DDT was such a potent malaria control strategy by highlighting the non-linear relationship between increasing mosquito death rates and decreasing sporozoite positivity rates.

In the 1970's the WHO sponsored a project in the Garki district of Nigeria to investigate if malaria could be eliminated from a region of intense transmission using effective treatment with chloroquine and spraying with DDT [145]. Mathematical models had a key role in the design and analysis of the Garki project. Dietz, Molineaux and Thomas constructed a model of malaria transmission incorporating human immunity and the effect of malaria control interventions, and validated it against data from the project [146]. As well as providing important insights into the successes and failures of the project, the model identified key indices of malaria transmission, including the vectorial capacity and the human blood index.

The foundations laid by the classical Ross-MacDonald and Garki models have been built upon by contemporary modelling groups, aided by increased computing power and more sophisticated statistical methods for validating models against data. In addition to methodological improvements, modern malaria transmission models incorporate improved scientific understanding of both human immunity and mosquito behaviour. Mathematical models have been developed to investigate many aspects of the epidemiology of malaria transmission and the impact of malaria control interventions (reviewed in detail by Mandal *et al* [147]), some notable efforts being those by modelling groups at Imperial College London [63], the Swiss Tropical and Public Health Institute [64, 148], Dave Smith and colleagues [149, 150], and Intellectual Ventures [151].

1.6.1. The Ross-MacDonald model

The Ross-MacDonald captures the key parts of the malaria transmission cycle, and can be used to predict basic malariological quantities such as the entomological inoculation rate (EIR), the parasite rate (*Pf*PR) and the basic reproductive number (R_0). It is defined by the following set of ordinary differential equations.

$$\frac{dS_H}{dt} = -mabI_M S_H + rI_H$$
$$\frac{dI_H}{dt} = mabI_M S_H - rI_H$$
$$\frac{dS_M}{dt} = \beta - acI_H S_M - \mu S_M$$
$$\frac{dE_M}{dt} = acI_H S_M - acI_H(t-\tau)S_M(t-\tau) - \mu E_M$$
$$\frac{dI_M}{dt} = acI_H(t-\tau)S_M(t-\tau) - \mu I_M$$

A flow diagram of the model and explanation of the parameters is shown in Figure 1.9. An expression for the basic reproductive number can be derived analytically for the Ross-MacDonald model by calculating the dominant eigenvalue of the next generation matrix, or more heuristically as described in Table 1.3. R_0 is a key quantity for malaria transmission – if control measures can reduce transmission such that $R_0 < 1$ then malaria will no longer be able to persist. R_0 has been estimated in several African populations, resulting in estimates varying by an order of magnitude from around one to more than 3,000 [40]. A key insight derived from the Ross-MacDonald model, is the exponential dependence of R_0 on the mosquito death rate μ_M, as a mosquito taking an infectious blood meal must survive the τ days of duration of sporogony in order to become infectious to humans. During each of these τ days, the mosquito is liable to die at rate μ_M. This sensitive dependence of malaria transmission on mosquito death rate highlights the importance of vector control tools such as LLINs and IRS which kill adult mosquitoes.

The Ross-Macdonald model has been extended to consider age structure [152, 153], acquired immunity [154, 155], spatial heterogeneity [156, 157], individual-based models [158], strain theory [159] and other phenomena.

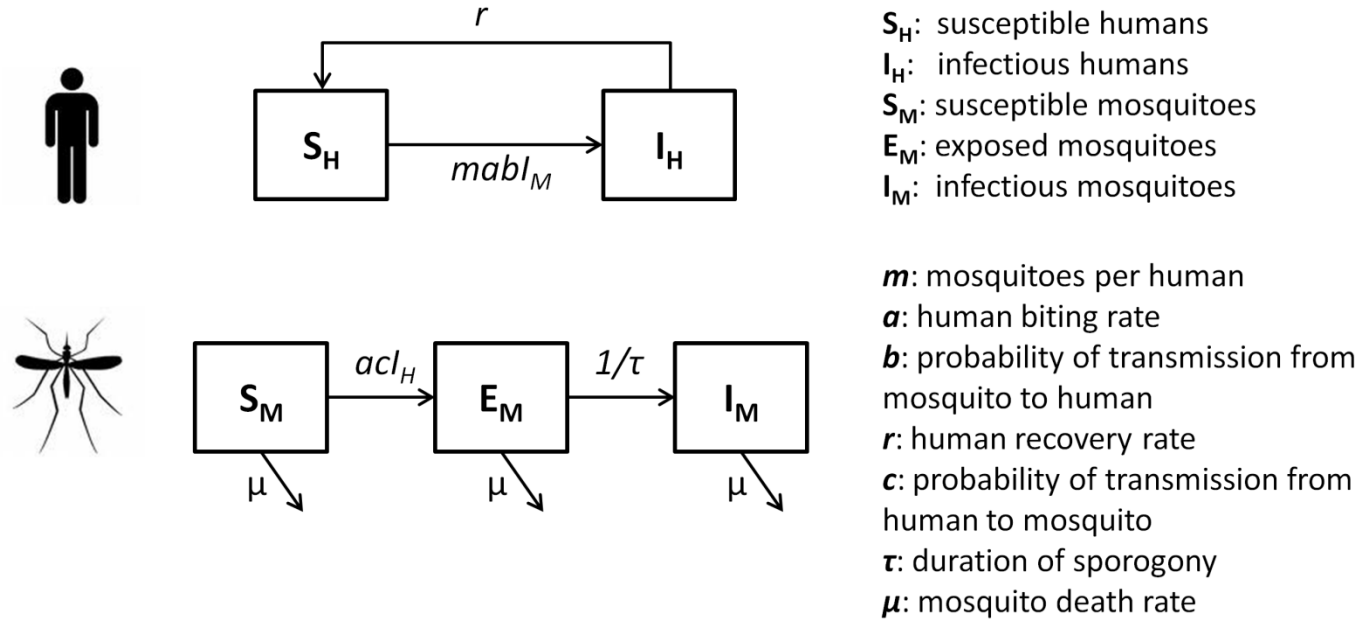

Figure 1.9: Flow diagram and parameters of the Ross-MacDonald model.

Table 1.3: Derivation of R_0 for the Ross-MacDonald model.

	Probability infections become infectious	Contacts per day	Duration of infectivity	R_0 contribution
human to mosquito	1	mac	$1/r$	mac/r
mosquito to human	$e^{-\mu\tau}$	ab	$1/\mu$	$abe^{-\mu\tau}/\mu$
total				$R_0 = \frac{ma^2bce^{-\mu\tau}}{r\mu}$

1.6.2. The Imperial College malaria transmission model

An individual-based simulation model for *Plasmodium falciparum* transmission for estimating the impact of packages of malaria control interventions [63] has been developed by the malaria modelling group at Imperial College London and collaborators at the London School of Hygiene and Tropical Medicine. The model has been extensively fitted to parasitological and clinical data from a range of transmission settings. A flow diagram of the model is shown in Figure 1.10 and full details are given in Griffin *et al* [63]. Briefly, individuals begin life susceptible (*S*) to infection but with partial maternal immunity which decays in the first months of life. Individuals become infected at a rate determined by the force of infection (Λ) which depends on the number of mosquitoes per person, the proportion of infectious mosquitoes, and the person's level of anti-infection immunity. Once infected, a person passes through the pre-patent (liver) stage of infection, and then progresses to develop clinical disease (*D*) or asymptomatic (*A*) infection, depending on their level of clinical immunity ϕ. A proportion f_T of those with clinical disease will be treated (*T*). Untreated individuals with clinical disease recover to the asymptomatic stage at a rate r_D, and treated individuals recover at a rate r_T after which they are afforded a period of prophylactic protection (*P*) with average duration $1/r_P$. Asymptomatic individuals (patent infection – detectable by microscopy) will move to a sub-patent stage of infection (*U*) at a rate r_A, from which they eventually return to susceptible at a rate r_U. Malaria can be transmitted from human to human by three different vector species: *An. gambiae* s. s., *An. arabiensis* and *An. funestus*.

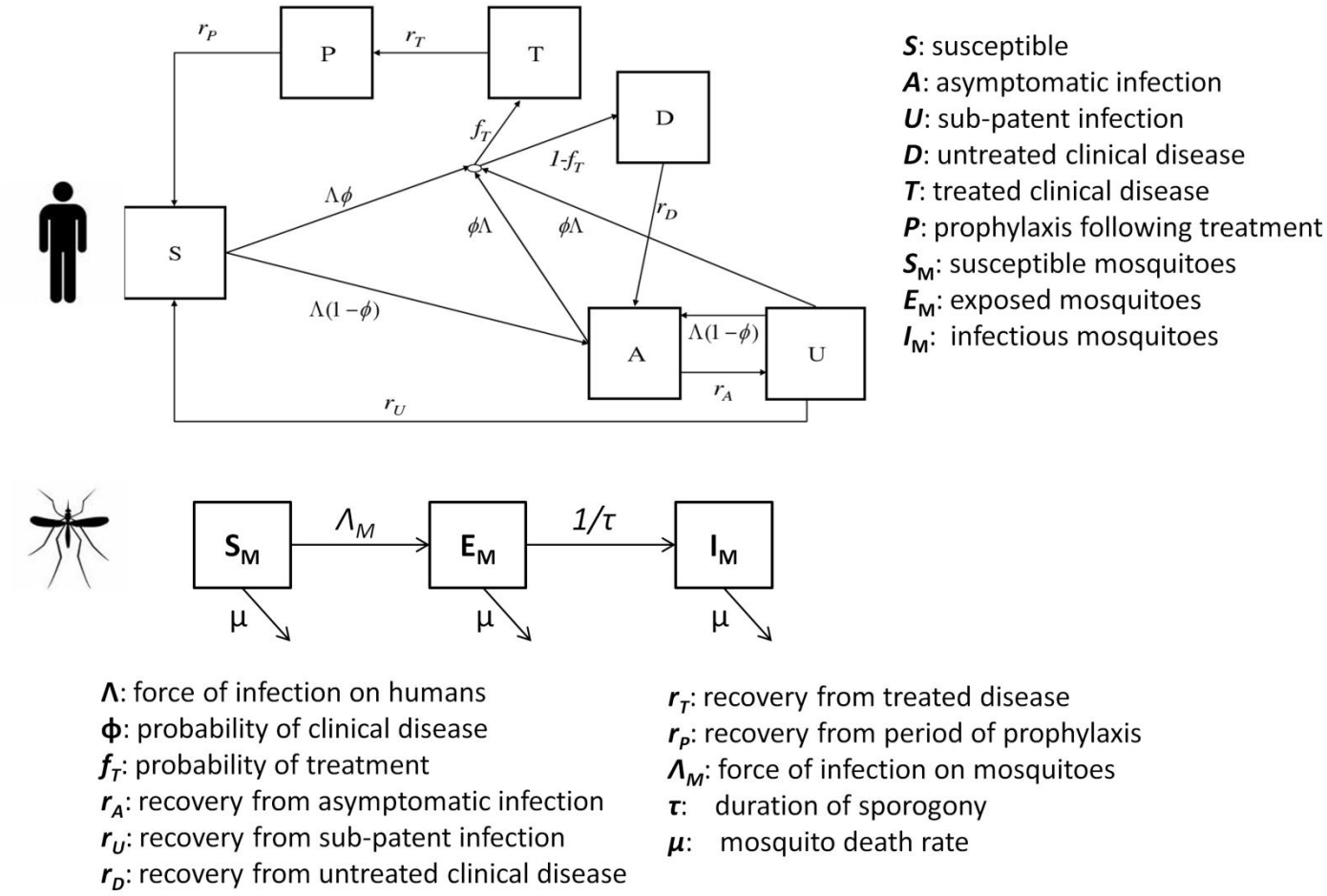

Figure 1.10: Flow diagram and parameters for the Imperial College malaria transmission model. Full details are available in Griffin *et al* [63].

1.6.3. The Swiss TPH Model

A group at the Swiss Tropical and Public Health Institute under the guidance of Prof. Tom Smith have developed a detailed micro-simulation model of the clinical epidemiology of malaria transmission. The model is described in detail in a supplement to *The American Journal of Tropical Medicine and Hygiene* [64] and summarised in Figure 1.11. It consists of multiple component modules for different aspects of the life history of malaria, each of which has been validated against epidemiological data. There are separate models for the relationship between EIR and the force of infection [160], acquisition of blood-stage immunity [161], infectiousness of humans to mosquitoes [162, 163], within-host dynamics of asexual *P. falciparum* parasites [164], incidence of clinical and severe malaria [165, 166], neonatal mortality [167], malaria-associated anemia [168], pre-erythrocytic vaccines [120, 169], and the costs of treatment [170]. The complexity of the model necessitates computationally expensive simulation distributed across volunteer computers.

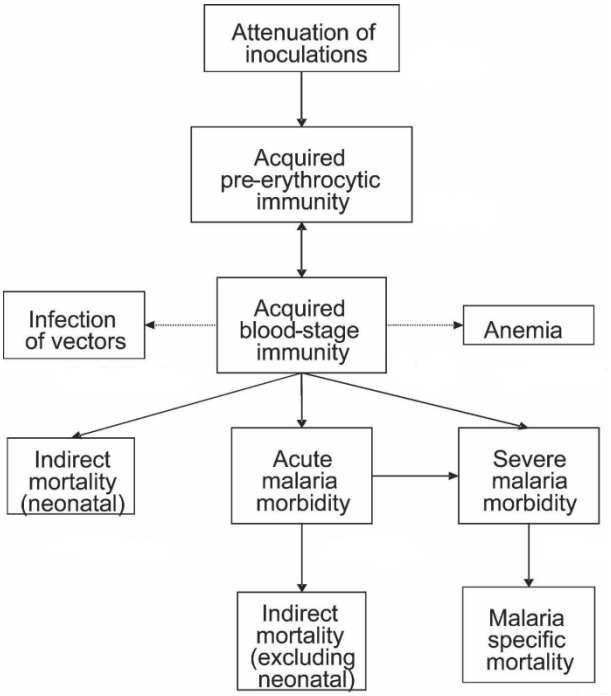

Figure 1.11: Key processes and relationships simulated by the Swiss TPH models of *Plasmodium falciparum* transmission and morbidity. Figure reproduced from Smith *et al* [64].

Although the Swiss TPH model includes components for multiple aspects of the life history of malaria, its main focus is on the evaluation of pre-erythrocytic vaccines on the clinical epidemiology of *P. falciparum*. One of the key results was the prediction that vaccines with efficacy similar to that of RTS,S/AS02 administered through the Expanded Program on Immunization would have a substantial impact on malaria morbidity and mortality, but have negligible effects on levels of malaria transmission typical of sub-Saharan Africa. Follow up publications from the Swiss TPH have further validated these findings [171], and extended model predictions to blood-stage and transmission-blocking vaccines [172].

1.6.4. Models of the impact of malaria vaccination on transmission

In the past, mathematical models have been used to explore the theoretical relationship between malaria transmission and pre-erythrocytic, blood-stage or transmission-blocking vaccines [173-176]. Following the progress of RTS,S through clinical trials, models are now being used as predictive tools as opposed to tools for purely theoretical investigations. A number of models have incorporated pre-erythrocytic vaccination with parameters chosen to reflect the properties of RTS,S [63, 177]. Following on from the Swiss TPH's findings, there has been a consensus across different models that although RTS,S/AS01 or RTS,S/AS02 administered through the EPI will have considerable public health benefits for vaccinated children, there is unlikely to be a substantial impact on malaria transmission. Similarly, it is predicted that mass vaccination with RTS,S/AS01 is unlikely to cause substantial reductions in malaria transmission, except in regions of low transmission [171]. In addition to estimating the impact

of vaccination campaigns, models can be used to investigate the interaction between vaccines and other malaria control interventions [63], and the likely cost-effectiveness of vaccination campaigns [178, 179].

1.6.5. Within-host models of malaria infection

There is an extensive literature on within-host models of malaria in both mice and humans, predominantly of the erythrocytic stage of malaria infection [180], with some models also investigating the sexual stages [181]. The first published model of the within-host dynamics of *P. falciparum* parasites was described by Anderson *et al* [176] who used a system of differential equations to model blood-stage infection as a population of merozoites infecting and replicating within a population of red blood cells. Anderson *et al* demonstrate that a blood-stage immune response that increases in proportion to parasite density can predict non-linear parasite dynamics similar to those observed in natural infections [182]. This approach of modelling blood-stage malaria using continuous differential equations has been extended to investigate gametocyte densities [183], antigenic variation [184] and the effects of anti-malarial drugs [185].

A key characteristic of blood-stage *P. falciparum* dynamics is the discrete 2 day replication cycle. Continuous differential equation models can recreate this periodicity, but at considerable computational expense [186]. An elegant solution to capture this periodicity has been to use difference equations with a 2 day time step, first utilised by Kwiatkowski and Nowak [187]. The most sophisticated and biologically realistic difference equation model is that developed by Dietz and Molineaux [188] which includes intra-clonal antigenic variation based on differential expression of *Pf*EMP1 antigens by parasitized red blood cells, between individual variation in parasite replication rates, innate auto-regulation of asexual parasite density, acquired variant-transcending immunity, and acquired variant-specific immunity. The model was validated against data from patients artificially infected with *P. falciparum* malaria for extended periods of time as part of malaria therapy to cure neurosyphilis [182]. A version of Dietz and Molineaux's discrete time model has been used to consider the effect of a hypothetical malaria vaccine that induces blood-stage antibodies which inhibit asexual parasite replication [164].

Although several within-host models have been developed for the erythrocytic stage of the malaria parasite's lifecycle, no published models have considered the pre-erythrocytic stage – the key stage that must be understood in the development of vaccines to prevent infection with *P. falciparum*.

1.7. Thesis aims

The aim of this thesis is to use mathematical models to provide insight into a number of pressing problems in the field of malaria vaccine development, ranging from the interpretation of results from clinical trials currently underway in Africa, to the relationship between antibody titres and protection from infection, and the effectiveness and cost-effectiveness of vaccination campaigns. Specific objectives in each chapter are:

- **Chapter 2:** I consider the effect of heterogeneity in malaria exposure and vaccine response on the results of vaccine efficacy trials.
- **Chapter 3:** I review the role of naturally acquired pre-erythrocytic immune responses in protection from infection based on sources from the published literature.
- **Chapter 4:** I explore the relationship between naturally acquired antibody titres and protection from infection using dose-response curves, and estimate the potential efficacy of multi-component pre-erythrocytic vaccines based on data on naturally acquired immune responses.
- **Chapter 5:** I estimate the relationship between anti-CSP antibody titres, numbers of CSP-specific $CD4^+$ T cells and protection from infection in malaria-naïve volunteers vaccinated with either RTS,S/AS02 or RTS,S/AS01.
- **Chapter 6:** I develop simple models of antibody kinetics and the maintenance of immunological memory, and consider the implications for duration of vaccine efficacy.
- **Chapter 7:** I estimate the effectiveness and cost-effectiveness of vaccination campaigns across a range of transmission settings, and in combination with insecticide treated nets.

Throughout this thesis I aim to demonstrate that mathematical models incorporating an understanding of the dynamics of malaria transmission provide an ideal framework for synthesising knowledge and data from across different disciplines engaged in research into malaria vaccines. Thus, for example, insight gained from immunological studies can be incorporated in models for investigating epidemiological problems, which can in turn be used to address public health questions.

Chapter 2. Heterogeneity in malaria exposure and vaccine response: implications for the interpretation of vaccine efficacy trials

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Randomised clinical trials carried out under conditions of natural exposure to malaria remain the most important tool for assessing the safety, immunogenicity and efficacy of candidate malaria vaccines. With the majority of the evidence required for vaccine licensure coming from such clinical field trials, it is vital that we do not misinterpret their results. In this chapter, I discuss how heterogeneities in malaria exposure, vaccine response and waning of efficacy need to be considered when analysing data from clinical trials.

2.1. Introduction

There are many sources of bias that may arise when measuring an infection-blocking malaria vaccine's efficacy in clinical trials. A significant source of bias that is often overlooked is due to the effects of heterogeneity. Heterogeneity can arise in one of three ways. Firstly, heterogeneity in exposure occurs due to some individuals being bitten more frequently than others. Between-individual variation has been observed in experimental trials in which differential attractiveness to humans sleeping in tents was quantified using mosquito traps [45]. This heterogeneity in biting rate has also been attributed to age due to differences in surface area between adults and children [189], differences in human sweat components [190], proximity to larval breeding sites [191], bed net usage [192] and differences in house design [44].

Secondly, there can be heterogeneity in vaccine response, which occurs due to variation in the degree of protection (or take) of the vaccine between individuals. For example if a vaccine is reported to have 90% efficacy against infection, at one extreme this could mean that each infection will be blocked 90% of the time, whilst at the other extreme it could mean that 90% of vaccinated people will be completely protected from infection. Smith *et al* [116] and Halloran *et al* [119] have investigated this concept and coined the terms all-or-nothing and leaky vaccines. An all-or-nothing vaccine is one which offers complete protection to a subset of the vaccinated population and has no effect on the rest of the population whilst a leaky vaccine is one which offers the same level of partial protection to everyone. In a malaria vaccine trial it is possible that the candidate vaccine will induce a different level of protection in each of the trial participants. The level of protection given to a vaccinated person will be described by their vaccine response which will depend on the relative immunogenicity of the vaccine and the magnitude of the induced antibody titres. Individual vaccine response may further depend on genetic and nutritional factors [193] as well as age, past exposure and season [194].

Finally, for a candidate malaria vaccine offering partial protection from infection, waning of efficacy is also likely to be observed. Understanding how a vaccine's efficacy wanes over time is at least as important as measuring its initial efficacy. Waning of efficacy can be estimated from clinical trials by measuring a vaccine's half-life: the time taken for the initial efficacy to be halved. However, a vaccine's half-life is a crude measure of waning, as the effects of a vaccine may not decay at a constant rate, and thus some people may remain protected for longer than others. This type of heterogeneity is often neglected in the interpretation of trial results.

Mathematical models have been used to investigate the impact of heterogeneity in exposure [149, 156, 172], heterogeneity in vaccine response [120, 172] and waning of efficacy [172] on malaria transmission. In this chapter theoretical arguments are used to understand how these three types of heterogeneity can affect measurement of vaccine efficacy in clinical trials. Examples are motivated by the RTS,S vaccine and hence focus on a pre-erythrocytic vaccine which is assumed to act by partially blocking infection. In the first section the basic methods currently used to estimate vaccine efficacy in Phase II and Phase III trials are described. The effects of the three types of heterogeneity on the estimates of vaccine efficacy from clinical trials are then investigated.

2.2. Measurements of vaccine efficacy

For a vaccine with efficacy VE, if the probability of an unvaccinated person becoming infected after an infectious bite is b (also referred to as transmission efficiency [195]) then the probability that a vaccinated person becomes infected is $(1-VE)b$. This efficacy VE, hereafter referred to as the individual efficacy, can be defined as the proportion of infectious bites on a vaccinated population that are blocked by the vaccine. This proportion can be directly estimated in laboratory based Phase IIa trials where healthy adult volunteers are exposed to bites from infectious mosquitoes and then monitored for blood-stage malaria infection [196, 197].

In field-based Phase IIb clinical trials in malaria endemic regions, it is impossible to directly estimate this proportion. Instead vaccine efficacy must be estimated by measuring malaria-related events in both the vaccine and control arms of the trial. Vaccine efficacy can then be calculated as

$$\text{efficacy}=1-\frac{\text{events with vaccine}}{\text{events without vaccine}}$$

Malaria infection has a complex life history giving rise to a choice of events to measure [198]. These include prevalence of infection as detected by the presence of parasitaemia, time to first infection, episodes of febrile malaria, episodes of severe malaria or multiple episodes in fixed time periods. Each of these end-points will be relevant in different settings. For example, infection-blocking efficacy would be most important to a traveller to a malaria endemic region, whereas efficacy against severe disease would be most important for a child living in an area of high transmission. Vaccine efficacy against time to first infection and prevalence of infection are focused

upon as these are the outcomes directly affected by pre-erythrocytic vaccines, although they can also be expected to have a downstream effect on morbidity and mortality.

Following the notation of Smith *et al* [116], the vaccine efficacy against cumulative incidence of infection, also known as risk-based infection-blocking efficacy, is denoted VE_r. This gives a measure of the risk of having become infected by the end of a trial of duration T for the vaccine group compared to the control group. Note that the cumulative incidence of infection is different to the prevalence of infection as it measures the proportion who have ever become infected and not the proportion infected at a given time. Vaccine efficacy against time to first infection, also known as rate-based infection-blocking efficacy, will be denoted VE_f. This gives a measure of the rate at which the vaccine group becomes infected compared to the control group.

In a trial of a vaccine with individual efficacy VE, of length T, in a setting with constant force of infection Λ, estimates for the proportion infected and the person years at risk (PYAR) can be calculated using the formulae in Table 2.1.

Table 2.1: Expected values for the observed infected proportion *I*(*T*), and expected person years at risk (PYAR) in a clinical trial with follow-up period *T*, of a vaccine with individual efficacy *VE*, in a region with force of infection Λ.

Group	Proportion infected $I(T)$	Person years at risk (PYAR)
Control	$1-e^{-\Lambda T}$	$\frac{1-e^{-\Lambda T}}{\Lambda}$
Vaccine	$1-e^{-(1-VE)\Lambda T}$	$\frac{1-e^{-(1-VE)\Lambda T}}{(1-VE)\Lambda}$

The risk-based efficacy can be calculated as

$$VE_r = 1-\frac{\text{proportion infected with vaccine}}{\text{proportion infected without vaccine}}$$
$$= 1-\frac{1-e^{-(1-VE)\Lambda T}}{1-e^{-\Lambda T}} = \frac{e^{VE\Lambda T}-1}{e^{\Lambda T}-1}$$

Thus the risk-based efficacy, VE_r, as measured in a field trial can be estimated from the individual efficacy. This measure approaches 0 as T increases as it is assumed that everyone will become infected if the follow-up is sufficiently long. In addition, for small T we have $VE_r \approx VE$.

In a field trial the rate at which a cohort becomes infected can be estimated by dividing the infected proportion at the end of the follow-up period by the total person years at risk. The rate-based efficacy can then be calculated as

$$VE_f = 1 - \frac{\text{infection rate with vaccine}}{\text{infection rate without vaccine}}$$
$$= 1 - \frac{1 - e^{-(1-VE)\Lambda T} \Big/ \left(\frac{1 - e^{-(1-VE)\Lambda T}}{(1-VE)\Lambda} \right)}{1 - e^{-\Lambda T} \Big/ \left(\frac{1 - e^{-\Lambda T}}{\Lambda} \right)} = 1 - \frac{(1-VE)\Lambda}{\Lambda} = VE$$

Therefore the rate-based efficacy is equal to the individual efficacy. In particular it is independent of the force of infection Λ and the length of the trial T.

VE is an unbiased measure of vaccine efficacy, in contrast to VE_r and VE_f which are measured in field trials and are potentially subject to bias. VE is the efficacy that would be estimated from a large scale Phase IIa trial where all volunteers are equal and receive the same number of infectious bites. The risk-based definition of efficacy is commonly used for diseases in which natural infection with the causative agent provides near complete protection against a second infection with the same agent, for example measles. However the high frequency at which people living in endemic regions are challenged with malaria infection makes risk-based infection-blocking efficacy uninformative as most trial participants are likely to have become infected during a trial. In order to overcome this difficulty a consensus has formed amongst vaccine trialists to primarily use rate-based measures based on time to first infection [198, 199].

2.3. Heterogeneity in transmission

In a site where a malaria intervention is being trialled, the transmission intensity is usually measured using the entomological inoculation rate (EIR). In the analysis of trial results it is often assumed that all participants experience the same homogeneous force of infection. Making this assumption and ignoring the effects of heterogeneity in transmission can bias trial results.

If the average force of infection is measured to be $\Lambda = b\varepsilon$, where b is the transmission efficiency (the probability that a bite from an infectious mosquito results in infection), and ε is the measured EIR, then the heterogeneity in malaria transmission can be modelled using some distribution f. A proportion $f(x)$ of the population under observation will experience a force of infection $x\Lambda$. In order for the average force of infection across the entire population to be Λ, the distribution f must have mean 1. Examples of distributions that will be considered are given in Table 2.2.

Table 2.2: Examples of distributions describing heterogeneity in exposure in order of increasing heterogeneity.

Distribution	Description
constant	All individuals receive the same number of infectious mosquito bites.
gamma	The distribution of infectious mosquito bites follows a gamma distribution with parameter 4.2 as suggested by Smith *et al* [149].
80/20	The distribution of infectious bites follows an 80/20 rule as suggested by Woolhouse *et al* [200] where 20% of people receive 80% of the bites.
extreme	A hypothetical example of extreme heterogeneity as might be observed in a localised epidemic, in this case modelled as 5% of people receiving 95% of infectious bites.

In an area with constant force of infection Λ (i.e. homogeneous transmission), the average proportion infected by time T will be $I(T) = 1 - e^{-\Lambda T}$. In an area with heterogeneity in transmission described by a distribution f, the average proportion infected will be

$$I_f(T) = 1 - \int_0^\infty e^{-x\Lambda T} f(x) dx$$

Figure 2.1A shows the average infected proportion in a trial as a function of the follow-up time T for the heterogeneity distributions listed in Table 2.2. As heterogeneity in exposure to infection increases, the proportion of individuals infected in any given setting decreases. Furthermore, for a given force of infection, homogeneous exposure, whereby everyone receives the same number of infectious bites, always leads to the highest proportion infected (see 2.9. Appendix 1). To understand this result, consider the problem from the hypothetical point of view of a mosquito population trying to infect as many humans as possible with malaria. In order not to waste any bites on people already infected, the mosquitoes should evenly distribute their bites on the entire population. If there is heterogeneity in the bite distribution, then many bites will be wasted on humans who are already infected.

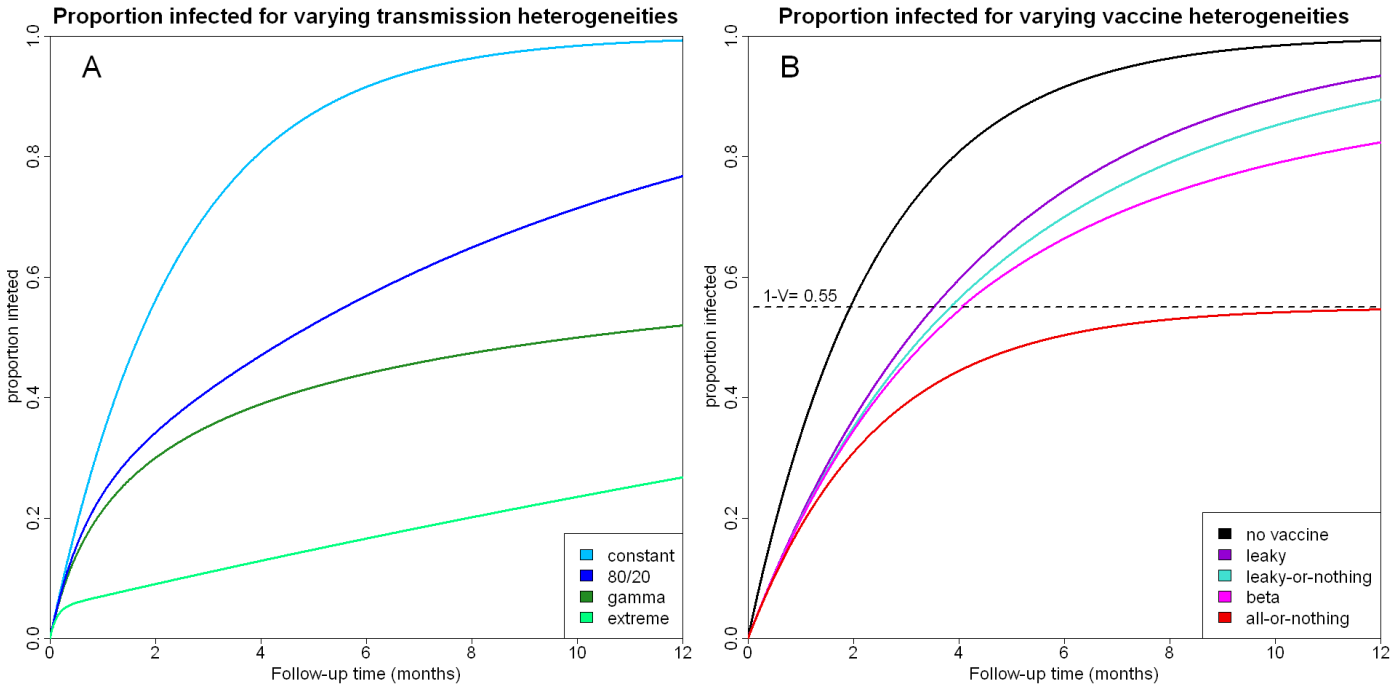


Figure 2.12: Cumulative proportion of infected trial participants for an infection-blocking vaccine with an average individual efficacy of 45.0% based on RTS,S, and in a similar setting to the Mozambique trial site described by Alonso *et al* [133]. (A) Cumulative proportion of unvaccinated trial participants infected under a range of transmission heterogeneities. (B) Cumulative proportion of vaccinated trial participants infected for a range of vaccine types. The proportion infected for an all-or-nothing vaccine can never cross the dashed line marked 1-V=0.55 as the 45% of vaccinees with total protection will never become infected. Note that the 4 vaccines each have the same average efficacy but different heterogeneities in vaccine efficacy.

2.4. Heterogeneity in vaccine response

Leaky and all-or-nothing vaccines represent the extreme cases of a vaccine that has a completely homogeneous effect on the vaccinated population and a vaccine that has a very heterogeneous effect. In reality one would expect to observe a range of intermediate behaviours between these two extremes. A vaccine that has variable individual efficacy on the vaccinated population can be described by an efficacy distribution g. Let $g(x)$ be the proportion of the population on which the vaccine has individual efficacy x. These efficacy distributions have been described previously by Halloran and Longini using frailty mixing models [201, 202]. Some examples are given in Table 2.3.

Table 2.3: Examples of distributions describing vaccine response in order of increasing heterogeneity.

Vaccine	Description
leaky	Vaccination gives everyone the same level of partial protection.
leaky-or-nothing	Vaccination offers partial protection to some people but no protection to others, as described by Halloran *et al* [202].
beta	Vaccination offers a variable level of protection to all vaccinees. The effect of vaccination follows a beta distribution as described by Maire *et al* [120].
all-or-nothing	Vaccination offers full protection to some people but no protection to others.

For a vaccine with efficacy distribution g, the average individual efficacy can be calculated by averaging the individual efficacy for the entire population

$$\int_0^1 xg(x)dx = VE$$

For a population in an area with force of infection Λ, given a homogeneous (leaky) vaccine with individual efficacy VE, the average proportion infected after follow-up time T will be $I(T) = 1 - e^{-(1-VE)\Lambda T}$. If the same population is instead given a vaccine with the same average individual efficacy VE, but with vaccine response described by a distribution g the average proportion infected will be

$$I_g(T) = 1 - \int_0^1 e^{-(1-x)\Lambda T} g(x)dx$$

Figure 2.1B shows the average infected proportion as a function of the follow up time T for the efficacy distributions listed in Table 2.3 for a specified average individual efficacy. The all-or-nothing vaccine always protects more people from infection than the leaky vaccine regardless of follow-up time. Thus, the more heterogeneous a vaccine is (i.e. the more it resembles an all-or-nothing vaccine), the more people will be protected from infection. In fact it can be proved (see 2.9. Appendix 1) that if a vaccine has average individual efficacy VE, then it will always protect at least as many people as would be expected from a leaky vaccine with the same average individual efficacy, and at most as many people as an all-or-nothing vaccine. To understand this, compare a leaky vaccine with an all-or-nothing vaccine. Given long follow-up, everyone in a group given a leaky vaccine will eventually become infected as eventually one of the infectious bites will evade the vaccine response. In contrast, a group given an all-or-nothing vaccine will contain a subgroup that will always remain free from infection. The unprotected subgroup will become infected at a natural rate, but the number of infections in this subgroup will be less than the number of infections in the entire leaky group. Therefore there will be fewer infections in the entire all-or-nothing group than in the leaky group.

2.5. How heterogeneity affects estimates of vaccine efficacy

Consider an infection-blocking vaccine with constant individual efficacy being tested in a range of transmission settings described by the distributions in Table 2.2. Figures 2.2A and 2.2C show the risk-based and rate-based efficacies that would be observed as a function of increasing follow-up time. Comparing the curves for the risk-based (Figure 2.2A) and rate-based (Figure 2.2C) efficacies it is apparent that the rate-based efficacy is a more useful measure as it converges to the individual efficacy as the follow-up time increases. The estimates of rate-based efficacy in the presence of transmission heterogeneity (Figure 2.2C), show that the more heterogeneous the transmission setting, the lower the measured value for the rate-based infection-blocking efficacy. This means that if a vaccine is tested in an area with a high level of transmission heterogeneity, its efficacy will be underestimated.

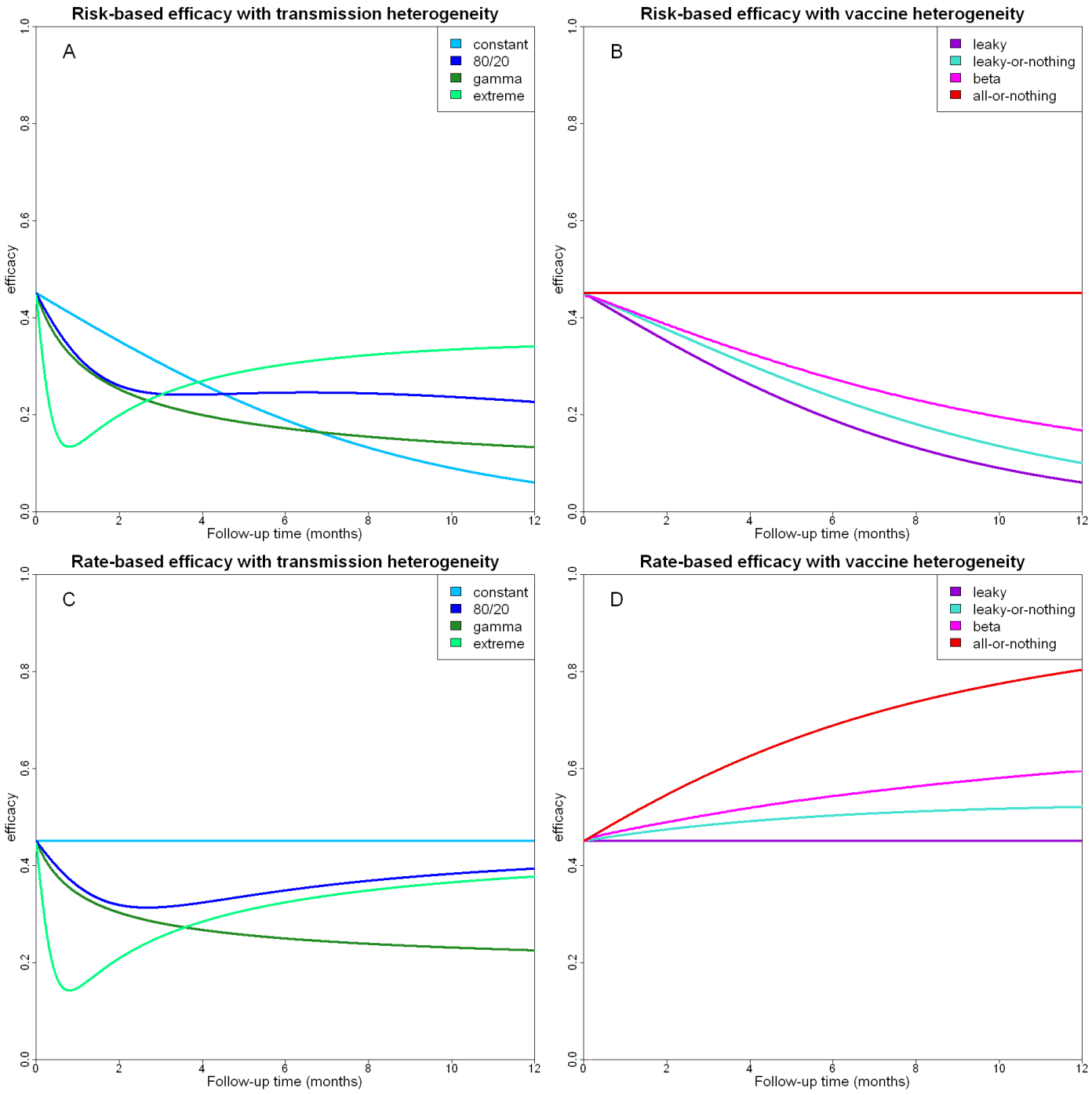


Figure 2.2: (A) Risk-based infection-blocking efficacy for a vaccine under the range of transmission heterogeneities in Table 2.2. (B) Risk-based infection-blocking efficacy for the four vaccines described in Table 2.3 with the same individual efficacy. (C) Rate-based infection-blocking efficacy under different transmission heterogeneities. (D) Rate-based infection-blocking efficacy for the four vaccines.

To understand this, consider a population where 20% of the population receive 80% of the infectious mosquito bites as suggested by Woolhouse *et al* [200]. In the unvaccinated control group, the high-risk 20% will almost certainly become infected whereas only a small proportion of the low-risk 80% will become infected. Now consider the vaccinated group. Despite being vaccinated a large proportion of the high-risk 20% will develop malaria infection, as they will be bitten so frequently that the vaccine will be unable to block all infections. In the low-risk group, only a small proportion will become infected due to the low number of infectious bites and the effect of the vaccine. Thus,

heterogeneity in the force of infection makes the infection profiles (who ends up infected) of the vaccine and control groups more similar, and hence a lower efficacy is measured.

Next consider an infection-blocking vaccine whose individual efficacy has been estimated from Phase IIa trials, but whose distribution of responses between individuals is unknown. Figures 2.2B and 2.2D show the risk-based and rate-based efficacies that would be observed for each of the response distributions described in Table 2.3. Note how the risk-based efficacies in Figure 2.2B decrease with follow-up time. This is because eventually everybody will become infected regardless of vaccination status. The exception to this is the case of an all-or-nothing vaccine where the subgroup which is fully protected will always remain uninfected. In contrast the rate-based efficacies in Figure 2.2D increase with follow-up time. This is because individuals who receive a high level of protection from the vaccine will remain susceptible for a longer time and significantly increase the total person years at risk used in the rate-based efficacy calculation. Finally, we observe that vaccines that induce more heterogeneous responses between individuals are estimated to be more efficacious than their homogeneous counterparts (Figure 2.2D), despite both vaccines having the same average individual efficacy. In particular, the upper limit for the observed rate-based efficacy is given by an all-or-nothing vaccine and the lower limit is given by a leaky vaccine. Therefore in a trial of a candidate malaria vaccine, if there is heterogeneity in vaccine response and this is ignored in the analysis, the vaccine efficacy will be overestimated.

2.6. Waning vaccine efficacy

RTS,S has been observed to induce long-lasting protection against malaria for up to 45 months [136, 203]. For example in a Phase II trial of children in Mozambique, Alonso *et al* [133] recorded a rate-based vaccine efficacy against first clinical episode of malaria of 29.9% (95% CI, 11.0-44.8) in a six-month follow-up period. In an extended follow-up of the Mozambique trial Sacarlal *et al* [203] recorded a vaccine efficacy of 16.8% (95% CI, -2.5-32.4) over months 21-33, and an efficacy of 11.8% (95% CI, -20.1-35.2) over months 33-45. These results could either be consistent with RTS,S inducing protection from infection over an extended period but with waning efficacy, or an artefact due to the apparent waning of efficacy due to the effects of heterogeneity.

Kanaan and Farrington [122] propose two models of waning vaccine efficacy; the selection and deterioration models. In the selection model, waning arises from heterogeneity in the duration of protection, i.e. a vaccinated person is protected for a period of time dependent on the vaccine half-life and then loses all protection. For example a person receiving a vaccine with initial individual efficacy 45% and a half-life of 1 year will have a 50% chance of still being protected after one year. In the deterioration model, waning arises from the gradual decline in the individual efficacy. For example a vaccine with initial individual efficacy of 45% and a half-life of 1 year will have an efficacy of 22.5% one year after it is administered. These two models are with-waning analogues of the all-or-nothing and leaky models. In both cases there can be additional heterogeneity between individuals in the rate of waning, with some

individuals remaining protected for longer than others. This would occur if a vaccine's efficacy is dependent on natural boosting through continuous exposure to infection.

Figure 2.3 shows the effects of waning efficacy for both leaky and all-or-nothing vaccines. The solid lines represent the cumulative infected proportion (Figure 2.3A) and the rate-based infection-blocking efficacy (Figure 2.3B) whilst the dashed lines represent these same quantities except with the addition of waning vaccine efficacy with a half-life of one year using the deterioration model for the leaky vaccine and the selection model for the all-or-nothing vaccine. As expected, waning efficacy reduces the observed rate-based vaccine efficacy for both leaky and all-or-nothing vaccines. If the effects of waning efficacy are ignored, estimates of rate-based efficacy can be prone to bias. For example, a waning vaccine with high initial efficacy could be confused with a non-waning vaccine with lower efficacy. Or a waning all-or-nothing vaccine could be mistaken for a non-waning leaky vaccine. Thus the only way to detect the presence of waning vaccine efficacy in clinical field trials is to ensure that there is extended follow-up of the trial participants.

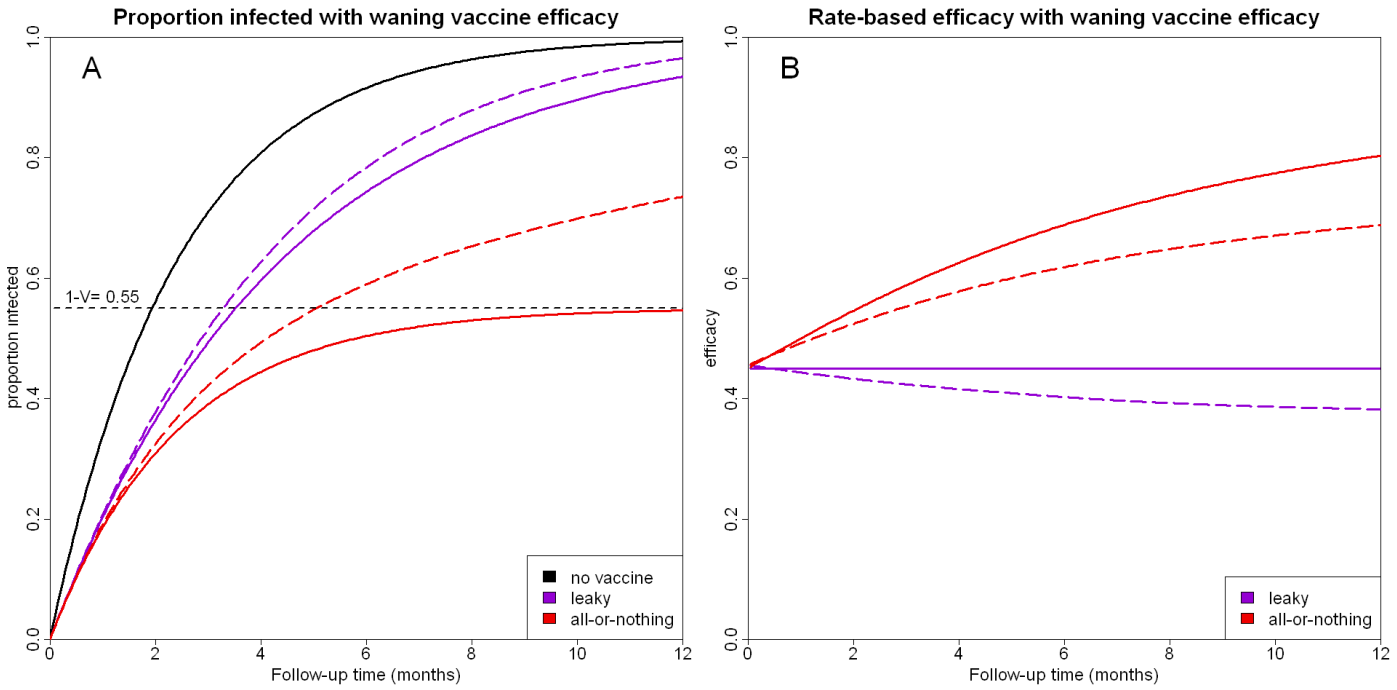


Figure 2.3: (A) Cumulative proportion of vaccinated trial participants infected for leaky and all-or-nothing vaccines. The dashed lines represent the infected proportion when vaccine efficacy wanes with a half-life of 1 year. (B) Rate-based efficacy for leaky and all-or-nothing vaccines. The dashed lines represent the expected rate-based efficacy with a waning vaccine.

2.7. Discussion

A measurement of vaccine efficacy may be biased if the way in which it is measured causes systematic differences between the estimated efficacy and the true efficacy. The results presented here demonstrate that several sources of heterogeneity can lead to biased estimates of vaccine efficacy if their effects are ignored. In general, heterogeneity in exposure to infectious mosquitoes will result in an underestimate of vaccine efficacy. In contrast, heterogeneity in individuals' response to the vaccine (often termed the take of the vaccine) will result in an overestimate of vaccine efficacy. In addition, waning efficacy of the vaccine will further complicate the observed patterns. Thus simple calculations that ignore these sources of heterogeneity can easily give biased results and it will be difficult to assess the extent to which these are biased upwards or downwards.

Whilst these results are intuitively clear, it is often assumed that randomization will diminish or remove their effect. Clearly randomization should at the least ensure that characteristics such as individual-level attractiveness to mosquitoes are balanced in the trial arms. Moreover, cluster-based designs in which villages or other population units are randomized should ensure a balance in village-level exposure between the arms. However, even if heterogeneity is balanced across trial arms through robust randomization, this will not counter-balance the biases identified here. For exposure-driven heterogeneity, in the control arm those that are highly exposed will almost certainly become infected whilst the low-risk population will avoid infection. Similarly, in the vaccinated arm a large, albeit lower, proportion of the highly exposed population will become infected but as in the control arm, the remainder will likely avoid infection. Because a large proportion of both the control and vaccinated group are unexposed, this will always result in an underestimate of vaccine efficacy.

In contrast, heterogeneity in vaccine response will only affect the vaccine arm of the trial. In this arm of the trial heterogeneity will cause some trial participants to receive a higher level of protection than others, whereas the control arm will be unaffected. The presence of a highly protected subgroup in the vaccine arm of the trial will cause the measurement of the rate-based infection-blocking efficacy to be greater than the individual vaccine efficacy. Thus the individual vaccine efficacy will be overestimated.

Given these heterogeneities, how can their impact on estimates of vaccine efficacy be minimized? The first step is to ensure that trial participants are as homogeneous as possible. For example children in a malaria vaccine trial should be of similar age, live in similar settings and have similar access to healthcare and anti-malaria interventions such as bed nets. This should occur not just through randomization but by ensuring that trials are undertaken in areas of relatively homogeneous transmission. However even with the most carefully selected study cohort there is likely to be residual heterogeneity in exposure and response. Thus analysis methods need to account for these possible biases. In studies of malaria immunity Bejon [204] and Kinyanjui [205] have suggested that the effects of heterogeneity be reduced by excluding individuals likely to be unexposed. However, when infection rather than blood-stage disease is the primary outcome this may be problematic given that infection is generally the most reliable marker of exposure. In addition Valim *et al* [206] recently proposed a statistical estimator of individual vaccine efficacy that corrects for the effects of heterogeneity in exposure by taking account of multiple exposures.

Whilst I am not aware of comparable estimators that account for heterogeneity in response, this may in fact not be a major problem for the RTS,S vaccine because in one of the Phase II trials of RTS,S [5], nearly all of the vaccinated group became infected with *P. falciparum* at some stage during the monitoring period. This suggests that the current RTS,S vaccine is leaky - although there may still be considerable heterogeneity in this leakiness. Thus further characterization of the heterogeneity in response is needed to ensure that estimates of vaccine efficacy are not substantially biased.

Extended follow-up of clinical trials of RTS,S have observed the apparent waning of vaccine efficacy against clinical malaria, as demonstrated by the coming together of Kaplan Meier plots comparing the infected proportions in vaccine and control groups [136]. This waning could be consistent with either a deterioration model where efficacy is gradually lost, or a selection model where efficacy is maintained for a random period of time dependent on the vaccine half-life, or may just be an artefact of heterogeneity in exposure and vaccine response. In addition there may be heterogeneity in waning where the vaccine has a variable half-life. Early efficacy estimates based on 6 or 12 month follow-up may be biased and this bias is difficult to quantify. For example, 12 month results could be consistent with a vaccine with high initial efficacy and a short half-life, or medium initial efficacy and a longer half-life. Extended follow-up, potentially spanning several years, is therefore required to obtain unbiased estimates.

2.8. Conclusion

RTS,S is currently undergoing extensive testing in Phase III clinical trials. These trials will enrol up to 16,000 children and infants across 11 sites in seven different African countries. The results presented here demonstrate that heterogeneity in exposure, response and vaccine waning can bias vaccine efficacy measures in ways that are not easily measurable. These factors need to be borne in mind when combining results from the wide range of transmission settings in these Phase III studies.

2.9. Appendix 1: Mathematical Supplement

2.9.1. Calculating the expected infection proportion

For an initially susceptible population in an area of constant, homogeneous malaria transmission with force of infection Λ, the expected cumulative infected proportion after time T, $I(T)$, can be described by the following simple equation

$$\frac{dI}{dt} = \Lambda(1-I)$$
$$I(0) = 0$$

That is people in the susceptible class $S = 1-I$ move to the infected class I at a constant rate Λ. This equation can be solved to give

$$I(T) = 1-e^{-\Lambda T}$$

2.9.2. Calculating the expected person years at risk

In a trial, person years at risk are computed for each individual as the time from entry into the study to malaria infection, or to the end of the study period for those who do not become infected. For a single unvaccinated trial participant under conditions of homogeneous transmission intensity the expected person years at risk (PYAR) is calculated as

$$PYAR = \int_0^T e^{-\Lambda T} dt = \frac{1-e^{-\Lambda T}}{\Lambda}$$

When there is heterogeneity in transmission described by some distribution f the mean PYAR can be calculated as follows

$$\begin{aligned} PYAR_f &= \int_0^T \int_0^\infty e^{-x\Lambda t} f(x)dx\ dt \\ &= \int_0^\infty \left[\frac{e^{-x\Lambda t}}{-\Lambda x} \right]_0^T f(x)dx \\ &= \int_0^\infty \left(\frac{1-e^{-x\Lambda T}}{\Lambda x} \right) f(x)dx \end{aligned}$$

When there is heterogeneity in vaccine response described by some distribution g the mean PYAR can be calculated as follows

$$PYAR_g = \int_0^T \int_0^1 e^{-(1-x)\Lambda t} g(x)dx\ dt$$
$$= \int_0^1 \left[\frac{e^{-(1-x)\Lambda t}}{-(1-x)\Lambda} \right]_0^T g(x)dx$$
$$= \int_0^1 \left(\frac{1-e^{-(1-x)\Lambda T}}{(1-x)\Lambda} \right) g(x)dx$$

2.9.3. Table of transmission heterogeneity distributions with calculated heterogeneities

A measure for the heterogeneity of a transmission distribution f, where f has mean 1, can be obtained by calculating the variance of the distribution.

$$\sigma_f^2 = \int_0^\infty (x-1)^2 f(x)dx$$
$$= \int_0^\infty x^2 f(x)dx - 1$$

Appendix Table 2.1: Transmission heterogeneity distributions with calculated heterogeneities.

Transmission	Description	Distribution	Heterogeneity – σ_f^2
constant	All individuals receive the same number of infectious mosquito bites.	$\delta(x-1)$	0
80/20	The distribution of infectious bites follows an 80/20 rule as suggested by Woolhouse *et al* [200] where 20% of people receive 80% of the bites.	$0.8\delta(x-0.25) + 0.2\delta(x-4)$	3.25
gamma	The distribution of infectious mosquito bites follows a gamma distribution with variance 4.2 as suggested by Smith *et al* [149].	$\frac{x^{1/4.2-1}\ {}^{1}/_{4.2}\ {}^{1/4.2}\ e^{-x/4.2}}{\Gamma(1/4.2)}$	4.2
extreme	A hypothetical example of extreme heterogeneity as might be observed in a localised epidemic, in this case modeled as 5% of people receiving 95% of infectious bites.	$0.95\delta(x-\frac{1}{19}) + 0.05\delta(x-19)$	18.053

2.9.4. Table of vaccine response distributions with calculated heterogeneities

A measure for the heterogeneity of a distribution of vaccine response g can be obtained by calculating the variance of the distribution

$$\begin{aligned}\sigma_g^2 &= \int_0^1 (x-V)^2 g(x)dx \\ &= \int_0^1 x^2 g(x)dx - V^2\end{aligned}$$

Appendix Table 2.2: Vaccine response distributions with calculated heterogeneities.

Vaccine	Description	Distribution	Heterogeneity – σ_g^2
leaky	Vaccination gives everyone the same level of partial protection.	$\delta(x-V)$	0
leaky-or-nothing	Vaccination offers partial protection to some people but no protection to others, as described by Halloran *et al* [119]	$\frac{3}{4}\delta(x-\frac{4}{3}V)+\frac{1}{4}\delta(x)$	$\frac{V^2}{3}$
beta	Vaccination offers a variable level of protection to all vaccinees. The effect of vaccination follows a beta distribution as described by Maire *et al* [120] ($\beta=1$)	$\frac{x^{\frac{\beta V}{1-V}-1}(1-x)^{\beta-1}}{B(\frac{\beta V}{1-V},\beta)}$	$\frac{V(1-V)^2}{\beta+1-V}$
all-or-nothing	Vaccination offers full protection to some people but no protection to others.	$(1-V)\delta(x)+V\delta(x-1)$	$V(1-V)$

2.9.5. Proof that heterogeneity in transmission reduces the infected proportion in a clinical trial

The heterogeneity in force of infection of malaria upon a population can be described by a distribution f . Let F be the family of such distributions, then $f \in F$ satisfies

$$\begin{aligned}&f:\mathbb{R}^+ \to \mathbb{R}^+ \\ &\int_0^\infty f(x)dx = 1 \\ &\int_0^\infty xf(x)dx = 1\end{aligned}$$

The expected cumulative proportion infected under a homogeneous force of infection will be $I(T)=1-e^{-\Delta T}$. Under a heterogeneous force of infection the expected infection proportion will be

$$I_f(T) = 1 - \int_0^\infty e^{-x\Lambda T} f(x)dx$$

To show that on average fewer people become infected in a heterogeneous setting, we need to prove $I_f(T) \le I(T)$.

For $a = \Lambda T > 0$ prove

$$e^{-a} \le \int_0^\infty e^{-ax} f(x)dx \le 1$$

First calculate the upper bound

$$U_f = \sup_{f \in F} \int_0^\infty e^{-ax} f(x)dx$$

Since $e^{-ax} \le 1 \forall x \in [0, \infty)$ we have

$$\int_0^\infty e^{-ax} f(x)dx \le \int_0^\infty f(x)dx = 1$$

so $U_f \le 1$. Now consider the family of mean-1 distributions

$$f_\gamma(x) = \frac{\gamma - 1}{\gamma}\delta(x) + \frac{1}{\gamma}\delta(x - \gamma)$$

with

$$\int_0^\infty e^{-ax} f_\gamma(x)dx = \frac{\gamma - 1}{\gamma} + \frac{1}{\gamma}e^{-\gamma a}$$

which tends to 1 as $\gamma \to \infty$. Thus

$$U_f = \sup_{f \in F} \int_0^\infty e^{-ax} f(x)dx \ge \sup_{\gamma \in \mathbb{R}^+} \int_0^\infty e^{-ax} f_\gamma(x)dx = 1$$

So $U_f = 1$. Note that this bound is not attained in F since $\lim_{\gamma \to \infty} f_\gamma(x) = \delta(x)$ which has mean 0, and therefore isn't in F.

Now calculate the lower bound L_f.

$$L_f = \inf_{f \in F} \int_0^\infty e^{-ax} f(x)dx$$

Let $T(x)$ be the tangent to e^{-ax} at $x = 1$.

$$T(x) = e^{-a}(1 + a - ax)$$

Since $T(x) \le e^{-ax} \forall x$ we have

$$\int_0^\infty e^{-ax} f(x)dx \ge \int_0^\infty T(x) f(x)dx = (1+a)e^{-a} \int_0^\infty f(x)dx - ae^{-a} \int_0^\infty xf(x)dx = e^{-a}$$

So $L_f \ge e^{-a}$. Now considering the mean-1 distribution $f(x) = \delta(x-1)$ we have

$$\int_0^\infty e^{-ax} \delta(x-1)dx = e^{-a}$$

so $L_f = e^{-a}$.

2.9.6. Prove that a vaccine with individual vaccine efficacy V will protect at least as many people as a leaky vaccine and at most as many people as an all-or-nothing vaccine.

The mean infected proportions for leaky and all-or-nothing vaccines with individual efficacy V will be given by

$$I_{\text{leak}}(T) = 1 - e^{-(1-V)\Lambda T}$$
$$I_{\text{aon}}(T) = (1-V)(1-e^{-\Lambda T})$$

The distribution of vaccine response for a vaccine with individual efficacy V can be described by a distribution g. Let G be the family of such distributions, then $g \in G$ satisfies

$$g : [0,1] \to \mathbb{R}^+$$
$$\int_0^1 g(x)dx = 1$$
$$\int_0^1 xg(x) = V$$

The mean infected proportion for a vaccine with response described by distribution g will be

$$I_g(T) = 1 - \int_0^1 e^{-(1-x)\Lambda T} g(x)dx$$

Prove that the mean proportion infected is always greater than it is for an all-or-nothing vaccine, and less than it is for a leaky vaccine, i.e.

$$I_{\text{aon}}(T) \le I_g(T) \le I_{\text{leak}}(T)$$

This is equivalent to proving

$$e^{aV} \le \int_0^1 e^{ax} g(x)dx \le 1 + V(e^a - 1)$$

for $a = \Lambda T > 0$. First calculate the upper bound

$$U_g = \sup_{g \in G} \int_0^1 e^{ax} g(x) dx$$

Let $U(x)$ be the straight line between $(0,1)$ and $(1, e^a)$

$$U(x) = 1 + (e^a - 1)x$$

Since $U(x) \geq e^{ax} \forall x \in [0,1]$ we have

$$\int_0^1 e^{ax} g(x) dx \leq \int_0^1 U(x) f(x) dx = \int_0^1 g(x) dx + (e^a - 1) \int_0^1 x g(x) dx = 1 + V(e^a - 1)$$

So $U_g \geq 1 + V(e^a - 1)$. Now consider the function $g_{\text{aon}}(x) = (1 - V)\delta(x) + V\delta(x - 1)$ which gives

$$\int_0^1 e^{ax} g_{\text{aon}}(x) dx = 1 + V(e^a - 1)$$

And hence $U_g = 1 + V(e^a - 1)$.

Now calculate the lower bound

$$L_g = \inf_{g \in G} \int_0^1 e^{ax} g(x) dx$$

Let $L(x)$ be the tangent to e^{ax} at the point $x = V$.

$$L(x) = e^{aV}(1 - aV + ax)$$

Since $L(x) \leq e^{ax} \forall x \in [0,1]$ we have

$$\int_0^1 e^{ax} g(x) dx \geq \int_0^1 L(x) g(x) dx = (1 - aV) e^{aV} \int_0^1 g(x) dx + a e^{aV} \int_0^1 x g(x) dx = (1 - aV) e^{aV} + a e^{aV} V = e^{aV}$$

So $L_g \leq e^{aV}$. Now consider the function $g_{\text{leak}}(x) = \delta(x - V)$ which gives

$$\int_0^1 e^{ax} g_{\text{leak}}(x) dx = e^{aV}$$

and hence $L_g = e^{aV}$.

Chapter 3. Methods for estimating the relationship between pre-erythrocytic immune responses and protection from *P. falciparum* infection in treatment re-infection studies

Vaccines to several diseases provide protection by inducing a similar or superior immune response to that generated following naturally occurring infection. However, despite considerable progress in the development of vaccines for preventing *P. falciparum* infection, the relationship between naturally acquired pre-erythrocytic immune responses and protection from infection remains poorly understood. In this chapter, I review published studies investigating the relationship between markers of pre-erythrocytic immunity and protection from infection and clinical malaria.

3.1. Introduction

Vaccines targeting the pre-erythrocytic stages of the *P. falciparum* parasite are likely to be an invaluable tool for preventing malaria associated deaths and reducing transmission in the future. There are several infection-blocking malaria vaccines currently under development [15, 16, 207], all of which confer protection by boosting some element of the pre-erythrocytic (PE) immune response, often to levels much higher than observed under conditions of natural exposure. Recent advances in vaccine development have led to renewed interest in the PE immune response [33], both naturally acquired and vaccine-induced.

When a *P. falciparum* infectious *Anopheles* mosquito takes a blood meal from a human, sporozoites are inoculated into the tissue surrounding the injection site [208]. Some sporozoites migrate to blood vessels where they are carried to the liver, and others are immobilised or drained to regional lymph nodes [209]. Upon reaching the liver sporozoites invade a hepatocyte where they differentiate into liver-stage parasites before finally releasing merozoites into the blood stream a few days later [29]. The PE immune response can prevent the successful development of sporozoites via antibody- or cell-mediated immune responses targeting sporozoite antigens: the prime targets being circumsporozoite protein (CSP), thrombospondin-related adhesion protein (TRAP) and liver-stage antigen 1 (LSA-1). The mechanisms underlying antibody-mediated protection include inhibition of hepatocyte invasion or opsonisation of sporozoites for uptake by macrophages and dendritic cells. It has also been observed that mosquitoes inoculate fewer sporozoites into immunized as compared to non-immunized mice, presumably due to the formation of antigen-antibody complexes in the proboscis [210]. Cell-mediated immunity is provided by $CD4^+$ or $CD8^+$ T cells, both of which have been observed to eliminate infected hepatocytes *in vitro* [211, 212]. Furthermore, $CD4^+$ T cells specific for liver-stage antigens produce a mixture of the cytokines IL-2, IFN-γ and TNF-α which have been shown to inhibit the intracellular stages of the parasite [213].

The failure of sporozoite inoculations to consistently progress to blood-stage malaria has been observed in both artificial challenge studies [214] and under conditions of natural exposure [145, 152]. In children exposed to malaria in Ifakara, Tanzania it was estimated that 93% and 99.6% of sporozoite inoculations failed to progress to blood-stage

infection in the low and high transmission seasons, respectively [215, 216]. The proportion of infectious mosquito bites progressing to blood-stage infection has been termed the transmission efficiency. Transmission efficiency can be defined on an individual level as the probability that an infectious mosquito bite leads to blood-stage infection, or on a population level as the proportion of mosquito bites on a population that cause new blood-stage infections. Smith *et al* [195] have reviewed studies reporting both the entomological inoculation rate (EIR) and the force of infection in young children and noted that transmission efficiency decreases with increasing EIR. Several explanations for this effect have been put forward; heterogeneity in the force of infection [195]; an infection-blocking PE immune response [217, 218]; density-dependent inhibition of sporozoite inoculation [160, 219]; and inhibition of intra-hepatic development by blood-stage parasites [220]. In addition, a strong blood-stage immune response that induces rapid clearance of parasites before detection can be conflated with a reduction in transmission efficiency [221].

Transmission efficiency is also dependent on age. In studies of the time taken for *P. falciparum* parasites to re-appear in the blood following treatment, the time until detection of re-infection for adults has been observed to be significantly longer than for children, suggesting a reduction in transmission efficiency with age [216, 222, 223]. This effect has been observed despite the fact that *Anopheles* mosquitoes bite adults more often than smaller children because of their larger surface area [189, 224].

Understanding the relationship between transmission efficiency and EIR and age is crucial for understanding the epidemiology of malaria transmission and the impact of PE vaccines. Smith *et al* [195] have explained the variation in transmission efficiency in children under six in terms of heterogeneity in the force of infection. If biting is homogeneous then infections are evenly distributed across the population resulting in a high transmission efficiency. If instead biting is heterogeneous, for example if 20% of people receive 80% of bites, then transmission efficiency will be low as most bites will be concentrated on the highly exposed 20% who are likely to already be infected.

Heterogeneity in the force of infection, density-dependent inhibition of sporozoite inoculation and inhibition of intra-hepatic development by blood-stage parasites cannot however explain the observed difference in transmission efficiency between children and adults. The most common explanation for this reduction in transmission efficiency with age is the acquisition of PE immunity [217]. However, attempts to quantify the magnitude of the PE immune response have been complicated by apparently contradictory results from studies investigating the relationship between markers of immunity and protection from infection. For example, the relationship between naturally acquired anti-CSP antibodies and protection from infection has been found to be positive [225], negative [226] and non-significant [227]. In order to explain these contradictory observations, we review existing published studies of the relationship between PE immune responses and protection from infection or clinical malaria, and describe how the results of these studies must be interpreted in terms of their study design and statistical power.

3.2. Methods

3.2.1. Review of studies of pre-erythrocytic immune responses

A search of the published literature using a systematic methodology for studies investigating the relationship between markers of naturally acquired PE immunity and protection from *P. falciparum* infection was conducted using the electronic online database PubMed. The search terms used were "(malaria OR falciparum) AND (pre-erythrocytic OR infection-blocking)". The results of this search were supplemented by iterative reviews of the reference lists of relevant published papers. 21 studies investigating the relationship between measures of antibody- and cell-mediated PE immune responses and protection from *P. falciparum* infection were identified (Table 3.3). A further 9 studies with clinical malaria as an endpoint were identified; these studies are summarised in Table 3.4.

There was substantial variation in the design of the studies identified. With a few exceptions the studies had either cross-sectional or longitudinal designs. In cross-sectional studies volunteers from a cohort are tested at the beginning and end of (and sometimes during) a study for parasites and immune responses. In longitudinal studies, active detection of infection (ADI) is performed by testing volunteers for parasites at a given frequency, for example every week. These study designs are summarised in Figure 3.1. Studies can be further classified according to how the immune response data are analysed. Some studies treat immune responses as a binary variable (e.g. high vs. low responders, or sero-positive vs. sero-negative), whereas some treat immune response as a continuous variable (e.g. antibody titre or number of antigen specific T cells per million). Based on study design and classification of immune response, the studies can be classified into four categories:

1. **Cross-sectional study with binary immune response.** Volunteers are split into two categories according to the measured immune response (e.g. antibody titre), and the presence of infection is tested at cross-sections. A common study design involves clearing existing infections and then taking a cross-section to test for new infections a number of weeks later. Logistic regression can be used to test for an association between immune response and protection from infection. Alternative suitable tests are a chi-square test or Fisher's Exact test.

2. **Cross-sectional study with continuous immune response.** Similar to the previous study design except that PE immune responses are analysed as a continuous variable. Logistic regression can be used to investigate the association between PE immunity and protection from infection. An alternative suitable test would be a Student t-test.

3. **Longitudinal study with binary immune response.** Volunteers are treated to clear existing infections and followed longitudinally with ADI every week or every fortnight. Immune responses, usually measured at the beginning of follow-up, are used to classify participants into high and low categories. Cox Proportional Hazards (PH) can be used to test for an association between immune response and protection from infection.

4. **Longitudinal study with continuous immune response.** Similar to the previous study design except that PE immune responses are analysed as a continuous variable. Cox PH is a suitable tool for statistical analysis.

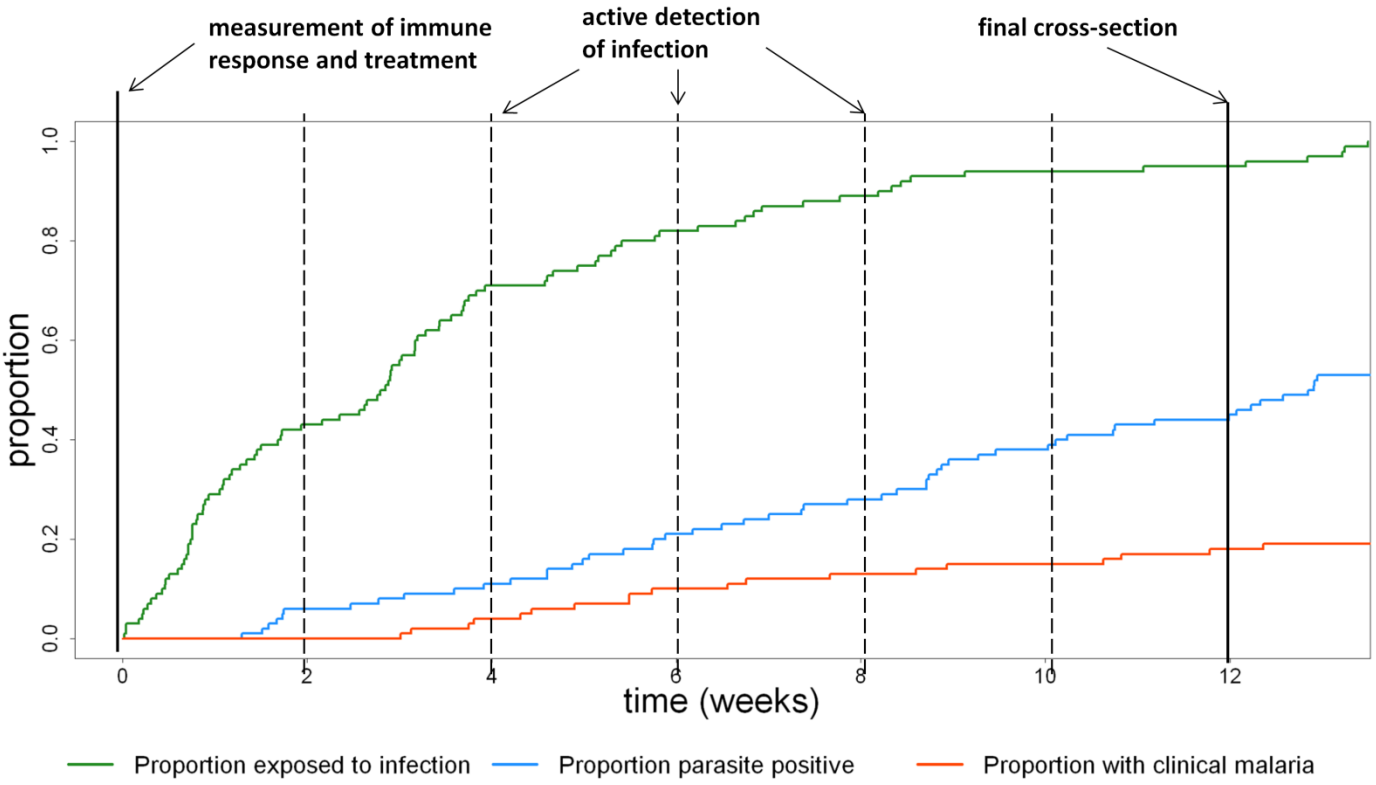


Figure 3.13: Schematic representation of a treatment re-infection study for measuring pre-erythrocytic immune responses. The solid black lines denote when samples are taken for a study with two cross-sections, and the dashed lines indicate when additional samples need to be taken for a longitudinal study. The difference between the cumulative proportion exposed to infection (green) and the cumulative proportion parasite positive (blue) is explained by pre-erythrocytic immunity and sporozoite inoculations that do not progress to blood-stage infection due to chance. The difference between the cumulative proportion parasite positive (blue) and the cumulative proportion with clinical malaria (red) is explained by blood-stage immunity.

Logistic regression and Cox PH analyses provide a convenient statistical framework for investigating the relationship between PE immunity and protection from infection, as it is straightforward to include interactions between multiple immune responses and to adjust for confounders such as LLIN use, age and location [226, 228]. Most treatment re-infection and PE vaccine studies using Cox PH to analyse data take the time of infection as the date of sampling when parasites were detected. However the true time of infection will have occurred at some time between the first positive sample and the previous negative sample. An interval censored analysis can be used to allow for the unknown infection time between consecutive samples. However, here, in keeping with the methodology of existing studies, we use the time to detection of parasites for analysis [117, 226].

In addition to differences in study design and statistical methodology, there was a great deal of variation in the characteristics of the identified studies, with the most notable differences being in the endpoint under investigation, the study size and duration of follow-up, the age range of the cohort and the transmission intensity. With such

variation in study characteristics it was not feasible to perform a meta-analysis of the effects of PE antibody- and cell-mediated immune responses on protection from infection.

In order to give an indication of a study's power to detect an effect, each study identified in the review was scored against four key characteristics relating to study design and statistical methodology:

- **Study design.** Studies with a longitudinal design with active detection for infection are scored 1 and cross-sectional studies scored 0.
- **Immune response**. Studies analysing immune response as a continuous variable are scored 1, and as a binary variable are scored 0.
- **Statistical test**. Studies using Cox proportional hazards for analysis of longitudinal data or logistic regression for analysis of cross-sectional data are scored 1.
- **Study size.** Studies where statistical tests are performed on populations of size greater than or equal to 100 are scored 1.

Based on these criteria, each study was assigned a Power Score between 0 and 4 as an indication of their power to detect an association between PE immune responses and protection (Table 3.3, Table 3.4). Similar metrics have been used with successful results in clinical medicine [229]. Although such a simple metric is somewhat arbitrary, it provides a useful tool for assessing evidence across multiple studies.

3.2.2. Relationship between immune response and protection

The relationship between a marker of PE immunity and protection from infection can be described by a dose-response curve [230, 231]. Standard statistical tests for an association between markers of PE immunity and protection from infection generally don't account for dose-response relationships, only assuming that greater immune responses are associated with increased protection.

Consider the hypothetical dose-response relationships between PE antibody titres and protection from infection depicted in Figure 3.2.When protection from infection is described by dose-response curve (A), the population under investigation will consist of a protected and an unprotected group. A statistically significant association between high antibody titres and protection is likely to be found whether antibody titres are analysed as a binary or a continuous variable. Dose-response curve (B) describes a continuous relationship between antibody titre and protection from infection. If antibody titre is analysed as a binary variable by splitting the cohort into low and high titre groups, an association between high titres and protection may be missed, as statistical tests will be based on comparisons between two partially protected groups. Analysing titres as a continuous variable will improve chances of detecting an association between antibody titres and protection. Dose-response curve (C) depicts a situation where there is a threshold for protection that is substantially higher than the median antibody titre observed in the population. Although there will be a small number of individuals protected by antibodies, the association is likely to be missed as the majority of the population are not protected despite some having high antibody titres.

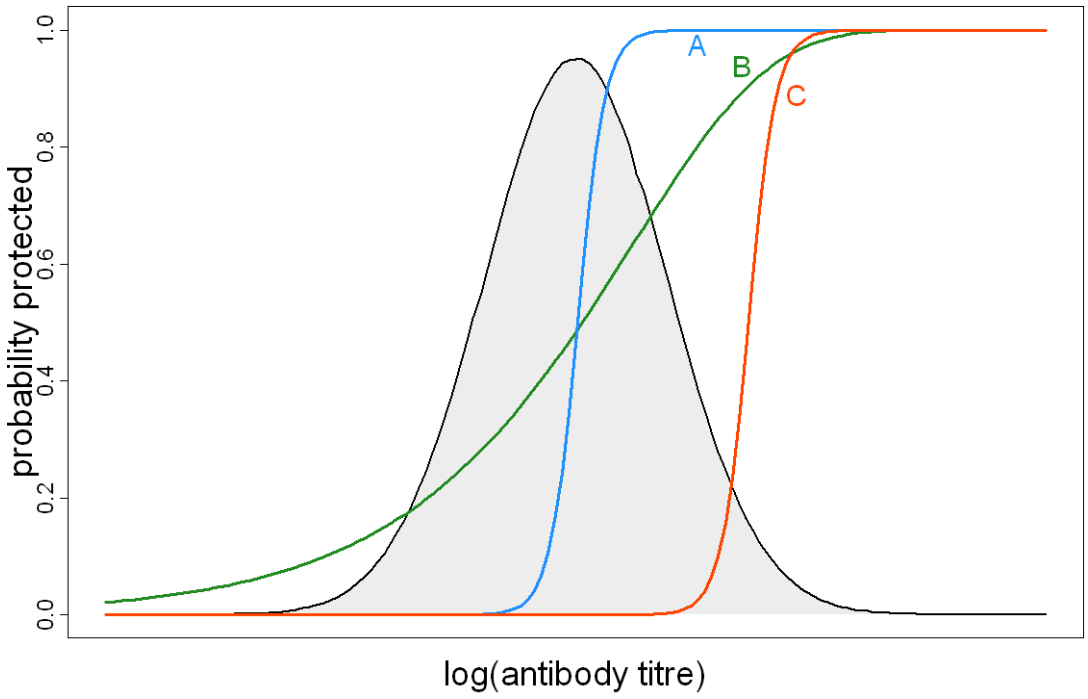


Figure 3.2: Distribution of antibody titres (grey) and hypothetical dose-response curves for the relationship between antibody titre and protection from infection. **(A)** Threshold for protection lies in the region of observed antibody titres. **(B)** No threshold for protection but there is a continuous increase in the probability of protection with increasing antibody titre. **(C)** A threshold for protection exists at a substantially higher titre than the median observed antibody titre.

3.2.3. Simulation of cross-sectional and longitudinal studies

To aid in the interpretation of study results we performed simulations to demonstrate the effect of study size, duration of follow-up, and transmission intensity on the statistical power to detect an effect of PE immunity.

In the absence of PE immunity, we assume transmission efficiency b_0, that is, a proportion b_0 of inoculations progress to blood-stage infection. Transmission efficiency can be reduced by PE antibodies (or cell-mediated immunity). We assume that antibody titre α confers protection from infection described by a dose response curve of the form $P(\alpha) = e^{-\log(2)\frac{\alpha}{\alpha_{50}}}$, where α_{50} is the antibody titre conferring 50% protection from infection (corresponding to curve B in Figure 3.2) [232]. The transmission efficiency of a person with antibody titre α is then reduced to $b(\alpha) = b_0 P(\alpha)$. In a region with entomological inoculation rate ε, the force of infection will then be given by $\Lambda(\alpha) = \varepsilon b_0 P(\alpha)$. After clearance of any existing infection with anti-malarial drugs, the probability that a person with antibody titre α will have been re-infected by time t is given by $I(t) = 1 - e^{-\Lambda(\alpha)t}$. We further assume that there is heterogeneity in the force of infection such that the infectious bites are Log-Normally distributed across the population at risk, independently of their antibody titre [233]. For simplicity we assume people don't clear parasites

before the detection of infection. We ignore sub-microscopic infections and issues of sensitivity and specificity, although the simulations can be extended to model these.

The outcome of 1 of 10,000 simulations, as well as the variation in antibody titres and force of infection in the study population is shown in Figure 3.3. The parameters used for the simulations are given in Table 3.1.

Table 3.1: Parameters for simulation of treatment re-infection studies.

Parameter description		30% protection	50% protection
duration of follow-up (weeks)	T	12	12
baseline transmission efficiency	b_0	0.55	0.55
mean Ab titre (arbitrary units)	μ_α	1	1
variance in Ab titre	σ_α^2	0.75	0.75
Ab titre for 50% protection	α_{50}	1.66	0.76
low transmission EIR (ibppy)	ε	5	5
high transmission EIR (ibppy)	ε	150	150
coefficient of variation in EIR	$\sigma_{\text{EIR}}/\varepsilon$	0.5	0.5

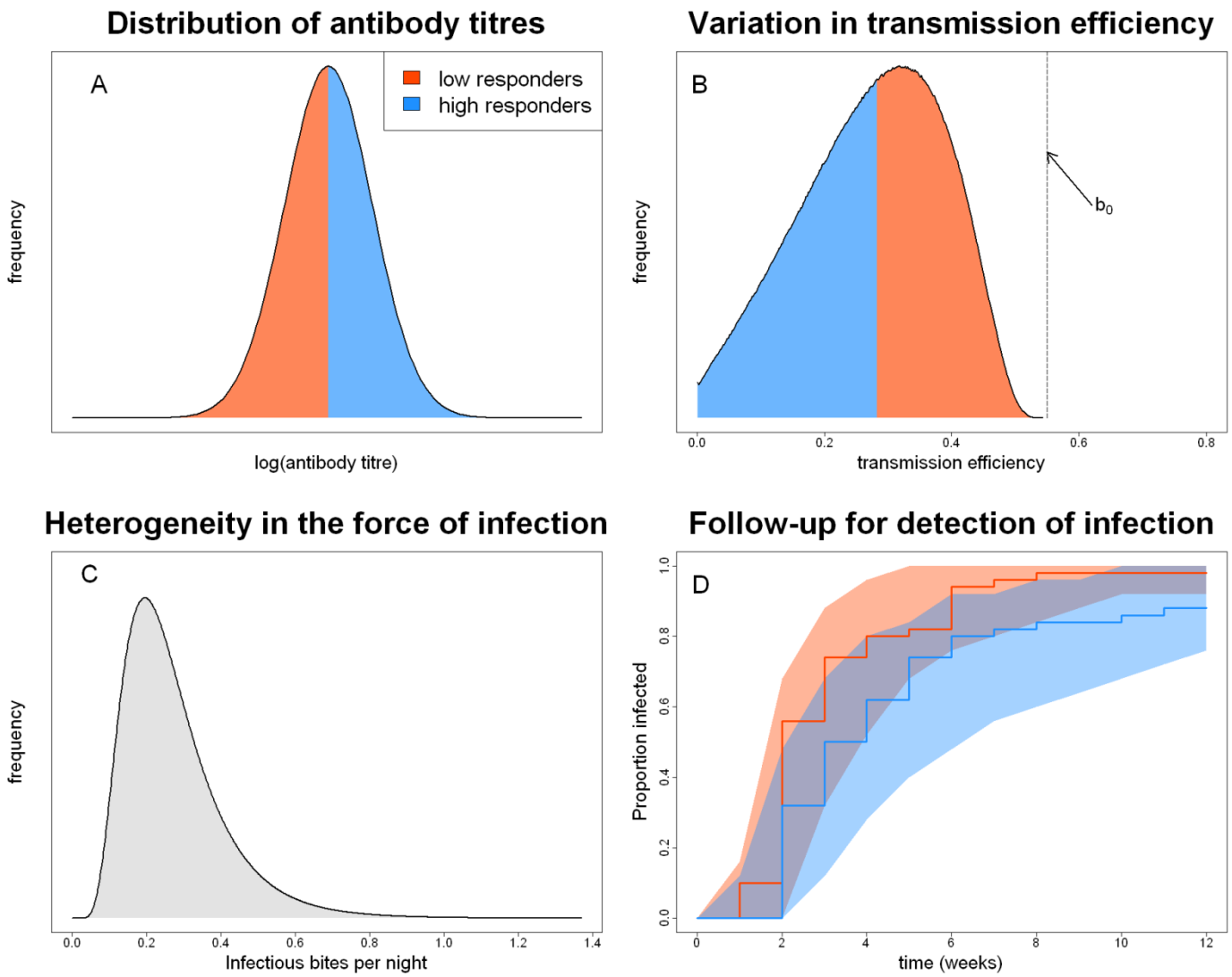


Figure 3.3: Simulated longitudinal study for evaluation of the relationship between protection from infection and pre-erythrocytic antibodies. It is assumed that 100 volunteers are followed up for 12 weeks with active detection of infection. **(A)** Log-normal distribution of antibody titres in the study cohort, split into high (blue) and low (red) responders. **(B)** Distribution of transmission efficiency in the study population, split into high (blue) and low (red) responders. **(C)** Heterogeneity in the force of infection: distribution in the number of infectious bites per night. **(D)** Sample Kaplan-Meier curves for high and low responders. The red and blue shaded regions represent 95% confidence intervals for the KM curves of low and high responders, respectively. The existence of a substantial overlap between the shaded regions illustrates the low statistical power to differentiate between high and low responders.

3.2.4. Estimation of statistical power to detect the effect of pre-erythrocytic immunity

We consider the effect of antibodies to two hypothetical PE antigens, causing a 30% and a 50% reduction in the force of infection, respectively (Table 3.4). We consider two forms of trial design: a longitudinal study with weekly ADI for 12 weeks, and a cross-sectional study with a cross-section at the time of parasite clearance and another cross-section 12 weeks later. Note that the cross-sectional trial design is equivalent to the longitudinal design without the measurements from ADI. The simulated data is analysed using either Cox proportional hazards or logistic regression.

3.3. Results

3.3.1. Review of studies of pre-erythrocytic immune responses

The review identified 21 studies of the association between markers of PE immunity and protection from infection (Table 3.3), and 11 studies of the association between markers of PE immunity and clinical malaria (Table 3.4). Although there was substantial variation in the study design and statistical methods used in the identified studies, there was a clear association between a study's Power Score and the likelihood that it reported a significant association between a marker of PE immunity and protection from infection or clinical malaria. This is demonstrated in Table 3.2 which shows the outcomes of statistical tests at the 5% significance level broken down by Power Score and endpoint. The results in Table 3.2 suggest that the probability of detecting a significant effect can be increased by increasing a study's Power Score, i.e. increasing study size, performing longitudinal follow-up, and analysing immune markers as continuous variable using Cox or Logistic regression.

Table 3.2: Proportion of tests from identified studies reporting a statistically significant association between markers of PE immunity and protection from infection or clinical malaria at the 5% significance level. Tests have been stratified according to their Power Score and endpoint.

	Power Score			
	1	2	3	4
endpoint				
infection	17% (2/12)	25% (6/24)	60% (3/5)	– (0/0)
clinical malaria	33% (2/6)	38% (5/13)	20% (1/5)	100% (3/3)
total	22% (4/18)	30% (11/37)	40% (4/10)	100% (3/3)

13 studies investigating the association between pre-erythrocytic antibodies and protection from infection were identified (Table 3.3); 2/12 found a significant association between anti-CSP antibodies and protection from infection, and 1/3 found a significant association between anti-TRAP antibodies and protection. No studies found a statistically significant association between anti-LSA1 antibodies and protection from infection. One study found a marginally significant association [234], however this association is likely due to correlation with other immune responses as LSA-1 epitopes are only expressed once a sporozoite has invaded a hepatocyte, at which stage immunoglobulin G (IgG) antibodies cease to be effective [235]. 8 studies investigating the effect of cell-mediated immune (CMI) responses directed against the PE stages were identified: 2/2 found a significant association between CSP-specific CMI responses and protection from infection, 2/2 found a significant association between TRAP-specific CMI responses and protection, and 4/6 found a significant association between LSA1-specific CMI responses and protection.

A larger proportion of studies found a significant association between cell-mediated immune responses and protection than between antibodies and protection suggesting that cellular responses may have a more important role in preventing infections than humoral responses [218]. However, an alternative explanation could be that studies of CMI responses were better designed than studies of antibody responses, as reflected in higher average

Power Scores for studies of CMI responses (mean Power Score 2.3 vs. 1.6). Better designed and more powerful studies will be more likely to detect a statistically significant association, if indeed there is a true relationship between PE immune response and protection from infection.

3.3.2. Simulated effect of study size on statistical power

The results of simulations to estimate the probability that a study will detect the effect of PE immunity at the 5% significance level are shown in Figure 3.4. As expected, statistical power increases with study size, and there is a higher probability of detecting the effect of an antibody that confers 50% protection from infection than one that confers 30% protection. Furthermore, greater statistical power is expected in high transmission settings when study data is analysed using Cox PH. Logistic regression is expected to have lower statistical power in high transmission settings as it is likely that all volunteers will develop infection during follow up.

When analysing the relationship between immune response and protection, more statistical power can be obtained by treating the immune response as a continuous variable (e.g. antibody titres, antigen specific T cells per million) than as a binary variable (e.g. high vs. low responders, sero-positive vs. sero-negative). This is because assigning continuous data to high and low categories reduces the amount of information captured in the data.

Importantly, even with a longitudinal design utilising a continuous measure of immunity, studies with 50 individuals have around 10% power to detect a 30% protection against infection in low transmission settings and 30% power in high transmission settings. Even for a larger, substantial effect size similar to that conferred by the RTS,S vaccine (50% protection against infection [133]), a study of 50 individuals would be under-powered in a low transmission setting. The simulation results suggest that cross-sectional surveys are likely to be underpowered to detect any likely association between individual immune responses and protection from infection except if the effect size is large (50% or more), the study population is >100 individuals, and the immune response is considered as a continuous variable.

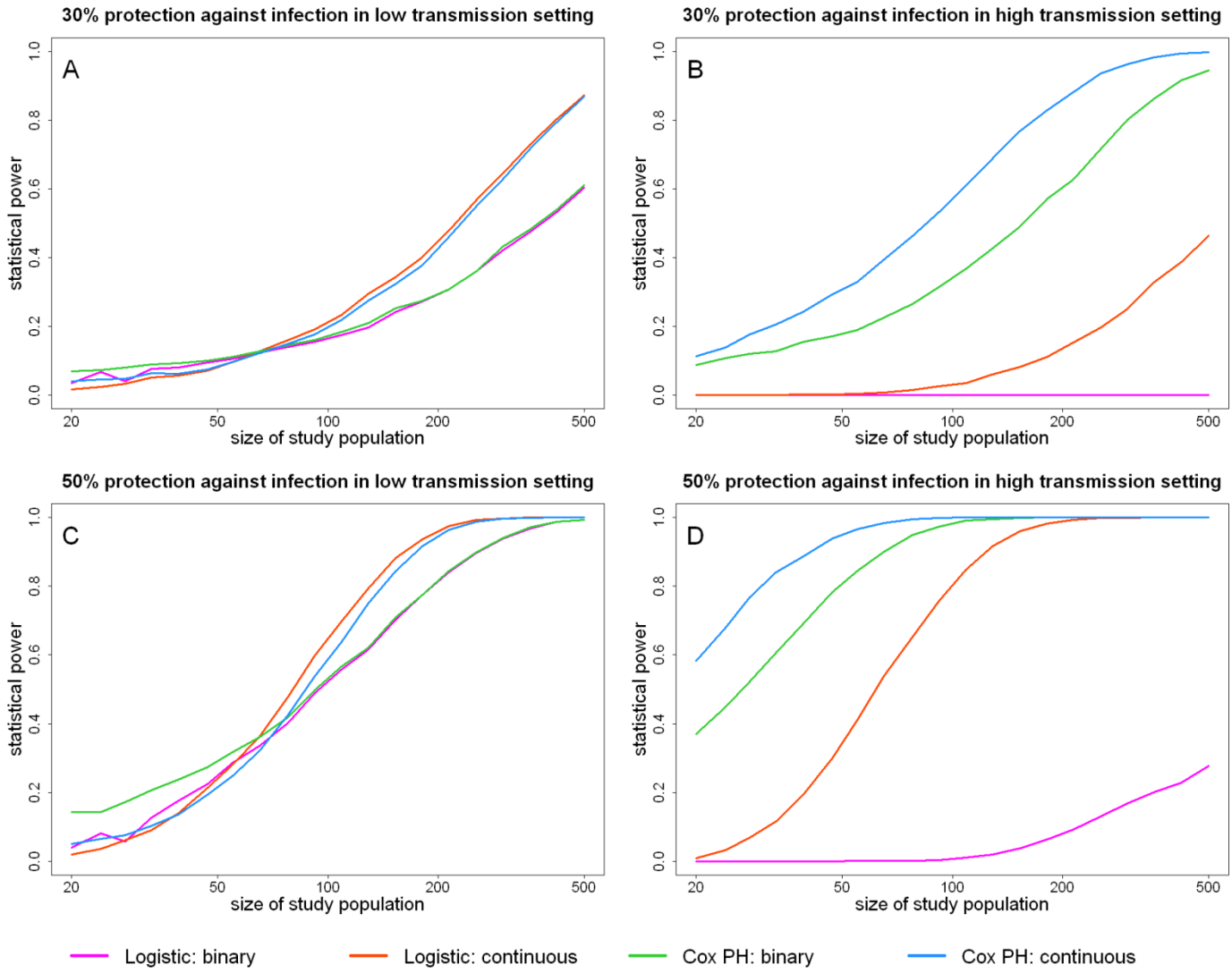


Figure 3.4: Probability of observing a statistical relationship at the 5% significance level between a PE immune response and protection from infection. **(A)** A study in a low transmission setting (EIR=5) where 30% of infections are prevented by PE immunity. **(B)** A study in a high transmission setting (EIR=150) where 30% of infections are prevented by PE immunity. **(C)** A study in a low transmission setting (EIR=5) where 50% of infections are prevented by PE immunity. **(D)** A study in a high transmission setting (EIR=150) where 50% of infections are prevented by PE immunity.

3.3.3. Simulated effect of sampling frequency on statistical power

Analysing trial data using Cox PH as opposed to logistic regression increases the statistical power to detect a PE immune response as Cox PH analysis utilises the additional information contained in the time of infection, whereas logistic regression only utilises the information on who becomes infected. In the extreme case of a study with a sufficiently long duration of follow up such that all participants become infected, a logistic regression analysis will be completely uninformative. The additional statistical power of the Cox PH analysis needs to be balanced against the cost incurred by ADI. A logistic regression analysis will require at least two cross-sectional blood smears at the start and end of the study, whereas a Cox PH analysis will require additional cross-sectional blood smears at weekly, fortnightly or monthly intervals throughout the study. Increasing the frequency of sampling for ADI will increase statistical power but at a cost of the resources needed for additional sampling.

In Figure 3.5 the statistical power of a longitudinal study as a function of sampling frequency is shown. In a low transmission setting, the benefit of increased sampling is negligible as little additional information is obtained by testing volunteers who are unlikely to become infected. In a high transmission setting, statistical power decreases with reduced sampling frequency. As power decreases slowly with sampling frequency, it may be most efficient to sample fortnightly or monthly if that would allow an increase in the total number of volunteers recruited to the study. In more intense transmission settings where all volunteers are likely to become infected sampling frequency will need to be increased to capture the information contained in the time to re-infection. Selecting the optimal sampling frequency will depend on study size and duration, the expected magnitude of the immune response under investigation, transmission intensity and seasonality, the cost of processing samples and the need to avoid taking too many samples from any one person.

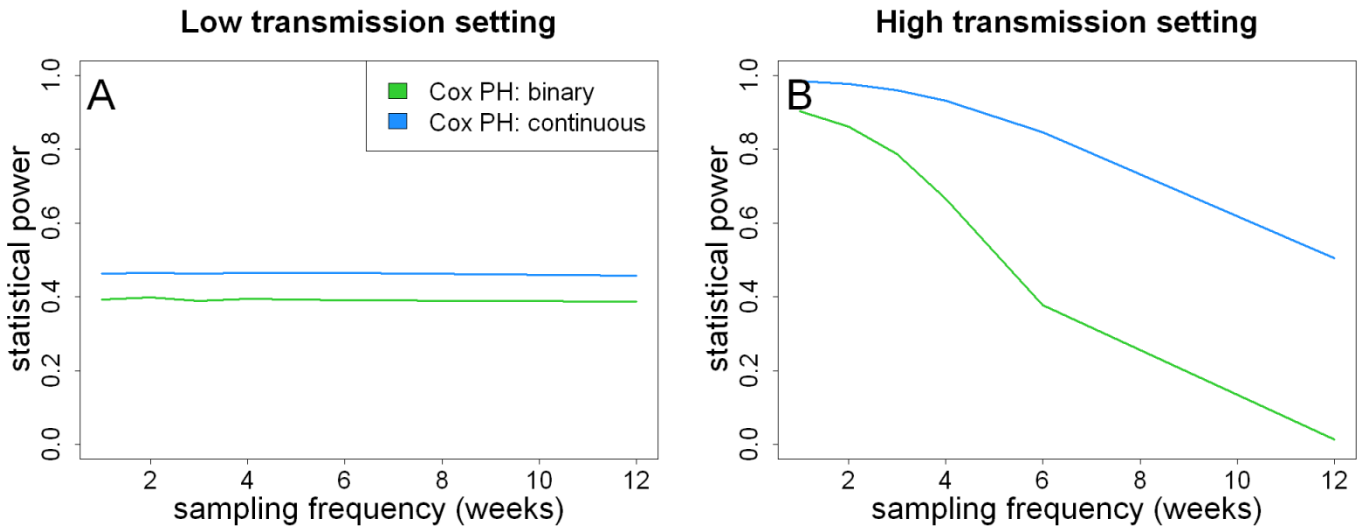


Figure 3.5: The effect of sampling frequency on the statistical power to detect the effect at the 5% significance level of a PE immune response that prevents 50% of infections in a study of 100 volunteers followed for 12 weeks. **(A)** Low transmission setting (EIR=5). **(B)** High transmission setting (EIR=150).

3.4. Discussion

The pre-erythrocytic immune response constitutes the first line of defence as the *P. falciparum* parasite enters the human host, and as such will impact upon malaria transmission, the incidence of clinical and severe malaria, and the efficacy of infection-blocking vaccines. Despites its key role in the clinical epidemiology of malaria, the magnitude of the PE immune response remains poorly quantified. In particular, identifying immune responses that significantly correlate with protection from infection has been difficult due to the low statistical power of treatment re-infection studies. Analysis of the results of a review of the published literature demonstrates that the majority of studies failed to detect a significant association between PE immune responses and protection from infection or clinical malaria (Table 3.3). However, our simple Power Score suggests that the likelihood of a study detecting statistically significant associations can be improved by increasing study size, implementing longitudinal follow-up of

participants, analysing immunological data as a continuous variable, and using Cox or logistic regression for estimation.

The results of our review demonstrate that there is a clear association between markers of cell-mediated immunity and protection from infection. However, comparable studies of PE antibodies do not demonstrate a consistent association between antibodies and protection from infection, despite the well-established role of antibodies as an immunological correlate of protection in vaccine studies [231]. This suggests that the cell-mediated component of the PE immune response confers greater protection than the antibody component. However an alternative explanation could be due to differences in study design, with existing studies of cell-mediated immunity having more power to detect an effect than studies of humoral immunity. This is demonstrated by our simple Power Score which shows that studies of the cell-mediated response had higher average power to detect an association than studies of antibody-mediated responses.

Designing a study to investigate the relationship between PE immune responses and protection from infection can be viewed as an optimisation problem with the aim of maximising the statistical power to detect an effect, and ensuring the effect size is estimated correctly, given real world limitations on time and resources. There will be a trade-off between the number of volunteers recruited, the frequency of sampling, the duration of follow up, and the number of immune responses measured. The optimal study design will depend on numerous local characteristics such as transmission intensity and the age of volunteers, but in general, statistical power can be maximised by increasing the number of study volunteers, and performing the study in a high transmission setting. The easiest thing to do to maximise statistical power to detect PE immunity is to use an appropriate statistical test. Treating the immune response as a continuous as opposed to a binary variable ensures that all collected data is utilised. Further adjusting for covariates such as LLIN use and location can improve the accuracy of statistical analyses. An example of a Cox PH analysis of the relationship between continuous antibody titres and clinical malaria adjusted for confounding variables can be found in the study by Greenhouse *et al* [226].

It may be beneficial to transform immune response data before analysis, for example antibody titres are often log-normally distributed in a cohort so it may be useful to investigate the relationship between the logarithm of antibody titre and protection from infection. Going beyond conventional statistical analyses such as logistic regression and Cox PH, biologically motivated models using dose-response curves for the relationship between immune response and protection from infection can be used to test for nonlinear phenomena such as threshold effects [139, 232].

Two of the most intractable problems encountered while investigating the relationship between immune responses and protection from malaria are correlation between immune responses, and the potential for immune responses to act as markers for exposure. A PE immune response to malaria is likely to be comprised of antibody- and cell-mediated responses directed against multiple parasite antigens [236]. These immune responses are likely to be substantially correlated as the acquisition of immunity depends on malaria exposure. Thus, if only a single immune response is measured, that immune response may be acting as a marker for the entire immune response. Measuring

multiple immune responses allows a more detailed investigation of the relationship between PE immunity and protection from malaria. However, even if multiple immune responses are measured, it may still be the case that immunity is conferred via some other undetected immune response correlated with the measured response. A potential solution to this problem may be to use microarray analysis where plasma from individuals exposed to malaria is tested against a large proportion of the *P. falciparum* proteome [71, 236].

Heterogeneity in malaria exposure will lead to heterogeneity in the rate of acquisition of immunity, with those individuals under the most intense exposure developing the strongest immune response [204]. Individuals identified as having a strong immune response may therefore be at increased risk of future infection compared to the rest of the cohort as their increased exposure may outweigh the benefits of a stronger immune response. This phenomenon may lead to a study identifying protective antibodies as a risk factor for future infection.

There will be variation in the measured naturally acquired PE immune responses across a cohort (Figure 3.3A). The ability of a statistical test to detect an effect of an immune response is conditional on there being enough variation in the immune response to detect corresponding variation in the outcome. For example, if all volunteers have strong immune responses with little variation, then a study will be unable to detect the effect of the immune response as there will be no volunteers with weak immune responses to compare against. The need to have sufficient variation in immune response to detect an effect isn't encountered in trials of vaccine-induced immune responses. Vaccine trials are designed to have a vaccine cohort with high vaccine-induced immune responses (if the vaccine is sufficiently immunogenic) and a control cohort with low immune responses.

3.5. Conclusion

The results of studies to investigate the association between markers of pre-erythrocytic immunity and protection from infection have often been inconclusive due to sub-optimal study design. The power to detect an effect of a pre-erythrocytic immune response can be increased by increasing study size, performing longitudinal follow-up and analysing immune responses as a continuous variable using Cox proportional hazards. In addition, mathematical models based on dose-response curves may be used to identify non-linear relationships between markers of pre-erythrocytic immunity and protection from infection that may be missed by conventional statistical analyses.

Table 3.3: Review of quantitative studies of the relationship between pre-erythrocytic immune responses and protection from malaria infection. Statistical results labelled * indicate that immune markers are positively associated with increased risk of infection.

Location	Cohort	Transmission	Immune response	Significance	Study details	Malaria endpoint	Notes	Score	Ref
Nyanza, Kenya	77 adults	EIR = 120	CSP IgG TRAP IgG LSA-1 IgG Multiple IgG	NS (68) NS (68) NS (68) P = 0.006 (68)	Weekly ADI for 12 weeks	infection	Participants split as high-low. High titres to 3 antigens: HR = 0.43 (0.23 – 0.80).	2	[237]
Uasin Gishu, Kenya	71 adults 37 children		CSP IgG TRAP IgG LSA-1 IgG CSP IgG TRAP IgG	NS (50) NS (50) NS (50) NS (32) P = 0.108 (32)	Weekly ADI for 10 weeks	infection	Participants split as positive/negative.	2	[222]
Ndiop, Senegal	110 all age groups		CSP IgG	NS (110)	Weekly ADI 11 weeks, clinical malaria for 21 weeks	infection	Participants split as high-low. CSP was significantly associated with duration of asymptomatic carriage.	3	[228]
Asembo Bay, Kenya	79 infants	EIR = 2-10	CSP IgG LSA1 IgG	NS (51) NS (51)	Monthly ADI 1 year	infection	Associations tested for were between maternal IgG and infection. Possible maternal IgG confounding.	1	[238]
Mali	39 young children, 66 older children, 88 adults		TRAP IgG	P=0.005 (66)	ADI every 2 weeks for 30 weeks	infection p/µL > 5000	PH linear regression	2	[239]
Thailand	135 adults		CSP IgG	NS (53)	Every 10 days for 2 months then PCD for clinical malaria	Infection	Comparison of titres in infected volunteers.	2	[240]
Saradidi, Kenya	83 adults	EIR≈135	CSP IgG	NS (83)	ADI every 2 weeks for 98 days	infection	Linear regression between titre and day of parasitemia	2	[241]

Table 3.3 (contd.): Review of quantitative studies of the relationship between pre-erythrocytic immune responses and protection from malaria infection. Statistical results labelled * indicate that immune markers are positively associated with increased risk of infection.

Location	Cohort	Transmission	Immune response	Significance	Study details	Malaria endpoint	Notes	Score	Ref
Farafenni, The Gambia	Children 1-11 years Adults 12+		CSP IgG CSP IgG	P = 0.01 (124) NS (64)	Weekly morbidity survey, fortnightly clinic, Cross-sectional comparison at beginning and end of season	infection infection	Participants split into sero-positive/sero-negative. Significance for parasitemia tested with χ^2 test.	2	[242]
Gabon	All ages		CSP IgG	NS (144)	2 cross sections	infection		1	[243]
Thailand	140 children 5-15 years		CSP IgG	P = 0.07 (17 +34)	17 cases identified, and 34 controls matched. Weekly ADI for 25 weeks	infection	Participants were split into low and high titres.	1	[244]
Farafenni, The Gambia	376 children	EIR=25 in adults, EIR<6 in children	CSP IgG	P < 0.01 (292)*	2 cross sections at beginning and end of rainy season	infection	CSP was positively associated with parasitemia but negatively associated with protection.	1	[245]
Burkina Faso	All ages – 3212 serum samples	Seasonal EIR≈3-300	CSP IgG	NS	3 cross sections, every 2 months	infection	Participants split into high low titres.	1	[246]
Dienga, Gabon	61 children		CSP IgG $(NANP)_5$ CSP IgG $(NAAG)_5$ LSA-1 Rep IgG LSA-1 J IgG	P=0.82 (n=25) P=0.84 (n=25) P=0.08 (n=25) P=0.051 (n=25)	ADI weekly then bi-weekly until 30 weeks	infection	Participants split by sero-positive/sero-negative	1	[234]
Uasin Gishu, Kenya	Children and adults		LSA-1 IgG LSA-1 cell proliferation (SI)	NS (93) NS (57)	Weekly ADI for 10 weeks	Infection	Participants split as positive/negative	2	[247]

Table 3.3 (contd.): Review of quantitative studies of the relationship between pre-erythrocytic immune responses and protection from malaria infection. Statistical results labelled * indicate that immune markers are positively associated with increased risk of infection.

Location	Cohort	Transmission	Immune response	Significance	Study details	Malaria endpoint	Notes	Score	Ref
Uasin Gishu, Kenya	Children and adults		LSA-1 IL-5 LSA-1 IL-10 LSA-1 IFN-γ LAS-1 TNF-α	NS (69) P=0.083 (77) NS (94) NS (54)	Weekly ADI 10 weeks	infection	Participants split as responder/non-responder	2	[248]
Asembo Bay, Kenya	107 children 6 months – 2 years	EIR = 100-200	CSP, TRAP LSA-1 IFN-γ	P=0.007 (107)	ADI	infection	Participants split into responders/non-responders.	2	[249]
Nyanza, Kenya	178 adult males, 12-35 years	EIR ≈ 300	LSA-1 IL-10	P=0.05 (137)	Weekly ADI for 16 weeks	infection	Participants split into responders/non-responders.	3	[250]
Kilifi, Kenya	217 all ages, 1 month to 81 years	EIR = 10 (3-15)	TRAP IFN-γ	P =0.05 (138) NS P=0.73 (138)	Weekly ADI 6 months	infection in 2 months infection in 6 months	Participants split into responders/non-responders.	3	[251]
Farafenni, The Gambia	391 children, 3-8 years		CSP-specific T cells	P=0.04	ADI weekly	infection	Th3R peptide, multiple logistic regression	3	[252]
Papua New Guinea	55 adults 17-88 years		LSA-1 IFN-γ LSA-1 IgG	P<0.001 (n=38) NS (n=38)	Cross-sections at 6 month intervals	infection	Only the aa 84-107 peptide tested significant. 3 others did not.	1	[253]
Gabon	100 children with mild malaria 100 children with severe malaria	EIR = 10-100 EIR = 10-100	LSA-1 IFN-γ LSA-1 IFN-γ	P<.001 (n=100) NS (n=100)	Case matching. ADI every 2 weeks after discharge from hospital.	infection		2	[254]

Table 3.4: Review of quantitative studies of the relationship between pre-erythrocytic immune responses and protection from clinical malaria. Statistical results labelled * indicate that immune markers are positively associated with increased risk of clinical malaria.

Location	Cohort	Transmission	Immune response	Significance	Study details	Malaria endpoint	Notes	Score	Ref
Farafenni, The Gambia	Children 1-11		CSP IgG	P = 0.08 (126)	Weekly morbidity survey, fortnightly clinic, Cross-sectional comparison at beginning and end of season	clinical malaria	Participants split into sero-positive/sero-negative. Clinical malaria tested with t-test.	2	[242]
Farafenni, The Gambia	376 children	EIR=25 in adults, EIR<6 in children	CSP IgG	NS (317)	2 cross sections at beginning and end of rainy season	fever + parasite mia		1	[245]
Nyanza, Kenya	86 children 3 months – 8 years		CSP IgG TRAP IgG LSA-1 IgG Multiple IgG	NS (83) NS (83) P=0.02 (83) P=0.009 (83)	ACD 52 weeks	clinical malaria	Participants split as high-low. High titres to 3 antigens: HR = 0.29 (0.10 – 0.81)	2	[255]
Kilombero, Tanzania	83 infants	EIR≈365	CSP IgG LSA-1 IgG	NS (83) NS (83)	PCD	clinical malaria	Cox PH using titration units. Possible maternal IgG confounding.	3	[256]
Balonghin, Burkina Faso	244 children 6 months 10 years	EIR<10	CSP IgG	P = 0.12 (124)	ACD for clinical malaria during peak transmission	clinical malaria	HR = 0.74 (0.50-1.09). Participants split into responder/non-responder.	3	[225]
Tensobentenga, Burkina Faso	142 children 6 months 10 years	EIR>100	CSP IgG	P=0.012 (29)			HR = 0.37 (0.17-0.80). Participants split into responder/non-responder.	2	
Bamako, Mali	327 children aged 1-12 years		TRAP 1.0 IgG TRAP C IgG CSP IgG	P=0.32 (n=327) P=0.003 (n=327) P=0.0735 (n=327)	case matching in hospitals	cerebral and clinical malaria	85 children admitted to hospital case-matched to 242 children with uncomplicated malaria. Comparisons made between seroprevalence of cerebral and uncomplicated cases.	1	[257]

Table 3.4 (contd.): Review of quantitative studies of the relationship between pre-erythrocytic immune responses and protection from clinical malaria. Statistical results labelled * indicate that immune markers are positively associated with increased risk of clinical malaria.

Location	Cohort	Transmission	Immune response	Significance	Study details	Malaria endpoint	Notes	Score	Ref
Bancoumana, Mali	285 children 1-9 years		TRAP IgG CSP IgG	NS NS	5 serological cross-sections and one longitudinal follow up.		Article in French.	2	[258]
Kampala, Uganda	414 Children 1-4 years		CSP IgG LSA-1 IgG	P=0.01* P=0.02*	Monthly ACD.	clinical malaria	Cox PH adjusted for covariates. Continuous antibody response.	4	[226]
Kisumu, Kenya	Adults and children	High trans: *Pf*Pr_{0-5}= 95%. Low trans: *Pf*PR_{0-5}=5%	TRAP IFN-γ LSA-1 IFN-γ	NS (38) P=0.02 (38)	ACD 36 weeks	clinical malaria	Participants split as responder/non-responder. LSA-1 T3 peptide measured by ELISA, high trans	1	[259]
Ngerenya, Kenya	112 all ages, 1 months to 81 years	EIR = 10	Cultured TRAP Cultured CSP $CD4^{+}CD25^{high}$	P=0.028 (94) P=0.16 (80) P=0.039 (108)	Weekly ACD for 300 days	clinical malaria	Logistic multivariate analysis with continuous variable.	3 3 4	[260]
Dienga, Gabon	300 children		LSA-1 Rep IgG LSA-1 IFN-γ and IL-10 LSA-1 CTL IFN-γ and IL-10	P=0.065 (n=76) P=0.024 (n=78) P=0.036 (n=78)	ACD bi-weekly	clinical malaria	Relationships were significant in Dienga but not Pouma.	2	[261]
Pouma, Cameroon	186 children		LSA-1 IgG LSA-1 IFN-γ and IL-10	NS NS					

Chapter 4. Efficacy Model for Antibody-mediated Pre-erythrocytic Malaria Vaccines

Published as: White MT, Griffin JT, Riley EM, Drakeley CJ, Moorman AM, Sumba PO, Kazura JW, Ghani AC, John CC: *Efficacy model for antibody-mediated pre-erythrocytic malaria vaccines.* Proceedings of the Royal Society B-Biological Sciences 2011, 278(1710):1298-1305.

In the previous chapter I reviewed the association between markers of pre-erythrocytic immunity and protection from infection. In this chapter I will demonstrate how the relationship between antibodies to the sporozoite antigens circumsporozoite protein, thrombospondin-related adhesive protein and liver-stage antigen 1, and protection from infection can be estimated by fitting models to data from a treatment re-infection study in Kenyan adults. Using this relationship between naturally acquired antibodies and protection from infection, I estimate the effect of anti-CSP and anti-TRAP antibody boosting vaccines on protection from *P. falciparum* infection.

4.1. Introduction

The outcome of an infectious bite is often viewed as a binary event in which the host either does or does not develop blood-stage malaria. However every bite can inject from 0 to 100+ sporozoites [262, 263] with the probability of blood-stage infection increasing for larger doses. Sporozoites that have been deposited in the skin or capillaries will remain at the injection site for up to an hour before trickling into the blood stream and migrating to the liver [209, 264]. Sporozoites are susceptible to antibody opsonisation from immunoglobulin G antibodies recognising sporozoite antigens at any stage in this journey [66].

Antibodies to the pre-erythrocytic antigens circumsporozoite protein (CSP), thrombospondin-related adhesive protein (TRAP) and liver-stage antigen 1 (LSA-1) have been shown to correlate with protection from *P. falciparum* infection in field studies [222, 237, 255]. CSP covers the entire surface of the sporozoite and is found on the plasma membrane of liver-stage parasites [72]. Antibodies to CSP immobilise sporozoites and inhibit parasite invasion of hepatocytes [73]. TRAP is found primarily within the sporozoite's micronemes and on the sporozoite surface [74]. Antibodies to TRAP inhibit sporozoite gliding motility [265] and hepatocyte invasion [75], however there is some evidence to suggest that anti-TRAP antibodies do not inhibit sporozoite infectivity *in vivo* [266]. LSA-1 is expressed soon after the sporozoite invades the hepatocyte in the liver [76]. As LSA-1 is only expressed inside the hepatocyte, which antibodies are unable to access, anti-LSA1 antibodies are not expected to provide protection from infection although LSA-1 is a likely target of cell-mediated immunity [76]. As pre-erythrocytic antibodies are directed at different aspects of sporozoite biology they are likely to interact cooperatively in the prevention of infection.

John *et al* [237] demonstrated a significant correlation between titres to the pre-erythrocytic antigens CSP, TRAP and LSA-1 and protection from infection. Individuals with titres to all three antibodies above a cut-off of 2 arbitrary units

(AU) were found to have a reduced risk of infection of 0.43 (95% CI, 0.23 – 0.80) compared to individuals with low antibody titres. Here we develop a mathematical model to characterise the relationship between pre-erythrocytic antibody levels and the probability of infection per infectious bite based on these data. This relationship is then used to explore the efficacy of antibody boosting vaccines based on combinations of the antigens CSP and TRAP.

4.2. Methods

4.2.1. Data

We analysed data on IgG antibody titres to CSP, TRAP and LSA-1, as well as the blood-stage antigens apical membrane antigen-1 (AMA-1), erythrocyte binding antigen-175 (EBA-175) and merozoite surface protein-1 (MSP-1), and time to re-infection with *P. falciparum,* from a study of 68 adults conducted in Kanyawegi, Nyanza Province, Kenya from August to December 2001. Approval for this study was obtained from the Ethical Review Board for the Kenya Medical Research Institute and University Hospitals of Cleveland at Case Western Reserve University. Full details of the trial can be found in John *et al* [237]. In brief, the population of Kanyawegi is approximately 3,500 and is located in a malaria-endemic region with an approximate entomological inoculation rate (EIR) of 120 infectious bites per person per year [267]. Individuals agreeing to participate were given quinine for five days and doxycyline for seven days to clear parasitemia, and then followed for malaria infection by microscopic inspection of blood smears obtained weekly for 14 weeks. *P. falciparum* infection was confirmed by PCR. Individuals who missed more than two weeks of blood smear testing were included in analysis up to the time of their last blood smear. Blood for laboratory studies to measure antibody titres was obtained by venepuncture prior to anti-malarial treatment. Antibody titres were measured in arbitrary units (AU) [237]. The IgG antibody titres to CSP, TRAP and LSA-1 were approximately Log-Normally distributed with mean and standard deviation of 6.83 (5.54) AU, 4.80 (4.31) AU and 8.60 (12.34) AU respectively.

4.2.2. Probability of infection per infectious bite

Data from experiments by Beier *et al* [262, 263] suggest that the number of sporozoites injected per bite follows an approximately Geometric distribution with arithmetic mean 10 and range 0-100+. For an infectious bite to progress to blood-stage malaria just one sporozoite must evade the pre-erythrocytic immune response. Denote the probability that a single sporozoite causes blood-stage malaria infection by $p_1 = r$. If sporozoites from an infectious bite act independently, the probability that *k* sporozoites cause blood-stage malaria is $p_k = 1-(1-r)^k$. If the distribution of the number of sporozoites per bite follows a Geometric distribution with mean *n*, then the probability that *k* sporozoites will be injected is $\left(1-\frac{1}{n}\right)^{k-1}\frac{1}{n}$. Thus the probability that a bite will cause blood-stage malaria infection, *b,* is:

$$b = \sum_{k=1}^{\infty} \left(1 - \frac{1}{n}\right)^{k-1} \frac{1}{n} \; 1 - (1-r)^k \; = \frac{nr}{(n-1)r+1}.$$

4.2.3. Dose-response curves

We assumed that IgG antibodies to each of the CSP, TRAP and LSA-1 antigens will induce the opsonisation and phagocytosis of invading sporozoites as they make their way from the skin to the liver, and hence antibody titres constitute a correlate of risk [268] with higher titres reducing the probability infection. The relationship between IgG antibody titre and the probability of a sporozoite evading the associated immune response is modelled using a dose-response curve. These fall into two qualitatively different categories: (i) convex where increases in dose lead to reduced increases in response; and (ii) threshold where the antibody response has a significant effect only once a threshold dose has been reached (Figure 4.1). A convex dose-response will result in leaky immunity and a threshold dose-response will give rise to all-or-nothing immunity.

Let x_{ij} be the IgG antibody titre to antigen *j* in individual *i*. Let r_{ij} be the probability that a sporozoite survives the immune response to antigen *j* in individual *i*. For a Hill function dose-response in individual *i* with antibody titre to antigen *j* of x_{ij} the probability that a sporozoite evades that antibody's immune response is

$$r_{ij} = \frac{1}{1 + \left(\frac{x_{ij}}{\beta_j}\right)^{\alpha_j}}$$

where β_j is the antibody titre that reduces the probability of a single sporozoite infection by half and α_j is a shape parameter. $\alpha_j \leq 1$ gives a convex dose response-curve and $\alpha_j > 1$ gives a threshold dose-response curve. For an exponential dose-response in individual *i* the probability that a sporozoite evades the immune response to antibody *j* is

$$r_{ij} = e^{-\frac{x_{ij}\log(2)}{\beta_j}}$$

where β_j is the antibody titre that reduces the probability of sporozoite infection by half. An exponential function is always convex.

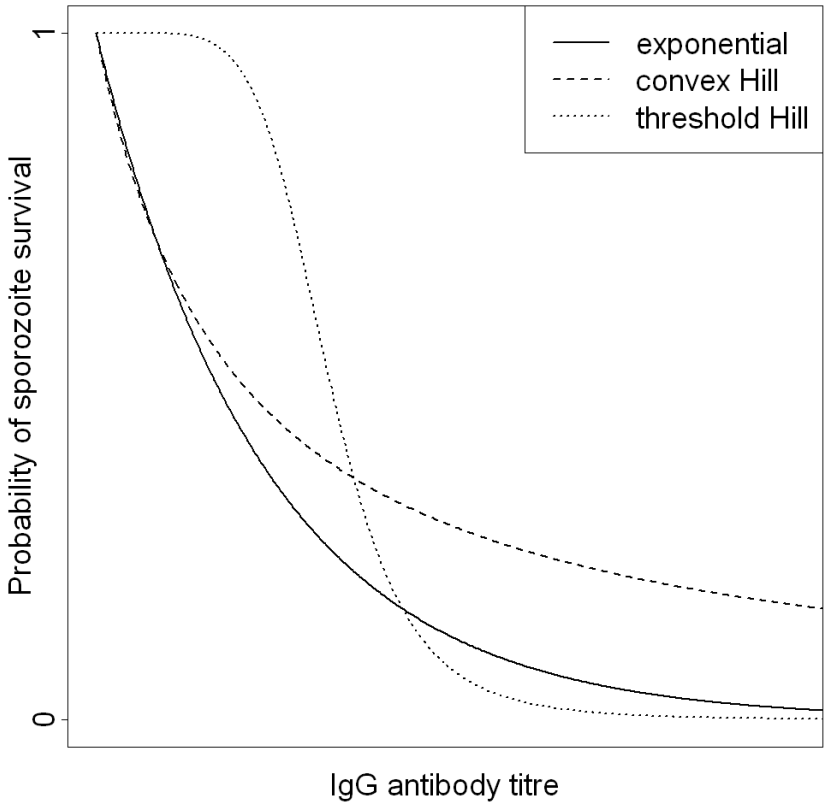


Figure 4.1: Example dose-response curves for the probability of sporozoite survival as a function of IgG antibody titre. The solid and dashed curves depict a convex dose-response relationship where the antibody is effective even at low levels. The dotted curve depicts a threshold dose-response curve where the antibody only becomes effective once a threshold has been reached.

4.2.4. Pre-erythrocytic immune response

For a sporozoite to successfully evade the pre-erythrocytic immune response it must survive the attack from each of the different antibodies and the cell-mediated immune response. Following the approach of Saul *et al* [269, 270] we assume each immune response is independent of the others so that $r_i = r_0 r_{i,\mathrm{CSP}} r_{i,\mathrm{TRAP}} r_{i,\mathrm{LSA}}$, where r_0 is the probability of a sporozoite evading the cell-mediated immune response in the absence of antibody. An alternative model incorporating interactions was also considered (see 4.6. Appendix 1). The probability that a bite from an infectious mosquito causes blood-stage infection is therefore

$$b_i = \frac{n r_i}{(n-1) r_i + 1}$$

Given a constant EIR ε and infection probability b_i, the probability that individual *i* will have developed blood-stage infection by time *t* will be $I(t) = 1 - e^{-\varepsilon b_i (t - t_L)}$ where $t_L = 10$ days is the time between sporozoite inoculation and the appearance of detectable blood-stage parasites.

4.2.5. Parameter estimation

The parameters α, β and r_0 were estimated by fitting this model to the data on time to re-infection using maximum likelihood methods. The data used are the antibody titres $x = (x_{\text{CSP}}, x_{\text{TRAP}}, x_{\text{LSA}})$, and the time to re-infection or final censoring *t*.

For individuals infected in the trial, the likelihood of them developing infection at time t_i is

$$\varepsilon b_i e^{-\varepsilon b_i (t_i - t_L)}$$

whilst for those censored at time t_i the likelihood of remaining free from infection is

$$e^{-\varepsilon b_i (t_i - t_L)}$$

If individuals $(1...m)$ become infected and individuals $(m+1...I)$ are censored, the data likelihood is

$$L(\alpha, \beta, r_0 \,|\, x, t) = \prod_{i=1}^{m} \varepsilon b_i e^{-\varepsilon b_i (t_i - t_L)} \prod_{i=m+1}^{I} e^{-\varepsilon b_i (t_i - t_L)} = \prod_{i=1}^{m} \varepsilon b_i \prod_{i=1}^{I} e^{-\varepsilon b_i (t_i - t_L)}$$

Model fits were compared using the Akaike Information Criterion (AIC). 95% confidence intervals for the model parameters were estimated by calculating the inverse hessian of the log-likelihood surface at the maximum likelihood estimate. When fitting the model using Hill functions, an exponential function was retained for the LSA-1 dose-response curve as this was better able to model the low level of protection offered by anti-LSA1 antibodies.

In the model described here the pre-erythrocytic immune response is directed towards sporozoites, with the assumption that individual sporozoites act independently of each other. A model where the immune response was directed towards the infectious bite as a whole was also fitted. This gave an inferior fit to the data and predicted similar results to the sporozoite model, but it was not possible to distinguish between the two assumptions with the available data.

4.2.6. Infection probability of humans

Let x_i be the vector of antibody titres from study participant *i* and assume that each data point x_i is sampled from a multivariate Log-Normal distribution. Then $y = \log(x)$ follows the distribution

$$\varphi(y) = \frac{1}{(2\pi)^{3/2} \det(V)^{1/2}} \exp\left(-\frac{1}{2} (y - \mu)^T V^{-1} (y - \mu) \right)$$

where $\mu = \text{mean}(\log(x_i))$ is the mean of the observed log antibody titres, and V is the covariance matrix of the data. The mean transmission efficiency b_{base} is obtained by integrating over the range of all possible titres to the three antibodies.

$$b_{\text{base}} = \int_{R^3} b(y)\varphi(y)dy$$

where $b(y)$ is the infection probability given log antibody titre y.

4.2.7. Antibody-boosting pre-erythrocytic vaccination

If antibody titres to antigen *j* in a population are Log-Normally distributed before vaccination, $x_j \sim \ln \text{N}\ \mu_{\text{base}}, V_{jj}$, then the mean antibody titre is $\text{Ab}_{\text{base}} = e^{\mu_{\text{base}} + V_{jj}^2/2}$. Assume that vaccination will boost the antibody titres so that they are log-normally distributed about a higher mean, $x_j \sim \ln \text{N}\ \mu_{\text{vac}}, V_{jj}$, with $\mu_{\text{vac}} > \mu_{\text{base}}$. The mean antibody titre after vaccination is then $\text{Ab}_{\text{vac}} = e^{\mu_{\text{vac}} + V_{jj}^2/2}$. The mean vaccine-induced antibody boost is therefore $\text{Ab}_{\text{boost}} = \text{Ab}_{\text{vac}} - \text{Ab}_{\text{base}} = e^{V_{jj}^2/2}\ e^{\mu_{\text{vac}}} - e^{\mu_{\text{base}}}$. Hence the log antibody titres of the vaccinated population are Normally distributed with a mean of μ_{vac} and variance matrix *V*.

$$\varphi_{\text{vac}}(y) = \frac{1}{(2\pi)^{3/2}\det(V)^{1/2}} \exp\left(-\frac{1}{2}(y-\mu_{\text{vac}})^T V^{-1}(y-\mu_{\text{vac}})\right)$$

The probability that a vaccinated person will become infected after a bite from an infectious mosquito is

$$b_{\text{vac}} = \int_{R^3} b(y)\varphi_{\text{vac}}(y)dy$$

and vaccine efficacy is given by

$$VE = 1 - \frac{b_{\text{vac}}}{b_{\text{base}}}.$$

4.3. Results

4.3.1. Relationship between antibody titre and probability of infection

The exponential dose-response curves provided a better and more parsimonious fit than the Hill function dose-response curves (Table 4.1). The fitted dose-response curves for CSP and TRAP indicate that increased antibody titres will provide increased protection against sporozoite infection (Figure 4.2). In contrast, and in accordance with biological expectations, the fitted dose-response curve for LSA-1 indicates no significant role for anti-LSA-1 antibodies in protection from infection.

In the absence of any antibody response we estimated the probability of sporozoite survival r_0=0.011 (95% CI, 0.004-0.017). This captures failure of a sporozoite to initiate blood-stage infection due to cell-mediated immunity, chance (e.g. migrating to tissues other than the liver), and the possibility that inoculated sporozoites are immature or damaged [271]. There is expected to be a substantial T cell-mediated immune response directed at liver stage parasites as the data were collected from semi-immune adults in a region of stable malaria transmission.

Table 4.1: Maximum likelihood estimates for dose-response curve parameters (α, β) to each of the antigens CSP, TRAP and LSA-1, and the probability of sporozoite survival in the absence of antibodies (r_0). The Exponential dose-response model provides the most parsimonious fit based on the AIC.

Dose-response curve	MLE Parameter Estimates (95% confidence intervals)							Model Fit	
	r_0	β_{CSP}	β_{TRAP}	β_{LSA}	α_{CSP}	α_{TRAP}	α_{LSA}	$\log(L)$	AIC
Exponential	0.011 (0.005 – 0.017)	13.07 (0 – 27.96)	11.64 (0 – 27.75)	9085.91 (0 – 192936)	–	–	–	-223.7	455.5
Hill function	0.010 (0.000 – 0.033)	20.30 (0 – 274.3)	6.51 (0 – 49.3)	7777 (0 – 177461)	96.2 (0 – 8667)	0.77 (0 – 3.79)	–	-222.5	457.1

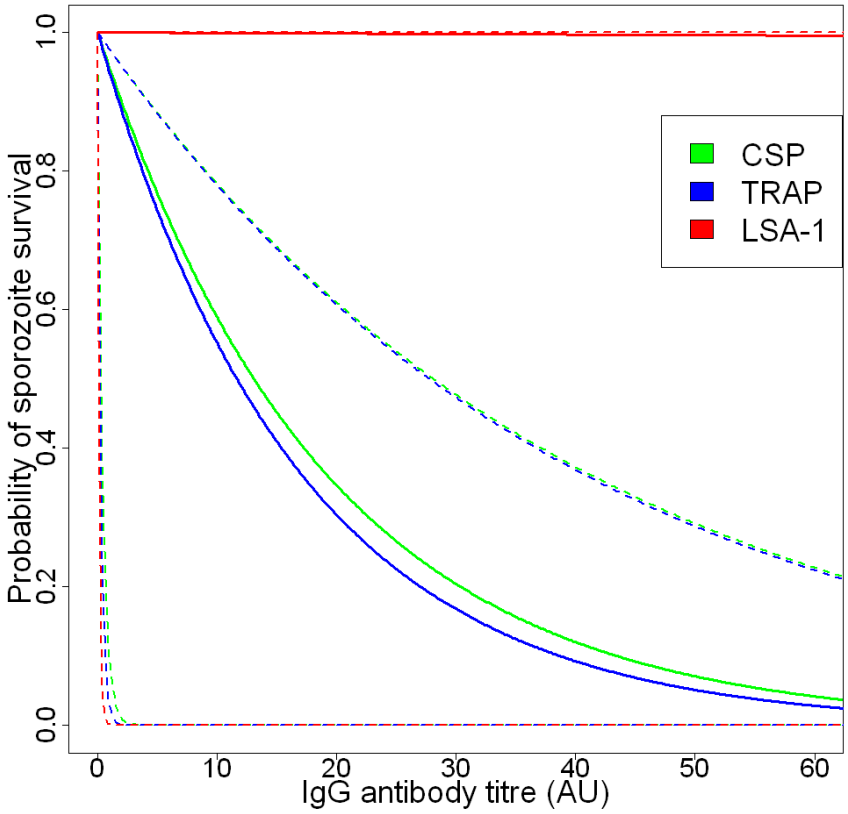


Figure 4.2: Estimated exponential dose-response curves for the relationship between probability of sporozoite survival and IgG antibody titre to each of the antigens CSP, TRAP and LSA-1. Dashed lines represent 95% confidence intervals for estimates.

4.3.2. Probability of infection per infectious bite

We estimate the mean transmission efficiency for this population to be b=0.056 which is in good agreement with the previously reported values of 0.012-0.086 [272] and 0.05-0.13 [273] for semi-immune adults in areas of intense malaria exposure. Figure 4.3 shows the estimated distribution of infection probability. Note that this variation is only due to variation in the IgG antibody titres as we assume the cell-mediated immune response is constant. The maximum infection probability is b=0.09 corresponding to the case of a person with very low IgG antibody titres and an average cell-mediated immune response.

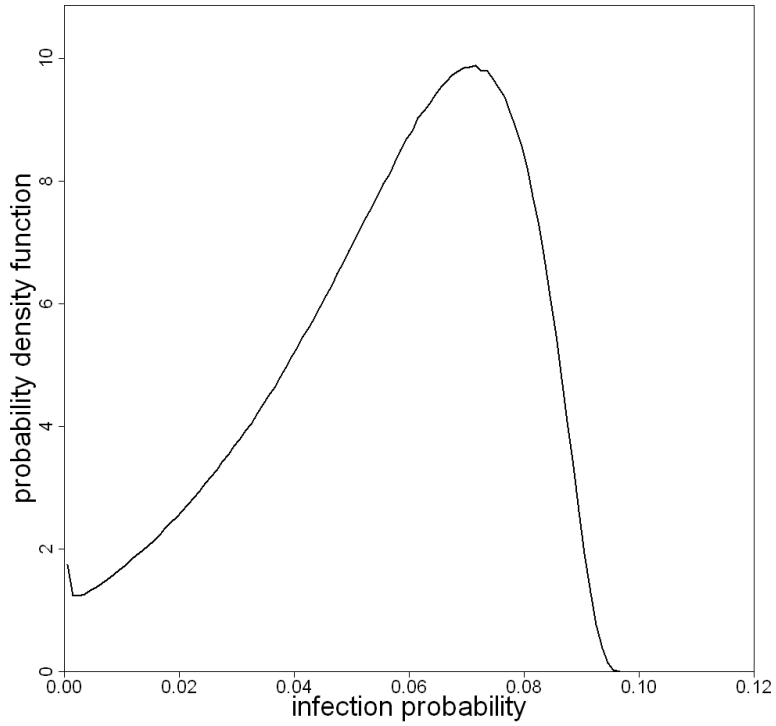


Figure 4.3: Estimated distribution of infection probability per infectious bite in the study population. The variation in infection probability represented here is due only to variation in the CSP, TRAP and LSA-1 IgG antibody titres.

4.3.3. Pre-erythrocytic vaccination

The maximum anti-CSP antibody titre observed in the study by John *et* al [237] was 27 AU, 4 times the mean anti-CSP titre. A vaccine that boosts average anti-CSP antibody titres above the baseline titre by this amount would have a predicted efficacy (VE) of 0.47 (Figure 4.4), assuming that the parameters estimated for the protection associated with naturally acquired antibody levels also apply to vaccine-induced antibodies. The maximum anti-TRAP antibody titre observed in the study was 22 AU, 4.5 times the mean anti-TRAP titre. A vaccine that boosts average anti-TRAP antibody titres by this amount would have a predicted efficacy of 0.52 (Figure 4.4). A multi-component vaccine combining CSP and TRAP is predicted to give an increase in efficacy of approximately 25% over a single component vaccine, with a predicted *VE* of 0.75 for a vaccine that gives a boost equal to the maximum anti-CSP and anti-TRAP antibody titres observed in the study (Figure 4.4).

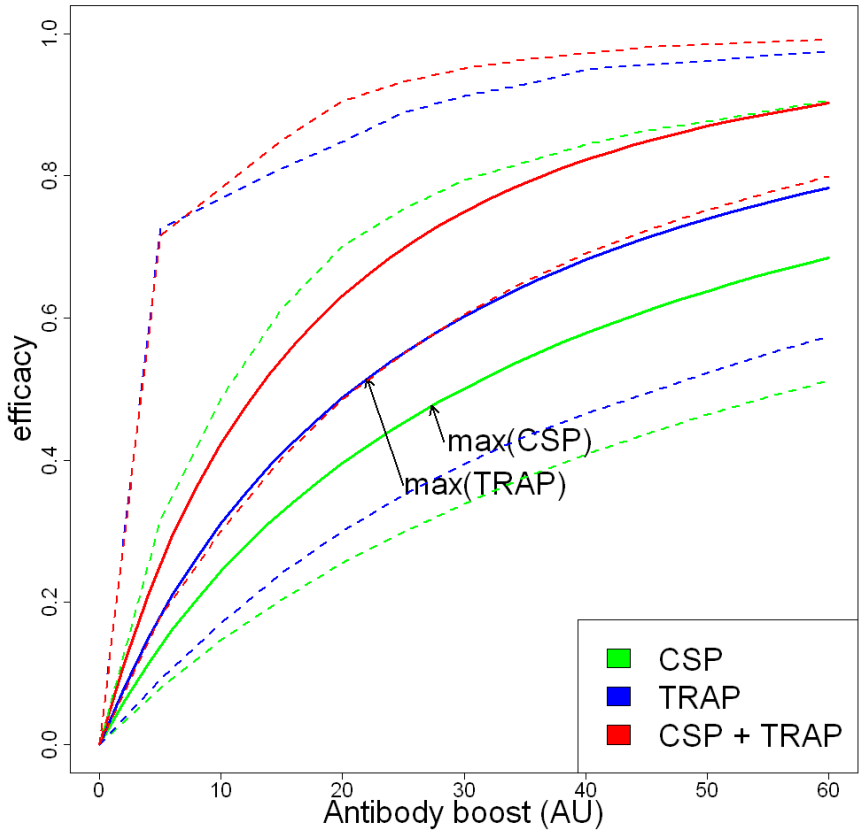


Figure 4.4: Predicted pre-erythrocytic vaccine efficacy using fixed boost vaccination. The maximum anti-CSP and anti-TRAP titres observed in the study participants are marked to indicate the vaccine efficacy that could be obtained by giving a boost equal to the maximum observed antibody titre. Dashed lines represent 95% confidence intervals.

4.3.4. Vaccine efficacy in different transmission settings

People living in a region of intense exposure to malaria will have a high baseline level of IgG antibodies. The benefit of a pre-erythrocytic vaccine that gives an antibody boost to a partially-immune individual is likely to depend on the vaccinee's baseline antibody level, as the relative increase in antibody levels due to boosting by vaccination may be highest in those people with a low baseline antibody level. Therefore when comparing the measured efficacy of a vaccine given in adults between regions of high and low malaria transmission, higher efficacy may be observed in the region of low transmission.

Using our model, the estimated additional efficacy over and above that already provided by naturally acquired immunity is observed to decrease monotonically as transmission intensity is increased (Figure 4.5). This result highlights the fact that if trials are undertaken in semi-immune adults with high baseline antibody titres, then the measured efficacy in terms of the proportion of infections averted could be lower than the efficacy measured in a population with lower baseline titres.

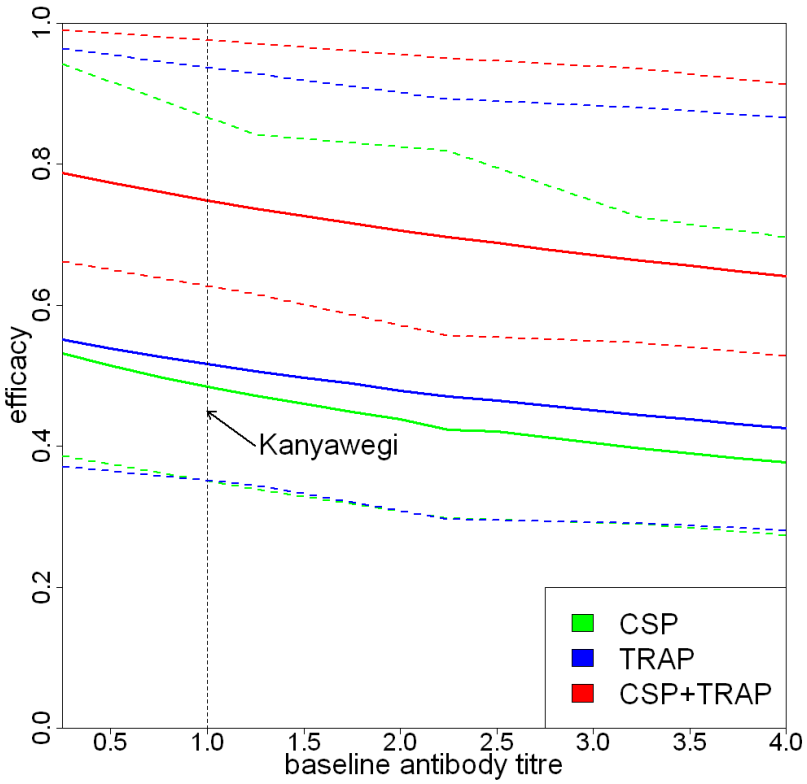


Figure 4.5: Predicted vaccine efficacy as a function of baseline antibody titre. The average baseline antibody titre relative to the study site at Kanyawegi is used as a marker of transmission intensity. Dashed lines represent 95% confidence intervals.

4.4. Discussion

Our results demonstrate a dose-response relationship between titres of antibodies to two of the pre-erythrocytic antigens, circumsporozoite protein (CSP) and thrombospondin-related adhesive protein (TRAP), and time to re-infection with *P. falciparum*. In contrast, we did not observe such a relationship between antibody titres for liver-stage antigen 1 (LSA-1) and time to re-infection. This is biologically plausible given that LSA-1 antigens are only expressed inside hepatocytes in the liver, where they are inaccessible to antibodies; the presence of these functionally redundant anti-LSA1 antibodies in the blood is likely due to immune recognition of the remains of eliminated parasites. The apparent correlation between anti-LSA1 antibody titres and protection from infection observed in field studies [237, 255] is therefore likely due to the correlation between anti-LSA-1 titres and titres of other effective pre-erythrocytic antibodies.

Despite the importance of the infection-blocking immune response, it is unclear whether it is primarily mediated by immune responses directed against sporozoites in the skin and liver, by a blood-stage response that clears emerging hepatic merozoites before replication, or by a blood-stage response that suppresses parasite density below detectable levels. The prevalence and titre of pre-erythrocytic and blood-stage antibodies are likely to be correlated with exposure to malaria, and hence with each other. We tested for such associations in our data by calculating the correlations between the pre-erythrocytic antibodies CSP, TRAP and LSA-1 and the blood-stage antibodies AMA-1, EBA-175 and MSP-1 (see 4.6. Appendix 1). Anti-CSP titres were not significantly correlated with antibody titres to the

other tested antigens suggesting that CSP has a genuine role in protection from infection. However, anti-CSP antibodies may be correlated with some other element of the pre-erythrocytic immune response not tested for [236]. Conversely, anti-TRAP titres were found to be highly correlated with anti-AMA-1 and anti-LSA-1 titres. Although AMA-1 is usually thought of as a blood-stage antigen, there is evidence that AMA-1 is expressed together with TRAP in the sporozoite micronemes during hepatocyte invasion [274]. Therefore anti-TRAP associated infection-blocking activity could be due to either direct anti-TRAP mediated inhibition of hepatocyte invasion, combined anti-TRAP and anti-AMA-1 inhibition of hepatocyte invasion, or simply reflect anti-AMA-1 mediated clearance of blood-stage parasites.

Several studies, including this one, have observed a role for naturally acquired pre-erythrocytic antibodies [222, 255] and naturally acquired cell-mediated immune responses [252] in preventing *P. falciparum* infection, although this association hasn't been observed in all studies [228]. On the other hand, some studies have concluded that pre-erythrocytic antibodies simply act as markers of exposure [242, 275], with protection from infection being mediated by correlated blood-stage immune responses Moreover, studies showing significant correlation between presence of parasitaemia before treatment and extended time to reinfection [216] or to symptomatic disease [276] suggest that blood-stage antibodies induced by previous infections are able to prevent subsequent infections. However, the observation that vaccine-induced pre-erythrocytic immune responses can prevent sporozoite infection [5, 129] provides strong evidence for the hypothesis that naturally acquired immune responses against the sporozoite prevent infection and are not just a marker for exposure.

Our model predicts that both CSP and TRAP-based malaria vaccines should be efficacious vaccine candidates. The most advanced candidate malaria vaccine, RTS,S, is a pre-erythrocytic CSP-based sub-unit vaccine inducing high anti-CSP IgG antibody titres [66, 129, 196] and moderate CSP-specific T cell responses [129, 277, 278] in vaccinated humans. Phase II trials have confirmed an association between anti-CSP IgG antibody titre and protection from infection in a challenge model [129, 279] and in the field [136]. Although the immunological mechanisms induced by RTS,S are not yet fully understood, it is believed that anti-CSP antibodies play a primary role in protection from infection, with a secondary role for CSP-specific $CD4^+$ T cell responses [231]. Our results agree with the observation from Phase II trials that RTS,S efficacy depends on the induced anti-CSP antibody titre. However, our model was fitted to antibody levels acquired under conditions of natural exposure and so care must be taken when extrapolating to the significantly higher titres observed after RTS,S vaccination. An additional caveat is that if an antibody is directly protective, we do not know how much of the protection is due to a direct causal mechanism, which is likely to confer protection in vaccine-induced boosting, and how much is due to the antibody's role as a marker of exposure and hence associated with other protective mechanisms, which would not confer protection in vaccine-induced boosting.

Our results suggest that a multi-component vaccine expressing both CSP and TRAP antigens could give an increase in efficacy of approximately 25% over a single component vaccine. A multi-component RTS,S/TRAP/AS02 vaccine has been trialled [280] but proved less efficacious than RTS,S/AS02 alone, possibly due to immunological interference

between the antigens. Despite this, considerable progress is being made in the development of a sub-unit pre-erythrocytic vaccine based on the TRAP sporozoite antigen. ME-TRAP, a heterologous prime-boost vaccine encoding the TRAP antigen and a string of T cell epitopes from various pre-erythrocytic antigens, offered 10% efficacy against infection in Gambian adults in a phase II trial [207]. However, protection from *P. falciparum* infection due to ME-TRAP is associated with high levels of interferon-γ secreting T cells [281], rather than IgG antibody titres, which were not explicitly included in our model. One limitation of our current model is the assumption that non-antibody mechanisms of protection such as cell-mediated immunity can be described by a constant factor r_0. This assumption will be considered further in the next chapter.

Our model can be used to investigate properties of vaccines other than efficacy. For example, evidence from clinical trials of RTS,S indicates that it is a leaky vaccine offering partial protection to everyone [133, 134]. This may be due to the convex dose-response curve we observed linking anti-CSP IgG antibody titre with protection from malaria infection. This does not, however, exclude the possibility of a threshold existing at the antibody titres induced by RTS,S which are an order of magnitude higher than naturally occurring titres [131]. The mechanics of sporozoite inoculation may further define vaccine type. When sporozoites are injected into the skin they remain at the bite site for up to an hour [209, 264]. Anti-sporozoite antibodies may opsonise and remove those remaining in the skin but fail to protect against those that enter the bloodstream quickly. Furthermore, the number of sporozoites injected during an infectious mosquito bite is highly variable [262, 263], so a vaccine may fail to prevent infection from bites injecting a large number of sporozoites. Therefore, despite the high anti-CSP antibody titres induced by RTS,S some sporozoites may still invade hepatocytes in the liver and initiate blood-stage infection. A combination vaccine incorporating LSA-1 may provide added protection by reducing the probability that infected hepatocytes survive to produce viable merozoites [76]. Even if a pre-erythrocytic vaccine fails to prevent infection, it can still provide partial protection by reducing the number of exo-erythrocytic forms in the liver [282], thus reducing the number of merozoites emerging from the liver and reducing the severity of blood-stage disease [283]. The increased efficacy against severe as compared to uncomplicated disease in Phase II trials of RTS,S may support this contention [118, 133].

RTS,S is currently undergoing extensive testing in Phase III clinical trials, enrolling up to 16,000 children and infants across 11 sites in 7 different African countries [15]. Our results predict that lower efficacy may be measured in the trial sites with the highest transmission intensity. This does not mean that the vaccine will be biologically less effective, rather, that in areas of high transmission intensity the ratio of infections prevented by naturally acquired pre-erythrocytic immunity (or maternally acquired antibodies) to infections prevented by vaccination will be higher than in areas of low transmission intensity. Continued sporozoite boosting in populations with higher exposure may also result in greater long-term vaccine efficacy and a need for fewer doses of vaccine, although there has been no evidence to date for this phenomenon.

Should RTS,S satisfy the necessary criteria to progress from Phase III trials to licensure, it will become the first fully-licensed malaria vaccine. After licensure the route to a second generation vaccine with improved efficacy could be a

multi-stage, multi-component vaccine based on RTS,S encoding a selection of pre-erythrocytic antigens such as TRAP and LSA-1, or blood-stage antigens such as AMA-1 and MSP-1 [284]. Our results suggest that if the problems of immunological interference can be overcome, the addition of a TRAP component to a CSP-based antibody boosting vaccine could provide significant improvements in efficacy.

4.5. Conclusion

This analysis demonstrates that in certain populations exposed to *P. falciparum* it is possible to estimate dose-response relationships between antibodies to the pre-erythrocytic antigens CSP and TRAP, and protection from infection. The relationships identified at antibody titres observed under conditions of natural exposure have been extrapolated to estimate the efficacy of vaccines that boost naturally acquired antibody titres. However it remains to be investigated whether there is a dose-response relationship between vaccine-induced antibody titres and protection from infection.

4.6. Appendix 1

4.6.1. Interaction between anti-CSP and anti-TRAP antibodies

In our analysis of the effects of anti-CSP and anti-TRAP antibodies on the opsonisation and phagocytosis of *P. falciparum* sporozoites, our null hypothesis (independent model) is that anti-CSP and anti-TRAP antibodies act independently of each other. We test this against the alternative hypothesis (interaction model) that anti-CSP and anti-TRAP antibodies interact with each other, either synergistically or antagonistically.

4.6.1.1. Interaction Model

In a pharmacological review on the effects of combinations of interacting drugs, Greco *et al* [285]outline methods for modelling combinations of interacting drugs from a response surface perspective. The outlined framework allows both synergy and antagonism between pairs of drugs to be modelled. We adapt their equations so as to be applicable to exponential dose-response relationships between the probability of sporozoite survival and titres to anti-CSP and anti-TRAP antibodies. The relationship between anti-CSP and anti-TRAP antibody titres and the probability of sporozoite survival can be defined by the following equation:

$$1 = \frac{x_{\text{CSP}} \log(2)}{\beta_{\text{CSP}} \log(1/r)} + \frac{x_{\text{TRAP}} \log(2)}{\beta_{\text{TRAP}} \log(1/r)} + \gamma \frac{x_{\text{CSP}} \log(2)}{\beta_{\text{CSP}} \log(1/r)} \frac{x_{\text{TRAP}} \log(2)}{\beta_{\text{TRAP}} \log(1/r)} \tag{1}$$

where r is the probability of sporozoite survival, x_i is the titre to antibody i, β_i is the titre to antibody i which gives a 50% reduction in the probability of sporozoite survival, and γ is an interaction term.

Rearranging (1) gives

$$\log(1/r)^2 - \left(\frac{x_{\text{CSP}}}{\beta_{\text{CSP}}} + \frac{x_{\text{TRAP}}}{\beta_{\text{TRAP}}} \right) \log(2) \log(1/r) - \gamma \log(2)^2 \frac{x_{\text{CSP}} x_{\text{TRAP}}}{\beta_{\text{CSP}} \beta_{\text{TRAP}}} = 0 \tag{2}$$

Now solving for $\log(1/r)$ gives

$$\begin{aligned} \log(1/r) &= \frac{\log(2)}{2} \left(\frac{x_{\text{CSP}}}{\beta_{\text{CSP}}} + \frac{x_{\text{TRAP}}}{\beta_{\text{TRAP}}} \right) \pm \sqrt{\left(\frac{\log(2)}{2} \right)^2 \left(\frac{x_{\text{CSP}}}{\beta_{\text{CSP}}} + \frac{x_{\text{TRAP}}}{\beta_{\text{TRAP}}} \right)^2 + \gamma \log(2)^2 \frac{x_{\text{CSP}} x_{\text{TRAP}}}{\beta_{\text{CSP}} \beta_{\text{TRAP}}}} \\ \log(1/r) &= \frac{\log(2)}{2} \left(\left(\frac{x_{\text{CSP}}}{\beta_{\text{CSP}}} + \frac{x_{\text{TRAP}}}{\beta_{\text{TRAP}}} \right) \pm \sqrt{\left(\frac{x_{\text{CSP}}}{\beta_{\text{CSP}}} + \frac{x_{\text{TRAP}}}{\beta_{\text{TRAP}}} \right)^2 + 4\gamma \frac{x_{\text{CSP}} x_{\text{TRAP}}}{\beta_{\text{CSP}} \beta_{\text{TRAP}}}} \right) \end{aligned} \tag{3}$$

And isolating r and choosing the positive solution gives

$$r = e^{-\frac{\log(2)}{2} \left(\left(\frac{x_{\text{CSP}}}{\beta_{\text{CSP}}} + \frac{x_{\text{TRAP}}}{\beta_{\text{TRAP}}} \right) + \sqrt{\left(\frac{x_{\text{CSP}}}{\beta_{\text{CSP}}} + \frac{x_{\text{TRAP}}}{\beta_{\text{TRAP}}} \right)^2 + 4\gamma \frac{x_{\text{CSP}} x_{\text{TRAP}}}{\beta_{\text{CSP}} \beta_{\text{TRAP}}}} \right)} \tag{4}$$

We can test for consistency by setting $\gamma = 0$

$$r = e^{-\log(2)\left(\frac{x_{\text{CSP}}}{\beta_{\text{CSP}}} + \frac{x_{\text{TRAP}}}{\beta_{\text{TRAP}}}\right)} = e^{-\log(2)\frac{x_{\text{CSP}}}{\beta_{\text{CSP}}}} e^{-\log(2)\frac{x_{\text{TRAP}}}{\beta_{\text{TRAP}}}} = r_{\text{CSP}} r_{\text{TRAP}} \quad (5)$$

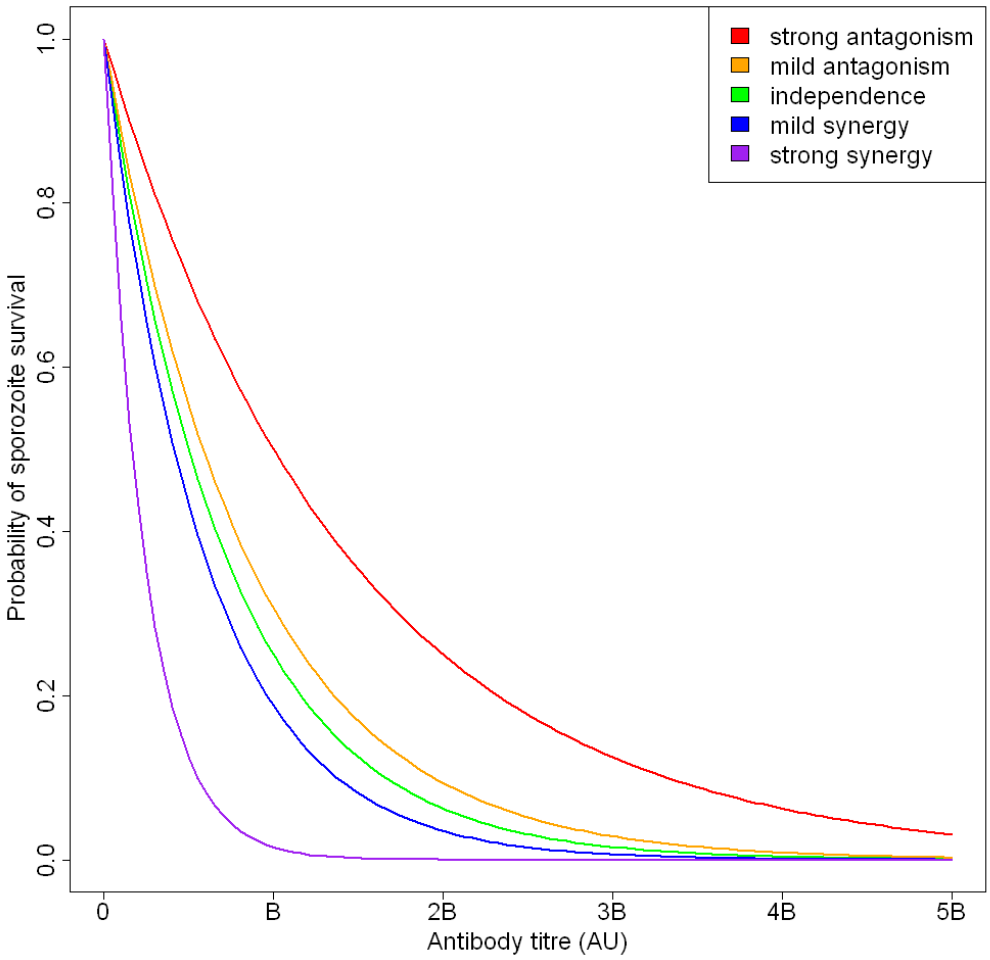
corresponding to our null hypothesis that anti-CSP and anti-TRAP titres act independently of each other.

4.6.1.2. Dependence of the probability of sporozoite survival on the interaction term γ

By looking at the special case where $x_{\text{CSP}} = x_{\text{TRAP}} = x$ and $\beta_{\text{CSP}} = \beta_{\text{TRAP}} = \beta$ we can investigate the dependence of the response on the interaction term γ

$$r = e^{-\log(2)\ 1+\sqrt{1+\gamma}\ \frac{x}{\beta}} \quad (6)$$

Setting $\gamma = 0$ gives $r = e^{-2\log(2)\frac{x}{\beta}}$ which once again corresponds to the assumption of independence of antibody titres. Setting $\gamma = -1$ gives $r = e^{-\log(2)\frac{x}{\beta}}$ which corresponds to the antagonistic case where a combination of titres is only as effective as each of the titres on their own. Note that mutual inhibition where each antibody actively inhibits the other resulting in a combined effect less than either of the individual effects cannot be modelled in this framework. Appendix Figure 4.1 shows the dose-response curves for a combination of two antibodies with interaction ranging from strongly antagonistic to strongly synergistic.



Appendix Figure 4.1: Dose-response curves for a combination of two antibodies of equal titre. B is the titre required for a single antibody response to kill 50% of sporozoites. γ > 0 results in synergistic interaction between antibodies, γ = 0 results in independent interaction between antibodies, and -1 < γ < 0 results in antagonistic interaction between antibodies.

4.6.1.3. Likelihood ratio test

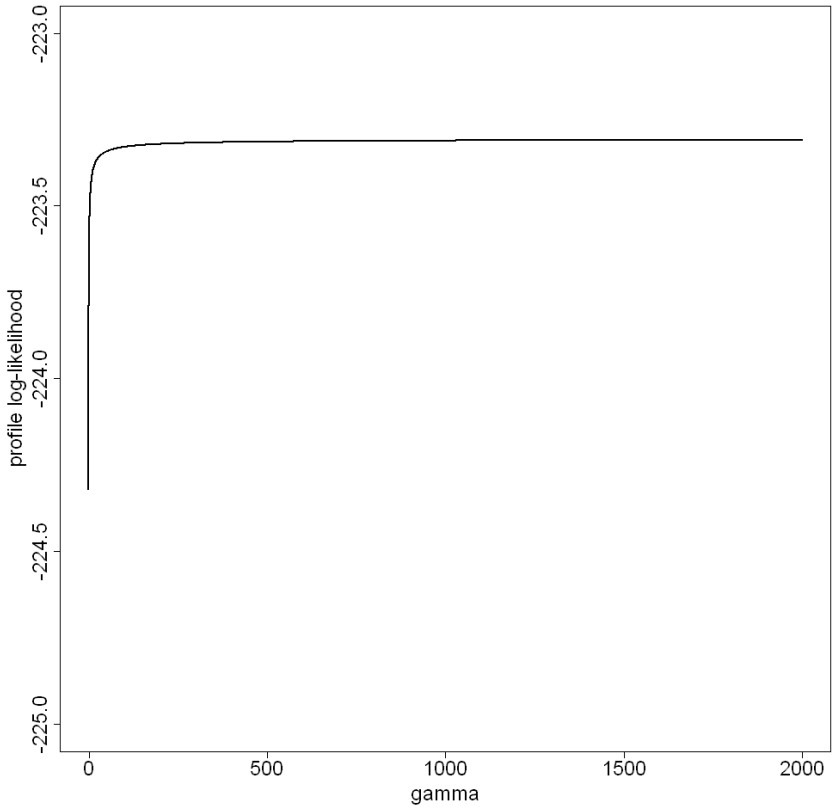
As the null hypothesis is nested within the alternative hypothesis, they can be compared using a likelihood ratio test. Fitting both models gives the results shown in Appendix Table 4.1. The likelihood ratio statistic is $\Lambda = 0.89$ (p=0.35) and thus we cannot identify an interaction effect from these data.

Appendix Table 4.1: Comparison of independent and interaction models.

Model	MLE Parameter Estimates				Model Fit	
	r_0	β_{CSP}	β_{TRAP}	γ	$\log(L)$	AIC
Independent	0.011	13.07	11.64	-	-223.7	453.5
Interaction	0.011	151.84	172.23	962.7	-223.2	454.6

Interestingly for the interaction model we get a maximum likelihood estimate of $\gamma = 962.7$ for the interaction term suggesting very strong synergy.

To further investigate the dependence of our model fit on γ we plot the profile log-likelihood in Appendix Figure 4.2. We see that the profile log-likelihood surface is very flat apart from a small fall in the region $-1 < \gamma < 0$. Thus it is not possible to identify significant interactions between the antibody responses and outcome in these data.



Appendix Figure 4.2: Profile log-likelihood for γ. Although the maximum value of the log-likelihood is given at γ = 962.7 the very flat shape of the profile log-likelihood demonstrates that there is very large uncertainty in this estimate.

4.6.2. Correlation between multiple antibody titres

Appendix Table 4.2: Analysis of Spearman rank correlation between antibody titres to the studied antigens. NS = not significant. [a] Not significant after Bonferroni correction.

	Relationship between antibody response and antigens					
Antigen	CSP	TRAP	LSA-1	AMA-1	EBA-175	MSP-1
CSP	_	_	_	_	_	_
TRAP	0.0629 NS	_	_	_	_	_
LSA-1	0.0641 NS	0.5033 1.21e-05	_	_	_	_
AMA-1	-0.1075 NS	0.3974 7.89e-04	0.3753 0.0016	_	_	_
EBA-175	0.1820 NS	0.0545 NS	0.1998 NS	0.1447 NS	_	_
MSP-1	0.1338 NS	0.3629 0.0023	0.3107 0.0099[a]	0.1499 NS	0.2599 0.0322[a]	_

Chapter 5. Mathematical modelling of the relationship between RTS,S vaccine-induced antibody levels, T cell mediated responses and protection against *Plasmodium falciparum* infection in malaria-naïve adults

In the previous chapter we investigated how the relationship between naturally acquired pre-erythrocytic antibodies and protection from infection can be estimated by fitting dose-response curves to data from treatment re-infection studies. In this chapter we use a similar methodology to estimate the relationship between anti-CSP antibodies, CSP-specific T cells and protection from infection in malaria-naïve volunteers vaccinated with RTS,S/AS02 and RTS,S/AS01.

5.1. Introduction

RTS,S comprises a chimaeric recombinant polypeptide encoding more or less the entire circumsporozoite protein (CSP) linked to recombinant S antigen from the hepatitis B virus (HBs); this polypeptide is formulated with additional HBs and one of two alternative adjuvant systems, AS02 and AS01. AS02 is an adjuvant system that contains an oil-in-water emulsion and the immunostimulants monophosphoryl lipid A (MPL) and QS21 [127]. AS01 is a related liposome-based adjuvant system that also contains MPL and QS21. Phase 2 field trials have indicated that RTS,S is more efficacious when formulated with the adjuvant AS01 than AS02 [130, 137, 138]. In field trials where RTS,S/AS01 and RTS,S/AS02 have been directly compared, RTS,S/AS01 has been found to be more immunogenic [132, 286, 287].

RTS,S induced immune responses will impact upon the within-host dynamics of pre-erythrocytic stage malaria infection. Sporozoites inoculated into the skin can be opsonised and immobilised by anti-CSP antibodies as they migrate through tissue before penetrating a capillary and entering the blood circulation [66]. Sporozoites that reach the liver will invade a hepatocyte where they will undergo hepatic development. Hepatocyte invasion could potentially be prevented by anti-CSP antibodies. Intracellular *Plasmodium* parasites can be targeted by vaccine-induced CSP-specific $CD4^+$ T cells leading to killing of the infected hepatocyte [211, 277]. After approximately 6.5 days of hepatic development [29, 288] hepatic merozoites will be released into the blood circulation to begin the erythrocytic stage of infection. If a cellular response does not kill an infected hepatocyte, it may still inhibit intracellular parasite development leading to substantially fewer merozoites being released into the blood. When released from the liver, merozoites undergo blood-stage replication causing an exponential increase in parasite numbers. Studies of early blood-stage *P. falciparum* infection in human volunteers have demonstrated that the smaller the liver-to blood inocula the longer the time taken for parasite density to reach a given threshold [282, 289].

Vaccination with RTS,S induces anti-CSP antibodies and CSP-specific $CD4^+$ T cells that produce a mixture of cytokines (such as IL-2, TNF-α, IFN-γ) and may also express the co-stimulatory molecule CD40L [277, 290]. Protection from

infection and clinical disease has been shown to be associated with both naturally acquired and RTS,S induced anti-CSP antibodies [139, 237]. CSP-specific T cells have been associated with protection from infection in RTS,S vaccinated children [291] and in children with naturally acquired immunity [252]. Characterising precise immunological correlates of protection in field trials is, however, complicated by heterogeneous exposure to malaria, temporal changes in immune markers, and interactions with naturally acquired immunity. In contrast, challenge trials in malaria-naïve adults provide an ideal opportunity to investigate the dose-response relationship between immune markers and protection from infection as the infectious dose can be controlled and the timing known, there is no naturally acquired immunity, and immune markers can be measured on the day of challenge. Kester *et al* [129] undertook such a challenge study for RTS,S/AS01 and RTS,S/AS02 in malaria naïve adults. In their study, the efficacy of RTS,S/AS01 and RTS,S/AS02 against infection was estimated to be 50% (95% CI, 32.9% - 67.1%) and 32% (95% CI, 17.6% - 47.6%), respectively.

Here we re-analyse the primary data from this challenge study using a novel approach based on mathematical models to determine the shape of the dose-response relationship between vaccine-induced anti-CSP antibody levels, CSP-specific T cells and protection from infection. Our results provide insights into the likely mechanism of action of the RTS,S vaccine as well as providing a more generalised framework for assessing the likely efficacy of vaccines in early stage development.

5.2. Methods

5.2.1. Challenge trial

Kester *et al* [129] evaluated the efficacy and safety of the RTS,S malaria vaccine in 138 malaria naïve adults when formulated with the AS01 and AS02 adjuvant systems. 52 volunteers were vaccinated with RTS,S/AS01 and 50 volunteers with RTS,S/AS02. 36 volunteers were recruited as infectivity controls and hence remained unvaccinated. Volunteers were challenged with the bites of five *P. falciparum* infectious *Anopheles stephensi* mosquitoes. After feeding the mosquito's salivary glands were dissected to examine for sporozoites. If salivary glands tested negative for sporozoites, additional mosquitoes were allowed to take a blood meal until the volunteer had received five infectious bites [292]. 36 of the volunteers receiving RTS,S/AS01 vaccination were challenged. 9 of those protected from infection were re-challenged 5 months later. 44 of the volunteers receiving RTS,S/AS02 vaccination were challenged. 9 of those protected were re-challenged 5 months later. 24 of the infectivity controls were challenged at the first round; the remaining 12 were challenged 5 months later. Following challenge, volunteers were assessed by blood smears taken twice daily starting on day 6.5 until day 14 and then once daily until the end of the study period at day 21. Volunteers who tested positive for malaria parasites were then treated with chloroquine, irrespective of symptoms.

Anti-CSP antibodies were measured by evaluating IgG responses to the *P. falciparum* CSP-repeat region measured using enzyme-linked immunospot assay (ELISA). Measurements of antibody titre were analysed in units of µg/mL. In

Phase II and Phase III field trials of RTS,S, antibody titres have been reported in ELISA units (EU/mL). The number of $CD4^+$ or $CD8^+$ T cells responding to CSP antigen and expressing the immune markers CD40L, IFN-γ, IL-2 and TNF-α per million $CD4^+$ or $CD8^+$ T cells were also measured (see [129] for further details). The measure of cell-mediated immunity (CMI) used in this analysis is the number of $CD4^+$ T cells expressing ≥2 immune markers per million $CD4^+$ T cells. Data from both challenge and re-challenge were analysed.

5.2.2. Model for *P. falciparum* infection

Malaria-naïve volunteers were challenged with the bites of five *P. falciparum* infectious mosquites. Data from mosquito feeding studies indicates that the number of inoculated sporozoites is highly variable [262, 263, 293]. We assume that the number of sporozoites inoculated into the skin by five mosquitoes follows a Geometric distribution. If a fixed proportion of these successfully develop in the liver, then the number of sporozoites initiating blood-stage infection will also be Geometrically distributed with some mean *n*. The probability that after challenge, *k* sporozoites will initiate blood-stage infection is then

$$P(\mathrm{SPZ}=k)=\frac{1}{n+1}\left(\frac{n}{n+1}\right)^k \tag{7}$$

Each sporozoite that survives liver stage development will initiate blood-stage infection by releasing merozoites into the blood stream after t_L days. We assume the number of merozoites is Normally distributed with mean μ and standard deviation σ. Once in the blood, parasites will begin replication increasing in number by a factor m = 3.8 per day [282]. Periodic fluctuations in parasite density due to sequestration, short term changes in innate immunity, and inter-individual differences in parasite replication rates are ignored. If blood-stage infection is initiated with Q parasites, then the parasite density at time t is

$$Pf(t)=\begin{cases}0 & t<t_L\\ Qm^{t-t_L} & t\geq t_L\end{cases} \tag{8}$$

Blood-stage infection can be diagnosed once parasite density reaches a threshold of P_T parasites/mL. If onset of parasitemia is observed on day T, the initial infectious dose of merozoites can be estimated as $Q=P_T m^{-(T-t_l)}$. Thus the delay in onset of parasitemia can be used to estimate the reduction in merozoites emerging from the liver, with greater delays corresponding to greater reductions. Other explanations for the variation in time to onset of parasitemia could be variation in within-host blood-stage replication rates, or variation in the density at which parasites are detected.

5.2.3. Model of infectivity controls

The initial dose of merozoites emerging from the liver in control *j*, Q_j, can be estimated from the time to onset of parasitemia T_j. If we assume the number of succesful sporozoites is Geometrically distributed, and the total number of merozoites is Normally distributed, then the likelihood of the parameters (n, σ) (the mean number of sporozoites initiating blood-stage infection and variation in the number of merozoites, respectively) is:

$$L\ n,\sigma|Q\ = \prod_{j=1}^{J}\sum_{k=1}^{\infty}\frac{1}{n+1}\left(\frac{n}{n+1}\right)^{k}\frac{1}{\sqrt{2\pi k\sigma^2}}\frac{e^{-\frac{1}{2}\frac{Q_j-k\mu}{\sqrt{k}\sigma}^2}}{1-\Phi\ 0\left|k\mu,k\sigma^2\right.} \quad (9)$$

where $j=\{1,...,J\}$ indexes the individuals in the study and *k* is the number of sporozoites injected. Φ is the cumulative normal distribution, with $1-\Phi\ 0\left|k\mu,k\sigma^2\right.$ being the area under a normal curve with mean $k\mu$ and variance $k\sigma^2$ in the region $[0,\infty)$.

5.2.4. Dose-response curves

Prevention of infection or reduction in parasite load in vaccinated volunteers following *P. falciparum* challenge will depend on anti-CSP antibody titres and the number of CSP-specific T cells as well as other factors such as inter-individual variation. Although it may not be possible to precisely characterise the relationship between immune markers and protection from infection, an approximate relationship can be estimated using a dose-response curve [231].

Given a dose x (e.g. anti-CSP antibody titre, CSP specific T cells) the response f (e.g. probability of infection, probability of sporozoite success, reduction in parasite load) can be estimated using dose-response curves, a method commonly used in the pharmacological literature [285]. The following dose-response curves are utilised:

1. Exponential $f(x)=e^{-\log(2)\frac{x}{\beta}}$ (10)
2. Hill function $f(x)=\frac{1}{1+\left(\frac{x}{\beta}\right)^{\alpha}}$ (11)
3. Restricted exponential $f(x)=\lambda+(1-\lambda)e^{-\log(2)\frac{x}{\beta}}$ (12)

where α is a shape parameter, and β and λ are scale parameters. A response such as infection probability may depend on the dose of two different quantities, say anti-CSP antibody titre x, and number of CSP-specific T cells y. If these immune mechanisms provide protection independently of each other then their dose-response curves can simply be multiplied to give

$$f(x,y) = e^{-\log(2)\frac{x}{\beta_x}} e^{-\log(2)\frac{y}{\beta_y}} \quad (13)$$

There may be synergistic interactions between vaccine-induced antibody-mediated and cell-mediated immunity, making a combination of anti-CSP antibodies and CSP-specific T cells more efficacious than the combined effect of each immune component acting in isolation [294]. Alternatively, antibody-mediated and cell-mediated responses may have a less than multiplicative effect when combined. For example, if an antibody response prevents 50% of sporozoites and a cellular response also prevents 50% of sporozoites, then a combined response may only prevent 60% of sporozoites, in contrast to the 75% that would be expected if the responses were multiplicative. Following the approaches of Greco *et al* [285] and White *et al* [232] interaction between immune responses can be modelled using the following dose-response function

$$f(x,y) = e^{-\frac{\log(2)}{2}\left(\left(\frac{x}{\beta_x}+\frac{y}{\beta_y}\right)+\sqrt{\left(\frac{x}{\beta_x}+\frac{y}{\beta_y}\right)^2+4\gamma\frac{xy}{\beta_x\beta_y}}\right)} \quad (14)$$

where β_x, β_y and γ are scale parameters. Here $\gamma > 0$ results in a dose-response surface with synergistic interaction, $\gamma = 0$ results in a dose-response surface where each immune component acts independently, and $-1 < \gamma < 0$ results in a less than multiplicative combination of immune responses.

5.2.5. Binary infection model

After *P. falciparum* challenge a volunteer will either become infected $I = 1$ or remain uninfected $I = 0$ depending on their anti-CSP antibody titre and number of CSP-specific T cells x. The likelihood that the parameters $\theta = \{\alpha, \beta, \lambda\}$ of a dose-response curve f generated the observed data can be written as

$$L(\theta | I, x) = \prod_{j=1}^{J} f(x_j)^{I_j} \left(1 - f(x_j)\right)^{1-I_j} \quad (15)$$

Equation (9) denotes the likelihood for a binary infection model, where volunteer j becomes infected $(I_j = 1)$ with probability $f(x_j)$, or is protected $(I_j = 0)$ with probability $1 - f(x_j)$.

5.2.6. Sporozoite infection model

In addition to the binary outcome of infection, there is also data on the delay in the time to detection of infection. The delay in onset of parasitemia can be modelled by assuming either a reduction in the number of sporozoites initiating blood-stage infection or a reduction in the number of merozoites per sporozoite. Given immune markers x the probability that a sporozoite successfully initiates blood-stage infection can be described by a dose-response

function $f(x)$, and the reduction in the number of merozoites per sporozoite can be described by the dose-response function $g(x)$. Combining equations (3) and (9), the likelihood of the dose-response parameters θ fitting the data can be written as

$$L\ \theta|I,Q,x\ =\prod_{j=1}^{J}\left(\left(\sum_{k=1}^{\infty}\frac{1}{nf(x_j)+1}\left(\frac{nf(x_j)}{nf(x_j)+1}\right)^{k}\frac{1}{\sqrt{2\pi kg(x_j)\sigma^2}}\frac{e^{-\frac{1}{2}\left(\frac{Q_j-\mu kg(x_j)}{\sqrt{kg(x_j)}\sigma}\right)^2}}{1-\Phi\ 0\left|k\mu,kg(x_j)\sigma^2\right.}\right)^{I_j}\left(\frac{1}{nf(x_j)+1}\right)^{1-I_j}\right) \quad (16)$$

The likelihood in equation (10) can be interpreted as follows. If volunteer j with immune marker x_j is protected ($I_j=0$) then 0 sporozoites will be successful with probability $\frac{1}{nf(x_j)+1}$. If volunteer j becomes infected $I_j=1$, then infection will have been initiated by $k\in[1,\infty)$ sporozoites with probability $\frac{1}{nf(x_j)+1}\left(\frac{nf(x_j)}{nf(x_j)+1}\right)^{k}$. The k successful sporozoites will release a number of merozoites into the bloodstream following a Normal distribution $\sim N(k\mu g(x_j),k\sigma^2 g(x_j))$.

5.2.7. Comparative role of the adjuvants

To investigate the hypothesis that the higher efficacy of RTS,S/AS01 can be explained by its superior immunogenicity over RTS,S/AS02, the data from vaccinated volunteers was split by adjuvant formulation: AS01 or AS02. The binary infection and sporozoite models were then fitted separately to each data set.

5.3. Results

5.3.1. Summary of anti-CSP antibody titres and numbers of CSP-specific T cells

On the day of challenge volunteers vaccinated with RTS,S/AS01 had significantly higher anti-CSP titres than volunteers vaccinated with RTS,S/AS02 (P=0.003, t-test) (Figure 5.1). In groups vaccinated with either RTS,S/AS02 or RTS,S/AS01, protected volunteers had significantly higher anti-CSP titres than those infected, (P=0.0059 and P=0.0066, respectively). For the sub-group of volunteers who became infected after *P. falciparum* challenge, there was no significant difference in anti-CSP titres between those receiving RTS,S/AS02 and RTS,S/AS01 (P=0.16, t-test).

RTS,S/AS01 induced a higher number of CSP-specific T cells per million than RTS,S/AS02 but this difference was not statistically significant (P = 0.46, t-test). Volunteers protected from infection had greater numbers of CSP-specific T cells than infected volunteers (P = 0.02, t-test). Anti-CSP antibody titres and number of CSP-specific T cells on the day of challenge or re-challenge were significantly correlated (ρ=0.24, P = 0.025).

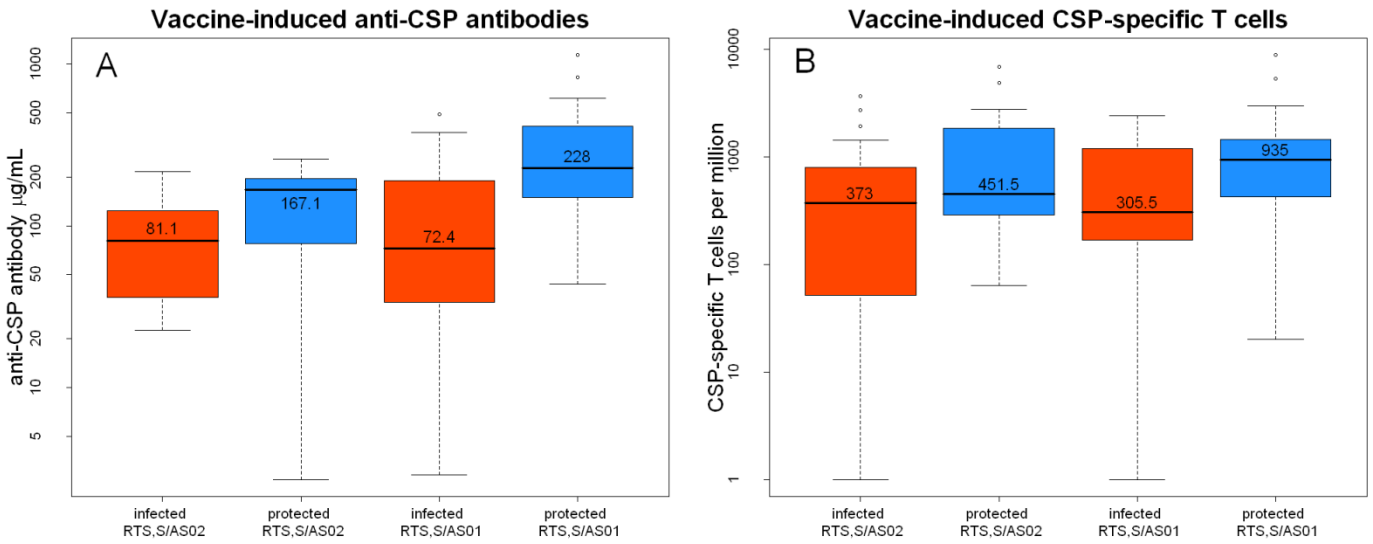


Figure 5.1: Anti-CSP antibody titres **(A)** and CSP-specific T cells per million **(B)** on day of challenge for volunteers vaccinated with RTS,S/AS01 or RTS,S/AS02.

5.3.2. Parameter estimates from the control cohort

All of the 36 volunteers in the control cohort developed detectable malaria infection, although the time to the detection of infection was variable. By fitting our model to these data, we estimate that the mean number of successful sporozoites is n = 16.1, and that the number of merozoites released per sporozoite follows a Normal distribution truncated at zero with µ = 20,000 and σ = 39,000, corresponding to a mean of 39,600 and a standard deviation of 27,300 (Table 5.1).

Table 5.1: Parameters describing biology of *P. falciparum* infection. Estimated parameters are shown with 95% confidence intervals.

Parameter	Description	Value	Reference
n	Mean number of successful sporozoites per challenge	16.1 (9.7 – 24.0)	estimated
t_L	Duration of liver-stage development	6.5 days	Murphy *et al* [29]
μ	Mean number of merozoites released per sporozoite	20,000	Meis *et al* [28]
σ	Standard deviation in number of merozoites per sporozoite	39,000 (15,850 – 112,500)	estimated
m	Daily blood-stage parasite multiplication rate	3.8 day^{-1}	Bejon *et al* [282]
Pf_T	Threshold parasite density for detection of infection	50,000,000 parasites/mL	Bejon *et al* [295]

5.3.3. Binary infection model

The maximum value of the likelihood in equation (9) was calculated for several dose-response curves for the relationship between anti-CSP antibody titres, numbers of CSP-specific T cells and protection from infection, nine of which are shown in Table 5.2. The best fit was given by exponential dose-response curves with 50% protection being conferred by an anti-CSP antibody titre of 253 (154 – 482) µg/mL or 3235 (1696 – 18465) CSP-specific T cells per

million (Table 5.5). 13 of 85 volunteers had anti-CSP antibody titres greater than 253 µg/mL, and 5 of 85 volunteers had cellular responses greater than 3235 CSP-specific T cells per million.

Table 5.2: Parameter estimates and model comparisons for 9 combinations of dose-response curves for the binary infection model. The models compared are based upon the dose-response curves (4), (5) and (6) applied to anti-CSP antibodies (Ab) or CSP-specific T cells (CMI). β_{ab} is the antibody titre required for 50% protection from infection. α_{ab} and λ_{ab} are shape parameters. β_{cmi} is the number of CSP-specific T cells per million required for 50% protection from infection. α_{cmi} and λ_{cmi} are shape parameters. γ is a parameter for the interaction between antibody-mediated and cell-mediated immune responses. Models are compared with maximum likelihood (ML) and the Akaike information criterion (AIC).

Model	Parameter estimates							ML	AIC	ΔAIC
	β_{ab} (µg/mL)	α_{ab}	λ_{ab}	β_{cmi} (cells/million)	α_{cmi}	λ_{cmi}	γ			
Ab (4) & CMI (4)	253.92	–	–	3235	–	–	–	-48.54	101.08	0
Ab & CMI (8)	359.46	–	–	9888	–	–	12.61	-48.46	102.92	1.84
Ab (6) & CMI (4)	253.78	–	8.9×10^{-8}	3237	–	–	–	-48.54	103.08	2.00
Ab (5) & CMI (4)	240.68	1.34	–	3168	–	–	–	-48.57	103.15	2.07
Ab (4) & CMI (5)	278.96	–	–	5182	0.69	–	–	-48.59	103.19	2.11
Ab (4) & CMI (6)	258.58	–	–	19.89	–	0.82	–	-49.35	104.70	3.62
Ab (4)	172.86	–	–	–	–	–	–	-52.08	106.16	5.08
Ab (5) & CMI (5)	268.65	1.73	–	6644	0.49	–	–	-48.34	106.69	5.61
CMI (4)	–	–	–	931	–	–	–	-61.21	124.42	23.34

Table 5.3 compares the observed and model predicted vaccine efficacy for the volunteers stratified into low, medium and high groups by anti-CSP antibody titre and number of CSP-specific T cells. It is predicted that volunteers with high anti-CSP antibody titres and high numbers of CSP-specific T cells will have 76.2% protection from infection.

Table 5.3: Comparison of predicted (black) and observed (blue) vaccine efficacy for the binary infection model. Volunteers have been stratified into low, medium and high groups according to their anti-CSP antibody titre and number of CSP-specific T cells per million.

		anti-CSP antibody titre (µg/mL)			
		2.7 – 78	78 – 183	183 – 1136	2.7 – 1136
number of CSP specific T cells	1 – 268	12.7% 12.5% (2/16)	29.9% 37.5% (3/8)	47.7% 25% (1/4)	22.6% 21.4% (6/28)
	268 – 820	18.8% 33.3% (2/6)	37.1% 14.3% (1/7)	58.3% 62.5% (10/16)	45.0% 44.8% (13/29)
	820–8798	40.1% 16.7% (1/6)	52.8% 64.3% (9/14)	76.2% 75.0% (6/8)	56.8% 57.1% (16/28)
	1 – 8798	20.0% 17.9% (5/28)	42.7% 44.8% (13/29)	61.9% 60.7% (17/28)	41.5% 41.2% (35/85)

The binary infection model can be used to compare the effects of antibody and cellular responses. In the absence of CSP-specific T cells, the model estimates that the average vaccine efficacy in the volunteers under investigation is 31% (95% CI, 19% – 43%). In contrast, in the absence of anti-CSP antibodies the average vaccine efficacy conferred by CSP-specific T cells is estimated to be 16% (95% CI, 3% – 25%). This suggests that antibodies have a greater role in protection from infection than cellular responses. Estimated vaccine efficacy as a function of anti-CSP antibody titre in the absence of T cells is shown in Figure 5.2.

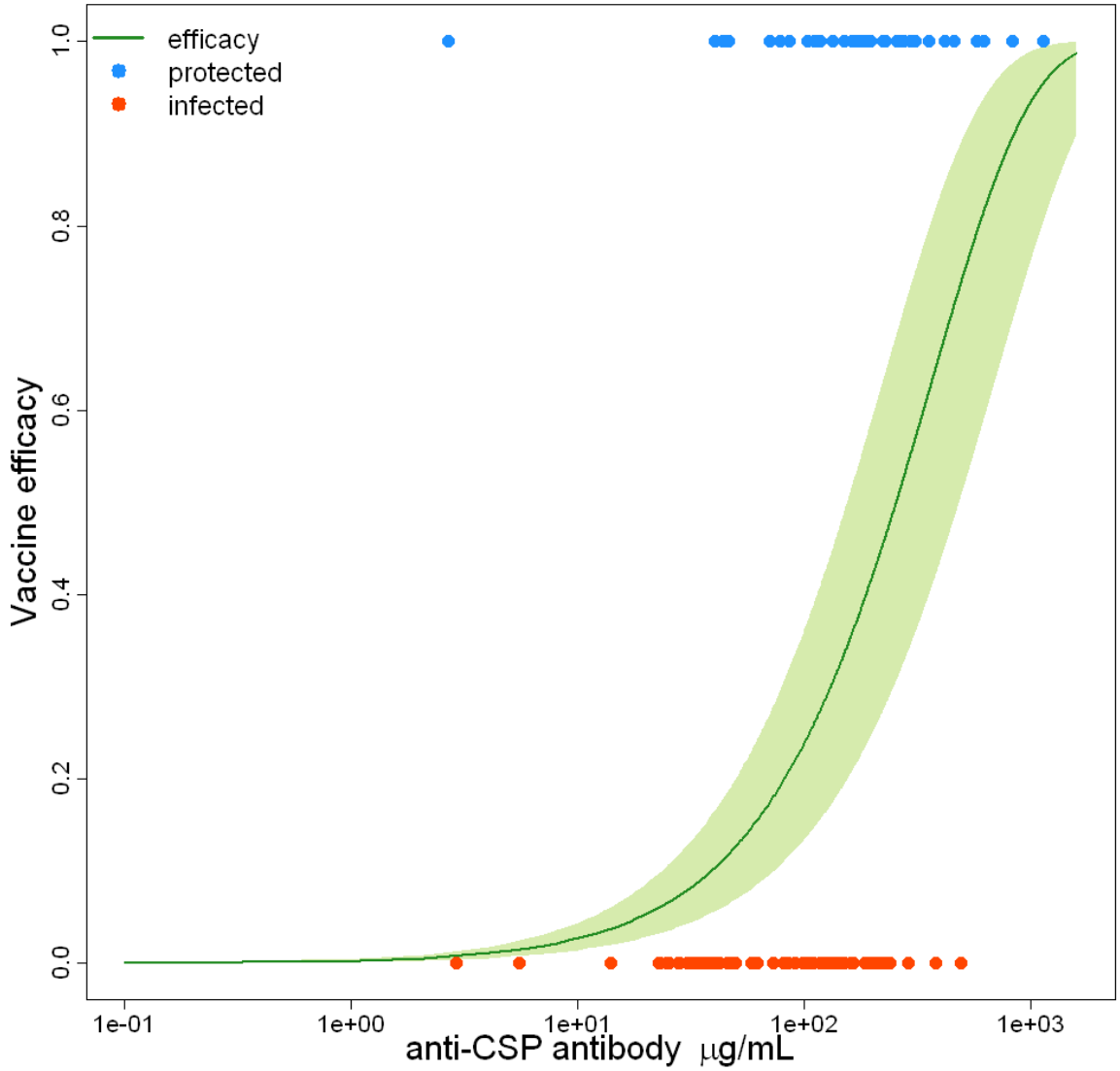


Figure 5.2: Estimated vaccine efficacy as a function of anti-CSP antibody titre in the absence of CSP-specific T cells. The shaded green region represents the 95% confidence interval. The anti-CSP antibody titres of protected (blue) and infected (red) volunteers are shown at the top and bottom, respectively.

5.3.4. Sporozoite infection model

If blood-stage infection is initiated by a single sporozoite releasing 20,000 merozoites 6.5 days after challenge, and parasite density increases exponentially due to blood-stage replication, then we estimate that the onset of parasitemia won't be detected until day 13. In the control group, the onset of parasitemia was detected on or before day 13 in 97% (35/36) of volunteers. In volunteers vaccinated with RTS,S who developed infection, the onset of parasitemia was detected on or before day 13 in only 52% (30/58), suggesting that many volunteers were infected with a dose of merozoites less than 20,000. This reduction in liver-to-blood inocula suggests that there is substantial inhibition of parasite development in the liver, possibly due to vaccine-induced cellular responses.

The reduction in sporozoite survival and inhibition of parasite development within the liver were modelled using dose-response curves for the dependence on antibody titres and cellular responses. Four models are compared in

Table 5.4. The model providing the best fit to the data assumes that the probability of sporozoite survival decreases with both increasing anti-CSP antibody titres and CSP-specific T cells according to exponential dose-response curves, and that the reduction in merozoites per sporozoite decreases with increasing anti-CSP antibody titres according to a restricted exponential dose-response curve. Although antibody-mediated inhibition of liver-stage development isn't a likely biological mechanism [235], we use it in our model as it proved to be the best marker of the effects of the vaccine-induced immune response on liver-stage inhibition. More detailed markers of cell-mediated immunity, in particular TNF-α, may better account for vaccine-induced liver inhibition.

The model predicts that the time to onset of parasitemia increases with increasing anti-CSP antibody titre (Figure 5.3). The time to onset of parasitemia is underestimated for a number of volunteers in Figure 5.3, as the plot doesn't capture the dependence on numbers of CSP-specific T cells. In addition, there is likely to be between-volunteer variation in innate immunity and blood-stage replication rates which are unaccounted for.

Table 5.4: Parameter estimates and model comparisons for the sporozoite infection model. β_{ab} and β_{cmi} are the antibody titre or number of CSP-specific T cells required to kill 50% of sporozoites. ω_{ab} and ω_{cmi} are the antibody titre or number of CSP-specific T cells required for a 50% reduction in the number of merozoites per sporozoite. λ is the maximum relative reduction in merozoites per sporozoite. Models are compared with ML and AIC.

Model	Parameter estimates					ML	AIC	ΔAIC
	β_{ab} (μg/mL)	β_{cmi} (cells/million)	ω_{ab} (μg/mL)	ω_{cmi} (cells/million)	λ			
Antibody liver inhibition	68.1	1240	8.3	–	0.02	-579.77	1167.54	0
Constant liver inhibition	63.1	1299	–	–	0.038	-582.09	1170.19	2.65
Cellular liver inhibition	57.2	1767	–	36.8	0.025	-592.89	1193.79	26.25
No liver inhibition	50.9	771	–	–	–	-649.36	1302.73	135.19

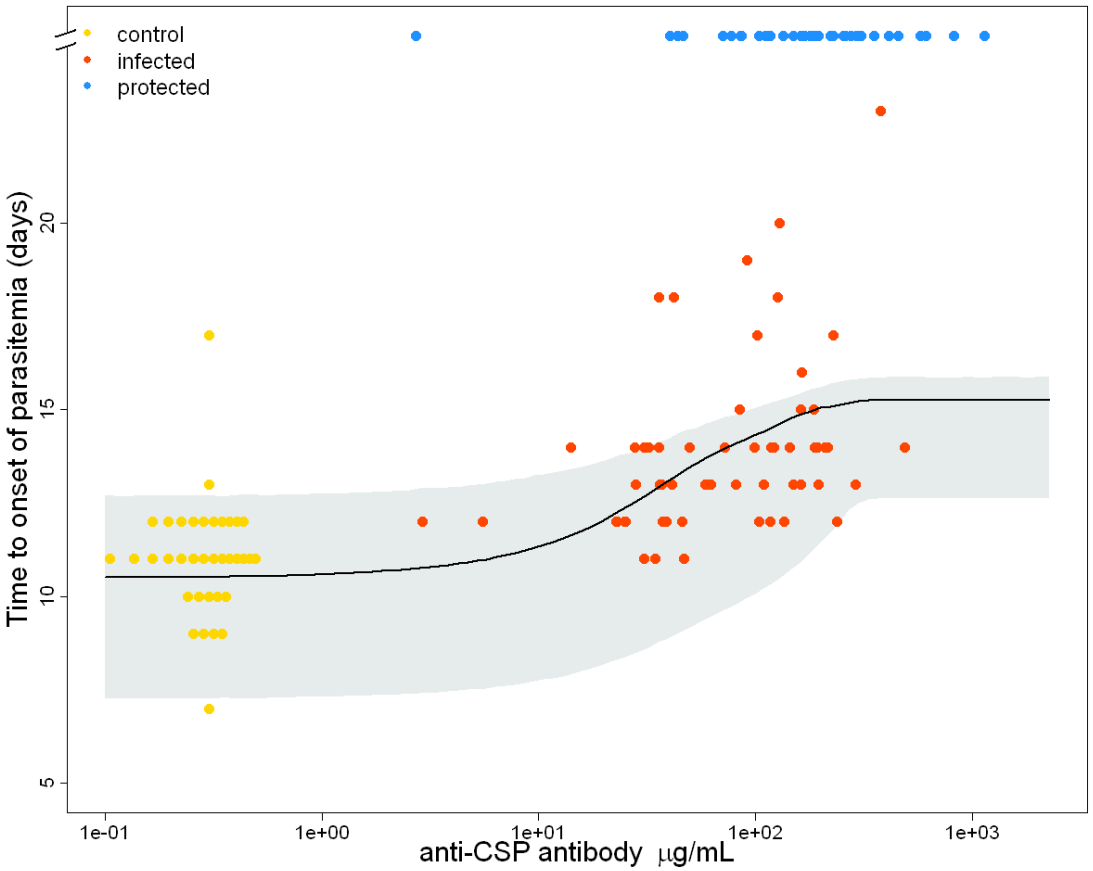


Figure 5.3: Estimated time to onset of parasitemia as a function of anti-CSP antibody titre (black line) with 95% confidence intervals (shaded grey region). The times to onset of parasitemia in the infectivity controls, who didn't have detectable anti-CSP antibody titres are plotted on the left (gold points). The anti-CSP antibody titres of protected volunteers in whom there was no onset of parasitemia, are shown at the top (blue points). The model doesn't capture all the variance in the data, with several data points lying outside the 95% confidence region.

Using the sporozoite infection model, we estimate that both infection-blocking efficacy and anti-parasite efficacy (defined to be the percentage reduction in merozoites entering the blood from the liver [296]) increase with increasing anti-CSP titres and numbers of CSP-specific T cells (Figure 5.4). The sporozoite model predicts that vaccination will prevent all sporozoites in 31% (95% CI, 23% – 38%) of challenges. At the titres induced by RTS,S vaccination the anti-parasite efficacy is predicted to be 97.7% (95% CI, 96.3% – 98.7%) suggesting that a very small number of parasites are responsible for breakthrough infection. The confidence intervals for anti-parasite efficacy are very wide at low titres where there is no data.

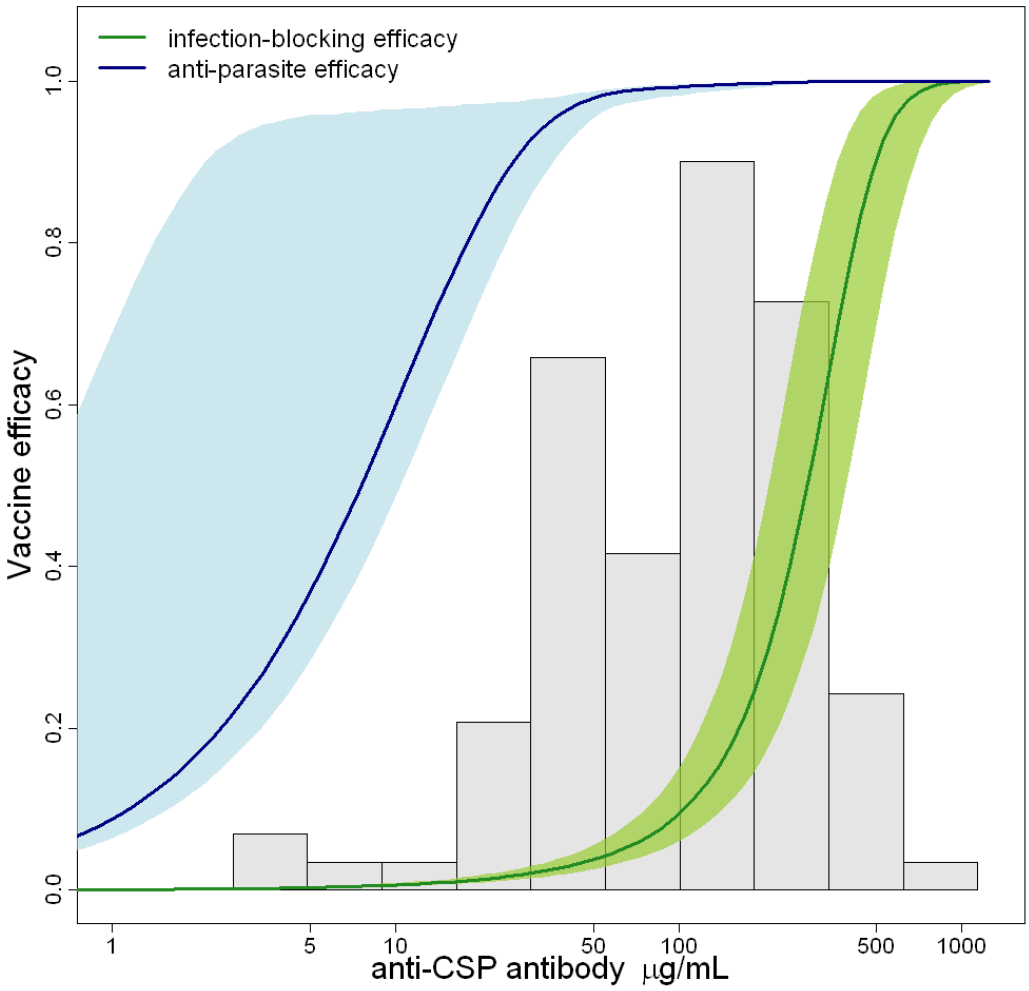


Figure 5.4: Estimated infection-blocking efficacy (green) and anti-parasite efficacy (blue) with 95% confidence intervals as a function of anti-CSP antibody titres obtained using the sporozoite model. A histogram of the distribution of anti-CSP antibody titres is shown in grey.

Table 5.5: Best fit parameter estimates with 95% confidence intervals for the binary and sporozoite infection models.

Parameter	Description	Value
Binary infection model		
β_{ab}	anti-CSP titre for 50% reduction in infection probability	253 (154 – 482) μg/mL
β_{CMI}	number of $CD4^+$ T cells for 50% reduction in infection probability	3235 (1696 – 18465) cells/million
Sporozoite infection model		
β_{ab}	anti-CSP titre for 50% reduction in sporozoite survival probability	68 (50 – 96) μg/mL
β_{CMI}	number of $CD4^+$ T cells for 50% reduction in sporozoite survival probability	1240 (741 – 3026) cells/million
β_{liver}	anti-CSP titre for 50% reduction in merozoites per sporozoite	8.2 (0.8 – 12.3) μg/mL
λ_{liver}	maximum reduction in merozoites per sporozoite	98% (95% – 99%)

5.3.5. Combined antibody and cellular response

The efficacy against infection estimated using the binary infection model with interaction between anti-CSP antibody responses and CSP-specific T cell responses is shown in Figure 5.5. All 3 vaccinated volunteers predicted to have greater than 90% vaccine efficacy were protected. 11 of 13 volunteers predicted to have greater than 70% vaccine efficacy were protected. The existence of a highly protected subgroup of volunteers suggests that vaccine efficacy in excess of 70% is possible if both anti-CSP antibody titres and numbers of CSP-specific T cells can be boosted to high enough levels.

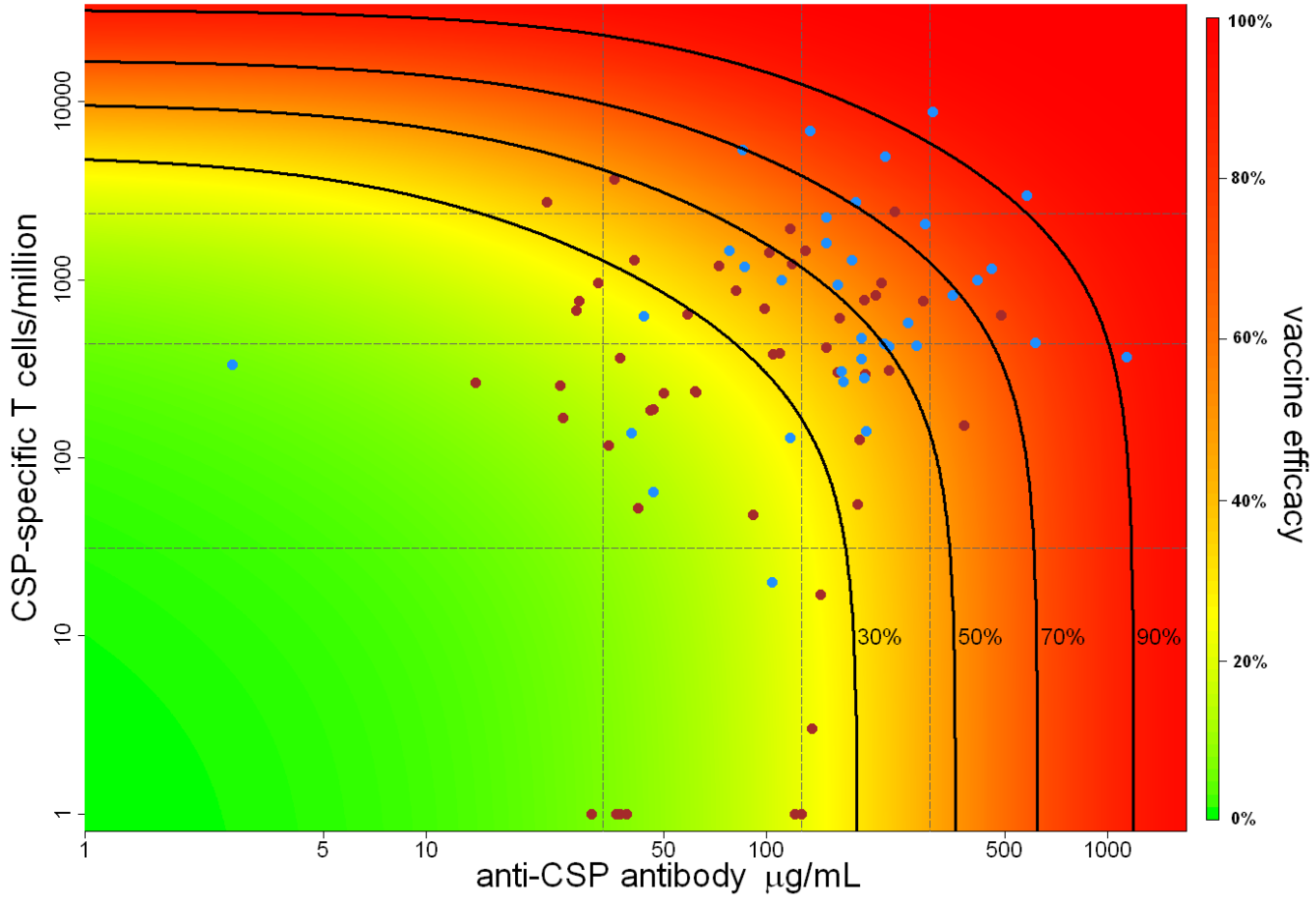


Figure 5.5: Estimated infection-blocking vaccine efficacy as a function of anti-CSP antibody titre and numbers of CSP-specific T cells per million obtained from the binary infection model. The vertical dashed grey lines denote the median and 90% ranges of the observed anti-CSP antibody titres, and the horizontal dashed grey lines denote the median and 90% ranges of observed numbers of CSP-specific T cells. The solid black lines denote the isoclines for 30%, 50%, 70%, and 90% vaccine efficacy against infection. The blue and brown points denote the anti-CSP antibody titres and numbers of CSP-specific T cells of protected and infected volunteers, respectively.

5.3.6. Effect of adjuvant formulation on vaccine efficacy

The volunteers were split into two groups according to adjuvant (AS01 or AS02) and both the binary and sporozoite infection models were fitted to the datasets. For volunteers vaccinated with RTS,S/AS01 the binary infection model estimated that 50% protection from infection could be conferred by an antibody titre of 264 (95% CI, 137 – 637) µg/mL or 2408 (95% CI, 1138 – 18706) CSP-specific T cells per million. In comparison, for volunteers vaccinated with RTS,S/AS02 it was estimated that 50% protection would be conferred by an antibody titre of 255 (95% CI, 143 – 587)

µg/mL or 3826 (95% CI, 1794 – 18450) CSP-specific T cells. A comparison of vaccine efficacy as a function of anti-CSP antibody titres is shown in Figure 5.6 for RTS,S/AS01 and RTS,S/AS02. Similarly, when the sporozoite model was fitted separately to volunteers vaccinated with RTS,S/AS01 or RTS,S/AS02 there was no significant difference between the estimated parameters. This suggests that the adjuvant formulation does not have a direct effect on vaccine efficacy, but contributes to protection only by increasing the magnitude of induced immune responses.

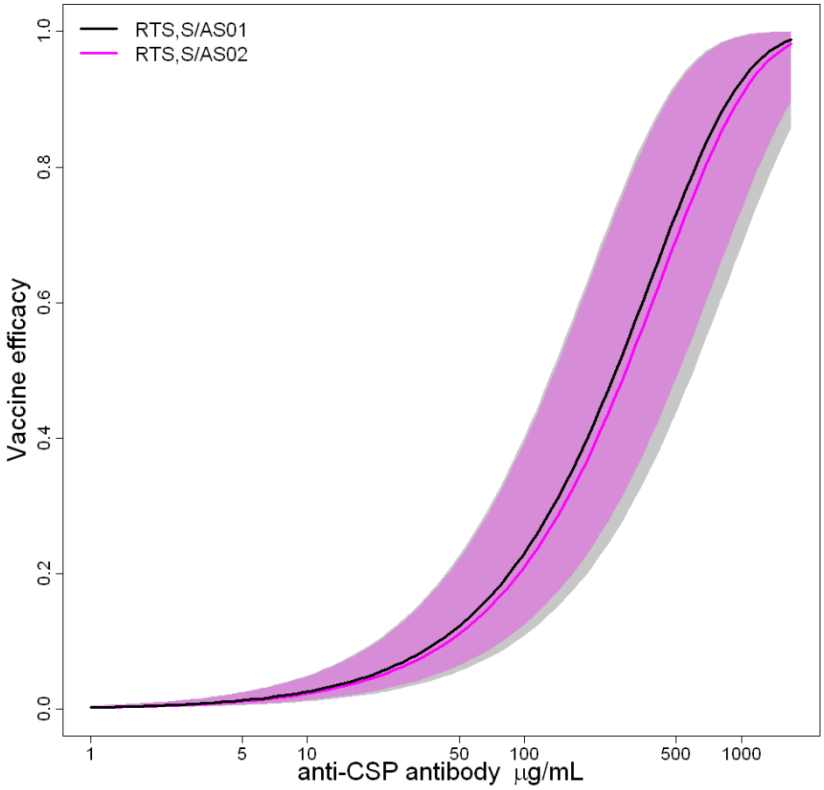


Figure 5.6: Comparison of vaccine efficacy as a function of anti-CSP antibody titre in the absence of CSP-specific T cells for RTS,S/AS01 (black) and RTS,S/AS02 (pink) based on the binary infection model. The grey and pink shaded regions denote 95% confidence intervals for the estimated efficacy of RTS,S/AS01 and RTS,S/AS02, respectively.

5.4. Discussion

Naturally acquired and vaccine-induced anti-CSP antibodies and CSP-specific T cells have been associated with protection from infection [237, 252, 278]. Using mathematical models to estimate the dose-response relationship between immune markers and protection from infection, we were able to estimate the probability of malaria-naïve volunteers vaccinated with RTS,S becoming infected after *P. falciparum* challenge, and if infected, the delay in onset of parasitemia due to vaccination.

Our results suggest that RTS,S prevents large numbers of *P. falciparum* parasites from surviving the pre-erythrocytic stage, with an estimated average anti-parasite efficacy of 97.7% (95% CI, 96.3% – 98.7%) from the sporozoite infection model. A comparably large level of anti-parasite efficacy of pre-erythrocytic vaccines has been suggested by the results of longitudinal PCR studies on challenged volunteers [282, 296]. Such high levels of anti-parasite efficacy are needed to obtain significant infection-blocking efficacy, given that, in theory, a single sporozoite evading

the vaccine-induced immune response can initiate blood-stage infection. The need for such high levels of anti-parasite efficacy to result in even a partially effective vaccine is a major challenge in developing highly efficacious pre-erythrocytic malaria vaccines. No definitive threshold for protection, in either anti-CSP antibody titres or $CD4^+$ T cell responses, was identified by our model. Instead, vaccine efficacy was estimated to increase continuously, albeit non-linearly, across the range of observed antibody titres and cellular responses.

The estimates of anti-parasite efficacy presented here are based on the reduction in the number of merozoites exiting the liver due to vaccination, in agreement with the measure used by Hill [296]. An alternative interpretation of anti-parasite efficacy would be the reduction in the number of sporozoites progressing to blood-stage infection, in which case we estimate 75% (95% CI, 68% – 79%). However, this measure of efficacy is sensitive to the mean number of inoculated sporozoites per infectious bite: a vaccine that prevents 75% of sporozoites will be much more effective at preventing infections if the mean number of successful sporozoites per bite is $n = 2$ than if it is $n = 50$. Our estimate of $n \approx 16$ successful sporozoites from five mosquito bites is compatible with Beier *et al*'s observation of a mean of 10 sporozoites per bite, corresponding to a mean of 50 sporozoites per challenge, only a fraction of which will progress to blood-stage infection. In a trial of several pre-erythrocytic vaccines by Bejon *et al* [282] with an experimental design similar to that of Kester *et al* [129], it was estimated that on average $n = 14$ sporozoites successfully progressed to blood-stage infection, a finding comparable with our estimate.

Our model results predict that RTS,S induced protection from infection is dependent on both anti-CSP antibodies and CSP-specific T cells. In the absence of cellular responses, vaccine-induced anti-CSP antibodies are estimated to provide 31% protection from infection. In contrast, in the absence of anti-CSP antibodies, CSP-specific T cells are estimated to provide 16% protection. This suggests that antibodies play a dominant role, accounting for approximately 2/3 of vaccine-induced protection from infection. However, this finding is only valid when the marker of cellular immunity is taken to be the number of CSP-specific T cells per million expressing ≥ 2 of the cytokines IL-2, TNF-α, IFN-γ or the co-stimulatory molecule CD40L [129]. More detailed analysis of cytokine responses may reveal a marker of cellular immunity with a stronger association with protection from infection. For example, CSP-specific $CD4^+$ T cells producing TNF-α have been associated with a reduced risk of clinical malaria in a trial of RTS,S/AS01 in children [291].

There may be synergistic interactions between vaccine-induced antibody and cellular responses [294]. There are two potential stages at which synergy can act: firstly, there could be synergy at the induction stage, such that when high antibody titres are induced it is more likely that high numbers of CSP-specific T cells will also be induced, or vice-versa [297]; secondly, there could be synergistic interactions between immune responses at the effector stage, such that an antibody response is more effective in the presence of a cellular response, and vice-versa. Mathematical models of the sort used here are unable to capture synergy at the induction stage, although evidence for this can be derived from the statistically significant correlation between anti-CSP antibody titres and numbers of CSP-specific T cells. Dose-response models with an interaction term suggest that there is synergistic interaction at the effector

stage, although this model did not provide a better statistical fit to the data than a model with independent antibody and cell-mediated responses (Table 5.2).

There was significant variation between volunteers in the anti-CSP antibody titres and numbers of CSP-specific T cells induced by RTS,S on the day of challenge. Under our sporozoite infection model, this variation in titres predicts substantial between-person heterogeneity in vaccine efficacy, that is, partial protection is induced in everyone but some individuals are more protected than others. The protection profile (the level of protection conferred on all vaccinees) is estimated to be intermediate between that of a leaky and an all-or-nothing vaccine, although this may change if immune effectors decay over time. The nature of infectious challenge with malaria may also contribute to variation in vaccine efficacy as infections arising from bites with a small number of inoculated sporozoites may be easily prevented, whereas infections arising from large doses of sporozoites may be difficult to prevent, however this hypothesis is likely to be difficult to test under field conditions.

RTS,S has been observed to be more immunogenic when formulated with AS01 compared to when formulated with AS02 [287]. The relationship between adjuvant formulation, induced anti-CSP antibody titres and CSP-specific T cells isn't fully understood. In this study, we have demonstrated that when adjusted for anti-CSP antibody titres and numbers of CSP-specific T cells, both RTS,S/AS01 and RTS,S/AS02 provide equivalent levels of protection from infection.

5.5. Conclusion

Our results suggest that the RTS,S vaccine acts through the induction of high levels of both anti-CSP antibodies and CSP-specific $CD4^+$ T cells, with the antibody response having a greater role in protection from infection. The existence of a subgroup of volunteers with high antibody and cell-mediated immune responses (and an estimated vaccine efficacy against infection greater than 70%) suggests that substantial increases in efficacy can be obtained if vaccine immunogenicity can be further improved.

Chapter 6. Estimates of the immune parameters determining the duration of antibody response to *Plasmodium falciparum* infection in African children

In the earlier chapters of this thesis I have investigated the relationship between markers of immunity and protection from *P. falciparum* infection or clinical malaria. In particular I focussed on static measures of pre-erythrocytic immunity such as antibody titres and $CD4^+$ T cell responses, ignoring the dynamic nature of immune responses. In this Chapter, in order to better understand the acquisition and maintenance of vaccine-induced and naturally acquired antibody responses, I develop simple models of the kinetics of naturally acquired antibodies which are fitted to data from young African children to obtain estimates of the parameters determining the duration of antibody response.

6.1. Introduction

In malaria-endemic areas, older children and adults acquire substantial immunological protection to severe malaria and death, but rarely - if ever - acquire sterile immunity [55]. Infants are particularly vulnerable to episodes of severe malaria in the period of time after innate resistance and maternally-acquired immunity has waned and before the acquisition of effective, adaptive immunity [56]. Although substantial protection from severe malaria and death is acquired after a small number of infections [57] episodes of acute febrile disease may continue for many years. In contrast to other pathogens such as measles, rubella and varicella, the immune parameters determining the acquisition and duration of the antibody response to *Plasmodium falciparum* remain poorly quantified [121].

Frequently cited explanations for the slow development of protective immunity include extensive antigenic diversity among parasite isolates [298] as well as low immunogenicity of malaria antigens and defective immunological memory. Although it is widely perceived that acquired immune responses to malaria are short-lived [55, 299], and that previously immune individuals suffer a loss of immunity when they move away from malaria endemic areas, the true picture is much more complex [300, 301]. Thus, while frequent reinfection is required to maintain high concentrations of anti-malarial antibodies, and while antibody responses can appear very transient – especially in young children [299, 302-304] –there is evidence that other components of the immune response to malaria may be long-lived [300]. For example, when epidemic malaria emerged in Madagascar after 25 years of malaria control, people born before the start of the control programme were much more resistant to clinical disease than younger people who lacked acquired immunity [305]. Similarly, parasite density, risk of severe clinical disease and death were all reported to be significantly lower among individuals exposed to malaria many years before than in malaria naïve individuals [306, 307]. Circulating memory B cells (MBC) specific for *P. falciparum* antigens can be detected at least 8 years after the most recent malaria infection [308, 309] and the best evidence suggests that they can persist for the lifetime of the individual [309]. Estimates of the half-life of antibody sero-positivity indicate that individuals remain

sero-positive for life for antibodies to conserved or relatively conserved antigens, even in areas of low ongoing malaria transmission [310].

Understanding the processes underlying the acquisition and loss of various immune effector mechanisms is crucial for understanding the epidemiology of the disease and the likely impact of vaccines. Here we investigate the acquisition and loss of humoral immune responses with biological models of immunological processes fitted to Immunoglobulin G (IgG) antibody titres to *P. falciparum* antigens using data from longitudinal cohort studies of Ghanaian and Gambian children [303, 304, 311]. We assume that exposure to *P. falciparum* antigen induces proliferation of antigen-specific naïve or memory B cells (MBC) and their differentiation into IgG antibody secreting cells (ASC), and that the duration of circulating IgG is dependent upon the half-life of individual IgG molecules, the half-life of IgG secreting cells and rates of reinfection which induce proliferation of new populations of MBCs and ASCs.

6.2. Methods

6.2.1. Longitudinal cohort data

Data from two longitudinal cohort studies of the antibody response to *P. falciparum* infection in African children were analysed. In the first study children from southern Ghana were followed from birth for over 2 years to investigate the association between maternal antibody and protection from malaria infection [311]. The entomological inoculation rate was approximately 5 to 10 infectious bites per year, 92% of which were due to *P. falciparum* [312]. Maternal blood samples were collected by venepuncture, and children's samples were collected at birth, 2, 4, and 6 weeks after birth, and then every 4 weeks by heel prick. Blood samples were tested for parasites by microscopy and PCR. Samples were analysed by enzyme-linked immunosorbent assays (ELISA) for the presence of antibodies to a number of *P. falciparum* antigens: apical membrane antigen 1 (AMA-1); the 19-kDa C-terminal fragment of merozoite surface protein 1 (MSP-1_{19}); two allelic sequences of merozoite surface protein 2 (MSP-2), FC27 and IC1, representing the two major families of MSP-2; and circumsporozoite protein (CSP). Data from 151 children followed from birth for up to 812 days were analysed.

In the second study children up to 6 years of age from the town of Farafenni in The Gambia were followed for 3 months during the dry season to investigate the decay in antibody titres following *P. falciparum* transmission during the wet season [303, 304]. Samples were taken every 2 weeks and tested for parasites by microscopy. Samples were analysed by ELISA for the presence of antibodies to a number of *P. falciparum* antigens: AMA-1, MSP-1, two allelic types of MSP-2, and erythrocyte binding antigen 175 (EBA-175). Data from 124 children were analysed.

6.2.2. Mathematical models

We describe a simplified model of the immunological processes underlying antibody generation (Figure 6.1). Upon recognition of *P. falciparum* parasites, antigen specific B cells proliferate and terminally differentiate into antibody secreting plasma B cells. Two populations of plasma B cells are created: short-lived ASCs with a half-life of the order of days or weeks, and long-lived ASCs with a half-life of the order of months or years. ASCs generate IgG antibodies which are used to combat ongoing malaria infection and protect against future infections. Individual IgG molecules have a half life of days or weeks.

Three models of the immunological processes underlying antibody kinetics are constructed to investigate the patterns observed in the Ghanaian and Gambian data (Figure 6.2). Antibody kinetics are assumed to be dependent on (i) the half-life of individual IgG molecules, (ii) the half-life of IgG secreting cells and (iii) rates of reinfection which induce differentiation of new populations of MBCs and ASCs. When antibody titres are boosted above some threshold between consecutive samples, antigen exposure is assumed to have occurred (which may or may not coincide with a new infection).

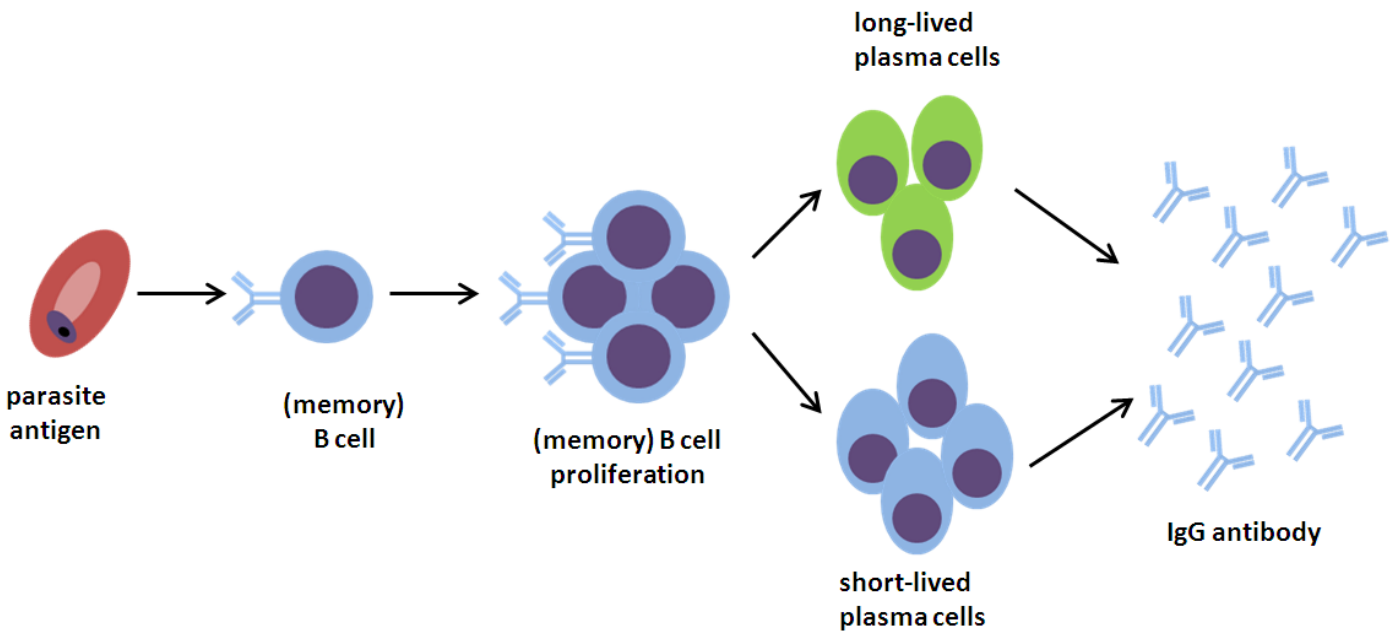


Figure 6.1: Schematic model of the immunological processes underlying the generation of IgG antibody.

Model 1

A simplified model of the immunological processes underlying antibody production is constructed so that IgG antibody, A, is generated in boosts following exposure to *P. falciparum* antigens and decays with half-life d_a days. When IgG antibody is generated at rate α the antibody kinetics can be described by

$$\frac{dA}{dt} = \alpha - rA \tag{17}$$

where $r = \frac{\log(2)}{d_a}$ is the rate of decay of antibody. If antibody is generated in boosts of size α_i following antigen exposure at time τ_i then equation (1) can be solved to give

$$\mathrm{A}(t) = \sum_i H(t,\tau_i)\alpha_i e^{-r(t-\tau_i)} \tag{18}$$

where H is a step function defined as

$$H(t,\tau_i) = \begin{cases} 0 & t < \tau_i \\ 1 & t \geq \tau_i \end{cases} \tag{19}$$

Model 2

Model 1 is adapted so that there is rapid proliferation of MBCs and differentiation into ASCs following exposure to *P. falciparum* antigens. These plasma cells, B, persist with half-life d_b days secreting IgG antibody, A, throughout their life. When plasma B cells are generated at a rate β, the antibody kinetics can be described by

$$\begin{aligned} \frac{dB}{dt} &= \beta - cB \\ \frac{dA}{dt} &= gB - rA \end{aligned} \tag{20}$$

where $c = \frac{\log(2)}{d_b}$ is the rate of decay of plasma cells, and g is the rate at which antibody is secreted by plasma cells.

If plasma cells are generated in boosts of size β_i at times τ_i then equation (4) can be solved to give

$$A(t) = \sum_i H(t,\tau_i)\beta_i \left(\frac{e^{-c(t-\tau_i)} - e^{-r(t-\tau_i)}}{r-c} \right) \tag{21}$$

Here the constant g has been absorbed into the boost size β_i.

Model 3

Model 2 is extended so that following antigen exposure, both short-lived plasma cells with half-life d_s days (of the order of days/weeks) and long-lived plasma cells with half-life d_l (of the order of months/years) are generated. Short-lived plasma cells are responsible for the rapid rise in antibody titres following initial antigen exposure and are assumed to combat the ongoing malaria infection. Long-lived plasma cells are responsible for maintaining antibody

titres after the infection has been cleared. If plasma cells are generated at rate β, a proportion ρ of which are short-lived and $1-\rho$ long-lived, then the antibody kinetics can be described by

$$\begin{aligned}\frac{dB_s}{dt} &= \rho\beta - c_s B_s \\ \frac{dB_l}{dt} &= (1-\rho)\beta - c_l B_l \\ \frac{dA}{dt} &= gB_s + gB_l - rA\end{aligned} \tag{22}$$

where $c_s = \frac{\log(2)}{d_s}$ is the rate of decay of short-lived plasma cells and $c_l = \frac{\log(2)}{d_l}$ is the rate of decay of long-lived plasma cells. If plasma cells are generated in boosts of size β_i at times τ_i then equation (6) can be solved to give

$$A(t) = \sum_i H(t,\tau_i)\beta_i \left(\rho \left(\frac{e^{-c_s(t-\tau_i)} - e^{-r(t-\tau_i)}}{r - c_s} \right) + 1-\rho \left(\frac{e^{-c_l(t-\tau_i)} - e^{-r(t-\tau_i)}}{r - c_l} \right) \right) \tag{23}$$

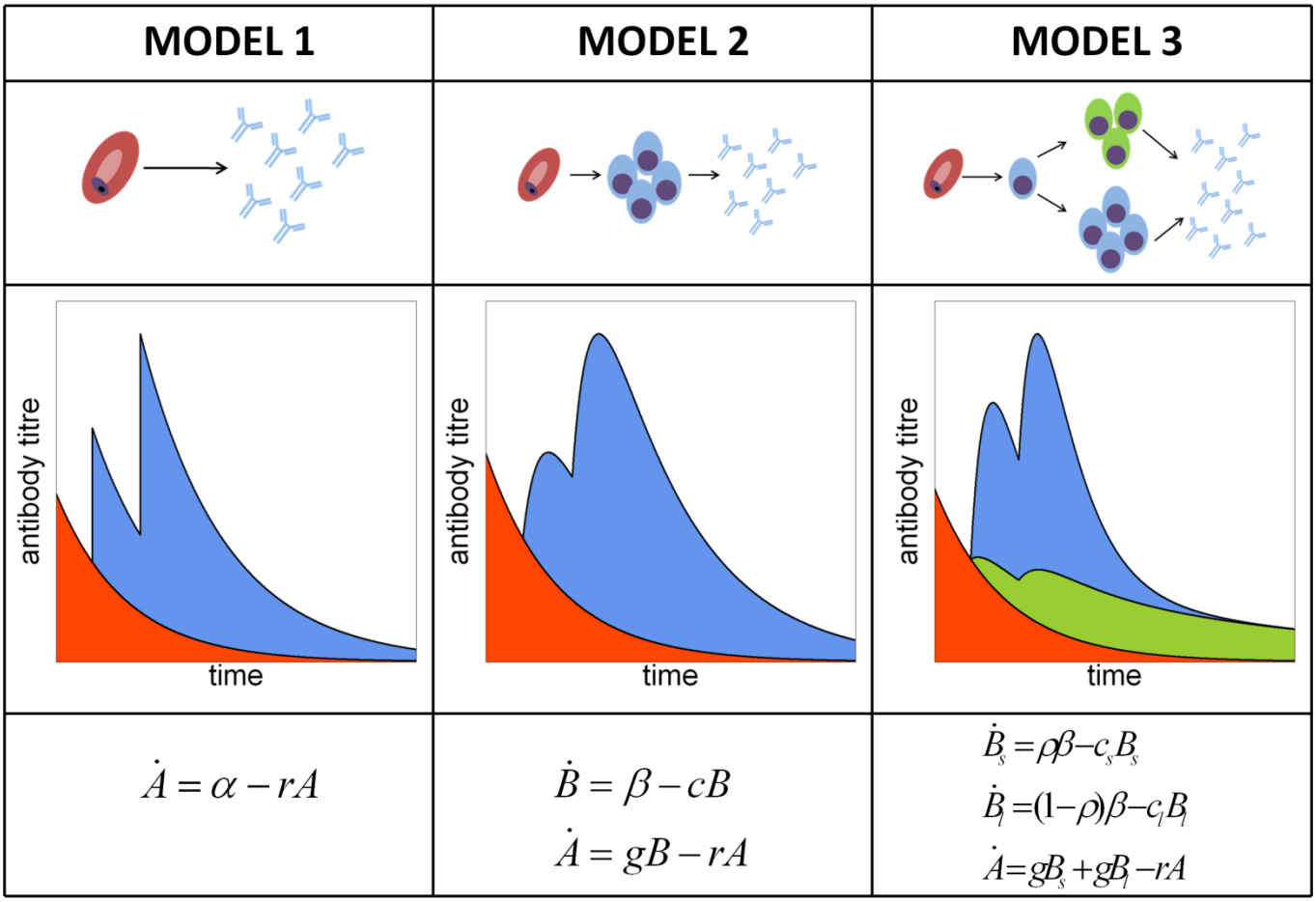


Figure 6.2: In Model 1 children are born with maternal antibody which decays exponentially (red). Upon exposure to *P. falciparum* antigen, antibodies are boosted and then decay exponentially (blue). In Model 2 ASCs are generated upon exposure to antigen and secrete antibody causing a gradual increase in titre followed by exponential decay. In Model 3 both short-lived and long-lived ASCs are generated upon antigen exposure. Short-lived plasma cells secrete antibody during their brief life time (blue) and long-lived plasma cells secrete antibody throughout their long life time (green).

6.2.3. Model fitting methods

Mixed effects model likelihood

Models 1 – 3 were fitted to the Ghanaian and Gambian longitudinal data sets using mixed effects methods such that local parameters are estimated for each child individually, with these local (or mixed effects) parameters being drawn from a global distribution [313]. For example, for each child k the half-life of maternal antibody may be estimated as d_m^k. These K estimates of the local parameters d_m^k will be drawn from a probability distribution. A Log-Normal distribution is suitable as it has positive support on $[0,\infty)$. Thus we have $\log(d_m^k) \sim N\ \mu_m, \sigma_m^2$. The mean d_m and the variance Σ_m^2 of the estimates of d_m^k are given by $d_m = e^{\mu_m + \frac{\sigma_m^2}{2}}$ and $\Sigma_m^2 = \ e^{\sigma_m^2} - 1\ e^{2\mu_m + \sigma_m^2}$.

Model 1

For child *k* we have data on observed antibody titres $\mathrm{A}^k = \ a_1, \ldots, a_J$ at times $T^k = \ t_1, \ldots, t_J$, from which we can estimate the times of antibody boosts $\Gamma^k = \ \tau_1, \tau_2, \ldots, \tau_I$ according to whether there is a boost to antibody titres between consecutive samples. The time of exposure τ_i is assumed to be the midpoint of the time period within which boosting of antibody titres was detected. We denote $D^k = \ A^k, T^k$ to be the vector of data for child *k*, and likewise *D* to be the vector of data for all *K* children. For child *k*, we estimate the half-life of maternal antibody d_m^k, the half-life of exposure-acquired antibody d_a^k, the initial titre of maternal antibody A_m^k, and the magnitude of boosts to antibody titres $\Delta^k = \{\alpha_1, \alpha_2, \ldots, \alpha_I\}$. We denote $\theta^k = \ d_m^k, d_a^k, A_m^k, \Delta^k$ to be the vector of parameters to be estimated.

Adapting equation (18) to include maternal antibody decaying at rate $m = \dfrac{\log(2)}{d_m}$ gives

$$\mathrm{A}(t) = \mathrm{A}_m e^{-mt} + \sum_{i \in I} H(t, \tau_i) \alpha_i e^{-r(t-\tau_i)} \qquad (24)$$

The predicted antibody titres will be $A(t_1), A(t_2), \ldots, A(t_J)$. We assume Normally distributed measurement error with standard deviation σ_{obs}. The difference between the observed antibody titre a_j and the predicted antibody titre $A(t_j)$ will be Normally distributed with variance σ_{obs}^2, truncated at zero as negative antibody titres are not allowed. The data likelihood of Model 1 for child k is given by

$$L^k_{\text{mod }1}\ \theta^k \left| D^k \right. = \prod_{j \in J} \frac{1}{\sigma_{\text{obs}}\sqrt{2\pi}} \frac{e^{-\frac{A(t_j)-a_j}{2\sigma^2_{\text{obs}}}^2}}{1-\Phi\ 0\left| A(t_j), \sigma^2_{\text{obs}} \right.} \tag{25}$$

Φ is the cumulative normal distribution (i.e. a normalizing factor), with $1-\Phi\ 0\left| A(t_j), \sigma^2_{\text{obs}} \right.$ being the area under a normal curve with mean $A(t_j)$ and variance σ^2_{obs} in the region $[0,\infty)$.

The parameters d^k_m and d^k_a are mixed effects parameters drawn from Log-Normal distributions with means d_m and d_a respectively. Including the contribution to the likelihood for the distance between the local and global estimates gives

$$L^k_{\text{mix}}\ \theta^k \left| D^k \right. = \frac{e^{-\frac{(\log(d^k_m)-\mu_m)^2}{2\sigma^2_m}} e^{-\frac{(\log(d^k_a)-\mu_a)^2}{2\sigma^2_a}}}{2\pi d^k_m d^k_a \sigma_m \sigma_a} L^k_{\text{mod }1}\ \theta^k \left| D^k \right. \tag{26}$$

where σ_m and $\mu_m = \log(d_m) - \frac{\sigma^2_m}{2}$ are the standard deviation and mean of the logarithm of the local estimates of d^k_m, and σ_a and μ_a are similarly defined. The total likelihood is obtained by multiplying the likelihood for each child

$$L_{\text{total}}\ \theta \left| D \right. = \prod_{k \in K} L^k_{\text{mix}}\ \theta^k \left| D^k \right. \tag{27}$$

where $\theta = d_m, d_a, \sigma_m, \sigma_a, \sigma_{\text{obs}}, \theta^1, ..., \theta^K$ is the vector of all parameters to be estimated, and $D = D^1, ..., D^K$ is the vector of all data.

The derivation of the likelihood of Model 1's fit to the Gambian data is similar to that outlined above, except without the inclusion of maternal antibody. Where observations of infants in the Ghanaian cohort begin with maternal antibody A_m, a Gambian child will begin with some titre A_0 at the end of the rainy season which is to be estimated.

Model 2

Under Model 2 a child from the Ghanaian cohort is born with maternally-acquired antibody titre A_m and receives boosts to plasma B cells $\Delta^k = \beta_1, \beta_2, ..., \beta_I$ at times $\Gamma^k = \tau_1, \tau_2, ..., \tau_I$ due to exposure to *P. falciparum* antigens. We denote $\theta^k = d^k_m, d^k_a, d^k_b, A^k_m, \Delta^k$ to be the vector of parameters to be estimated. Adapting equation (21) to include maternal antibody gives

$$A(t) = A_m e^{-mt} + \sum_{i \in I} H(t, \tau_i)\beta_i \left(\frac{e^{-c(t-\tau_i)} - e^{-r(t-\tau_i)}}{r-c} \right) \tag{28}$$

The data likelihood for child k under Model 2 is given by

$$L^k_{\text{mod }2}\ \theta^k \left| D^k \right. = \prod_{j \in J} \frac{1}{\sigma_{\text{obs}}\sqrt{2\pi}} \frac{e^{-\frac{A(t_j)-a_j}{2\sigma^2_{\text{obs}}}^2}}{1-\Phi\ 0 \left| A(t_j), \sigma^2_{\text{obs}} \right.} \tag{29}$$

Including the contribution to the likelihood for the difference between the local and global parameters gives

$$L^k_{\text{mix}}\ \theta^k \left| D^k \right. = \frac{e^{-\frac{(\log(d^k_m)-\mu_m)^2}{2\sigma^2_m}} e^{-\frac{(\log(d^k_a)-\mu_a)^2}{2\sigma^2_a}} e^{-\frac{(\log(d^k_b)-\mu_b)^2}{2\sigma^2_b}}}{2\pi^{\ 3/2} d^k_m d^k_a d^k_b \sigma_m \sigma_a \sigma_b} L^k_{\text{mod }2}\ \theta^k \left| D^k \right. \tag{30}$$

The total likelihood is obtained by multiplying the mixed effects likelihood for all K children.

$$L_{\text{total}}\ \theta \left| D \right. = \prod_{k \in K} L^k_{\text{mix}}\ \theta^k \left| D^k \right. \tag{31}$$

Model 3

Under Model 3 a child from the Ghanaian cohort is born with maternally-acquired antibody titre A_m and receives boosts to plasma B cells of $\Delta^k = \beta_1, \beta_2, ..., \beta_I$ at times $\Gamma^k = \tau_1, \tau_2, ..., \tau_I$ due to exposure to *P. falciparum* antigens. A proportion ρ of ASCs are assumed to be short-lived and a proportion $1-\rho$ long-lived. We denote $\theta^k = d^k_m, d^k_a, d^k_s, d^k_l, \rho^k, A^k_m, \Delta^k$ to be the vector of parameters to be estimated. Adapting equation (23) to include maternal antibody gives

$$A(t) = A_m e^{-mt} + \sum_i H(t, \tau_i)\beta_i \left(\rho \left(\frac{e^{-c_s(t-\tau_i)} - e^{-r(t-\tau_i)}}{r-c_s} \right) + \ 1-\rho \ \left(\frac{e^{-c_l(t-\tau_i)} - e^{-r(t-\tau_i)}}{r-c_l} \right) \right) \tag{32}$$

The data likelihood for child k under Model 3 is given by

$$L^k_{\text{mod }3}\ \theta^k \left| D^k \right. = \prod_{j \in J} \frac{1}{\sigma_{\text{obs}}\sqrt{2\pi}} \frac{e^{-\frac{A(t_j)-a_j}{2\sigma^2_{\text{obs}}}^2}}{1-\Phi\ 0 \left| A(t_j), \sigma^2_{\text{obs}} \right.} \tag{33}$$

The proportion of short-lived plasma cells ρ is constrained to be in the region [0,1] and thus we assume a Logit-Normal distribution for the mixed effects parameters ρ^k with mean μ_ρ and variance σ^2_ρ on the logit scale. There is

no analytical solution for the mean of a logit-normal distribution in terms of the mean and variance of the underlying Normal distribution, so we estimate the mean on the logit scale μ_ρ and then numerically calculate ρ.

Including the contribution to the likelihood for the difference between the local and global parameters gives

$$L^k_{\text{mix}}\ \theta^k \left| D^k \right. = \frac{e^{-\frac{(\log(d_m^k)-\mu_m)^2}{2\sigma_m^2}} e^{-\frac{(\log(d_a^k)-\mu_a)^2}{2\sigma_a^2}} e^{-\frac{(\log(d_s^k)-\mu_s)^2}{2\sigma_s^2}} e^{-\frac{(\log(d_l^k)-\mu_l)^2}{2\sigma_l^2}} e^{-\frac{(\log(\frac{\rho^k}{1-\rho^k})-\mu_\rho)^2}{2\sigma_\rho^2}}}{2\pi^{5/2} d_m^k d_a^k d_s^k d_l^k \sigma_m \sigma_a \sigma_s \sigma_l \rho^k (1-\rho^k)\sigma_\rho} L^k_{\text{mod 3}}\ \theta^k \left| D^k \right. \quad (34)$$

The total likelihood is obtained by multiplying the mixed effects likelihood for all K children.

$$L_{\text{total}}\ \theta \left| D \right. = \prod_{k \in K} L^k_{\text{mix}}\ \theta^k \left| D^k \right. \quad (35)$$

Markov chain Monte Carlo methods

The three models were fitted to the data on each of the four antigens from both cohorts using Markov Chain Monte Carlo (MCMC) methods. Parameters were updated at each MCMC iteration using a random walk Metropolis-Hastings algorithm with three update stages illustrated here for the case of Model 1 fitted to the Ghanaian data. A ' indicates an attempted update parameter. At iteration *n*, carry out the following parameter updates:

1. **Local parameter update.** For each child k, update the following local parameters in one step
   - Half-life of maternal antibody: $d_m^{k\prime} = N\ \ d_m^k, \lambda^k_{\text{loc}} V_{d_m^k}$
   - Half-life of exposure-acquired antibody: $d_a^{k\prime} = N\ \ d_a^k, \lambda^k_{\text{loc}} V_{d_a^k}$
   - Calculate updated mixed effects likelihood $L^k_{\text{mix}}\ \theta^{k\prime} \left| D^k \right.$
   - Accept the parameter update with probability

$$\min\left(1, \frac{L^k_{\text{mix}}\ \theta^{k\prime} \left| D^k \right.}{L^k_{\text{mix}}\ \theta^k \left| D^k \right.}\right)$$

   - If *n* < 5,000,000 update λ^k_{loc} using the Robbins-Munro algorithm for tuning acceptance rates
2. **Nuisance parameter update.** For each child k update the following parameters in one step
   - Initial titre of maternal antibody: $A_m^{k\prime} = N\ \ A_m^k, \lambda^k_{\text{nuis}} V_{A_m^k}$
   - Magnitude of antibody boosts $i \in \{1, \ldots, I\}$: $\alpha_i^{k\prime} = N\ \ \alpha_i^k, \lambda^k_{\text{nuis}} V_{\alpha_i^k}$

- Calculate updated mixed effects likelihood $L^k_{\text{mix}}\ \ \theta^{k\prime}\,\middle|\,D^k$
- Accept the parameter update with probability

$$\min\left(1, \frac{L^k_{\text{mix}}\ \ \theta^{k\prime}\,\middle|\,D^k}{L^k_{\text{mix}}\ \ \theta^{k}\,\middle|\,D^k}\right)$$

- If n < 5,000,000 update λ^k_{nuis} using the Robbins-Munro algorithm for tuning acceptance rates

3. **Global parameter update.** Update the following global parameters in one step
   - Half-life of maternal antibody: $d_m{}' = N\ \ d_m, \lambda_{\text{glob}} V_{d_m}$
   - Half-life of exposure-acquired antibody: $d_a{}' = N\ \ d_a, \lambda_{\text{glob}} V_{d_a}$
   - Standard deviation in half-life of maternal antibody: $\sigma_m{}' = N\ \ \sigma_m, \lambda_{\text{glob}} V_{\sigma_m}$
   - Standard deviation in half-life of exposure-acquired antibody: $\sigma_a{}' = N\ \ \sigma_a, \lambda_{\text{glob}} V_{\sigma_a}$
   - Observational variance: $\sigma_{\text{obs}}{}' = N\ \ \sigma_{\text{obs}}, \lambda_{\text{glob}} V_{\sigma_{\text{obs}}}$
   - Calculate updated total likelihood $L_{\text{total}}\ \ \theta'\,|\,D$ and the updated prior likelihood $P\ \ \theta'$
   - Accept the parameter update with probability

$$\min\left(1, \frac{L_{\text{total}}\ \ \theta'\,|\,D\ \ P\ \ \theta'}{L_{\text{total}}\ \ \theta\,|\,D\ \ P\ \ \theta}\right)$$

   - If n < 5,000,000 update λ_{glob} using the Robbins-Munro algorithm for tuning acceptance rates

The variances of the proposal distributions V were first estimated by performing 10 million MCMC iterations to estimate the variance of the posterior distributions. No prior distributions were placed on local or nuisance parameters, only on global parameters (Table 6.1). 200 million MCMC iterations were computed with calibration of acceptance rates using a Robbins-Munro algorithm [314]. All Markov chains were visually examined for appropriate mixing and convergence. Such large numbers of iterations were needed because of the large number of parameters to be estimated.

Model comparison

The median and 95% credible intervals for the posterior distributions for each of the estimated global parameters are shown in Table 6.2 for the Ghanaian data and in Table 6.3 for the Gambian data. For each model, the log-

likelihood and Akaike Information Criterion (AIC) at the median global parameter estimates are shown in Tables 6.2 and 6.3. The results of comparisons between mixed effects models should be interpreted with caution as the likelihood is composed of two components: the likelihood of the model fit to each individual child, and the likelihood that the mixed effects parameters are drawn from the global parameter distributions. Model 1 can be shown to be nested in Model 2 which can in turn be shown to be nested in Model 3, and hence we expect the likelihood of Model 3 to be greatest, followed by Model 2, when the models are fitted to the data from one child at a time. However, this ordering of likelihoods cannot be guaranteed in the mixed effects framework. In addition, counting the degrees of freedom in hierarchical, mixed effects models is not straightforward [315, 316]. The formulation of the AIC presented here counts each of the mixed effects and global parameters as a degree of freedom.

Prior parameter distributions

As the models were fitted in a Bayesian framework it was possible to incorporate prior information from the literature on the parameters under investigation (Table 6.1). No estimates of the half-life of maternal antibody were found in the literature. Riley *et al* [311] have estimated the rate at which infants become sero-negative due to waning of maternal antibody, but this information cannot be used to inform priors as it was derived from the same Ghanaian cohort under investigation in this study. Sehgal *et al* [317] found that in Papua New Guinean infants, most lost all detectable malaria-specific IgG between the ages of 5 and 7 months. This information was used to restrict the maximum half-life of maternal antibody.

Prior estimates of the half-lives of exposure-acquired IgG antibodies were obtained from the literature: Kinyanjui *et al* [299] estimated that combined responses of IgG1 to the merozoite antigens MSP-1, MSP-2, AMA-1 and EBA-175 had a half-life of 9.8 days (95% CI, 7.6 – 12.0). The half-life of IgG3 antibody was estimated to be 6.1 days (95% CI, 3.7 – 8.4). These estimates were derived from hospitalised children after an episode of clinical malaria. We used 9.8 days as a prior for the half-life of exposure-acquired antibody in Models 2 and 3.

No estimates of the relative proportions of antibody generated by short-lived and long-lived ASCs were found. Weiss *et al* [68] estimated the expansion and contraction of the MBC compartment to various merozoite antigens after *P. falciparum* infection. The proportion of short-lived MBCs can be estimated by measuring the peak number of antigen specific MBCs after an episode of clinical malaria and the number of MBCs 12 months later. Using data from Weiss *et al* we estimated that 86% of AMA-1 specific MBCs are short-lived and 20% of MSP-1 specific MBCs are short-lived. Given that we are estimating the relative proportions of ASCs and not MBCs we used these estimates as weakly informative priors. No estimates of the half-lives of short-lived ASCs in humans were found. Ndungu *et al* [318] report that short-lived ASCs generated following *Plasmodium chabaudi* infection in mice die within 3 – 5 days. We thus assumed a prior estimate of 4 days for the half-life of ASCs in humans.

Table 6.1; Prior distributions for parameters. The value shown is the mean of the probability distribution with the range containing 95% of the distribution, except in the case of the uniform distribution where the range denotes the minimum and maximum allowable values.

parameter	symbol	distribution	value (range)	model	Reference
half-life of maternal antibody	d_m	Uniform	(0 – 365) days	M1, M2, M3	Sehgal [317]
half-life of IgG antibody	d_a	Log-normal	9.8 (8.3 – 11.5) days	M2, M3	Kinyanjui [299]
half-life of short-lived plasma cells	d_s	Log-normal	4.0 (3.1 – 5.1) days	M3	Ndungu [318]
half-life of long-lived plasma cells	d_l	Log-normal	365 (208 – 596) days	M3	estimate
proportion of short-lived cells	ρ	Logit-normal	0.86 (0.46 – 0.98)	M3	Weiss [68]

6.3. Results

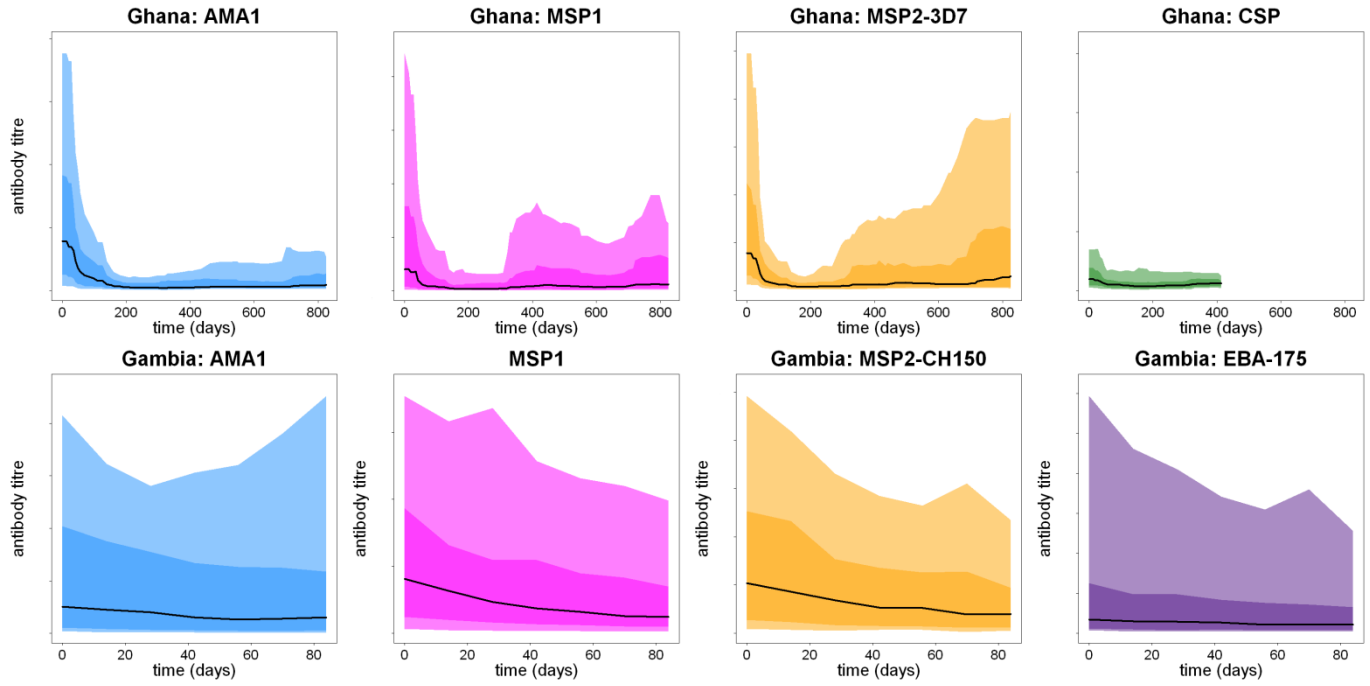


Figure 6.3: Aggregated antibody kinetics for Ghanaian and Gambian children. The black curve represents the median age-dependent antibody titre. The darkly shaded regions contain 50% of the data, and the lightly shaded regions contain 95% of the data. Ghanaian children are followed from birth for up to 812 days. Gambian children are followed from the start of the dry season for 84 days.

The aggregated age-dependent antibody kinetics for each of the antigens studied in both cohorts are shown in Figure 6.3. In the Ghanaian data the median antibody titre decreases substantially in the first 100 days, consistent with exponential decay of maternal antibodies, and then increases gradually as antibodies are acquired in response to malaria infection. In the Gambian data there is an observable decline in median antibody titres as antibody generated in the wet season decays during the dry season. Figure 6.3 captures the general time-dependent trends in antibody kinetics but not the individual level variation in immune parameters. In individual children, protective antibodies are not acquired at a continuous rate but rather in a stochastic manner in response to antigen exposure. Thus each child will have a unique antibody profile depending on the number of infections and individual-level variation in immune parameters. Figure 6.4 shows a sample of the antibody kinetics of four children from each cohort.

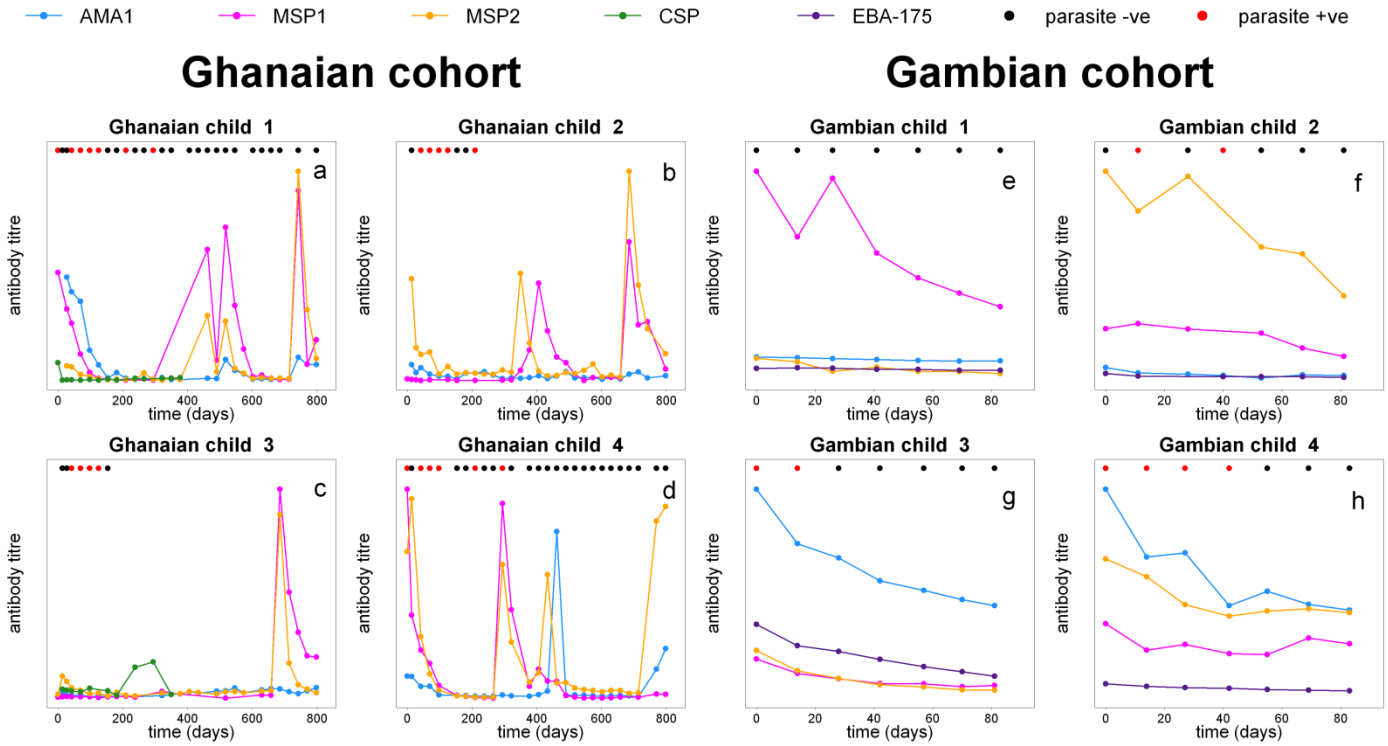


Figure 6.4: Measurements of antibody titres for a sample of 4 of 151 Ghanaian **(a-d)** and 4 of 124 Gambian children **(e-h)**. Where *P. falciparum* parasites were tested for, it is indicated at the top of each plot as present (red) or absent (black).

The Ghanaian children were born with maternally acquired IgG antibodies but no ASCs of their own, hence antibody titres decay at a constant rate in the first months of life (Figure 6.4 a-d). Throughout the period of follow-up children experienced sporadic boosts to antibody titres upon exposure to *P. falciparum* antigens, after which antibody titres decay again. In some children there is evidence for a bi-phasic decay, with antibody titres dropping rapidly immediately after boosting and then decaying at a much slower rate over a period of months to years. This bi-phasic decay may be due to generation of populations of short-lived and long-lived ASCs following antigen exposure. Short-lived ASCs produce large quantities of antibody needed to protect against current malaria infection, while long-lived ASCs maintain a sustained antibody response for protection against future infections.

The Gambian children ranged in age from 1 to 6 years, too old for maternal antibodies to be present in appreciable quantities [56]. Follow-up commenced after the end of the rainy season, during which the children are likely to have been exposed to *P. falciparum* parasites [303]. During follow-up a steady decay in antibody titres punctuated by occasional boosts was observed (Figure 6.4 e-h). Following a boost, titre appears to decay at a faster rate than before boosting (as seen in Gambian child 1, Figure 6.4e), possibly due to the decay of short-lived ASCs induced by recent antigen exposure.

In both cohorts longitudinal parasite presence data as detected by PCR or microscopy was available for most children. As antibody titres are boosted in response to antigen exposure, one would expect a new malaria infection to be accompanied by an increase in antibody titres. However, upon examination of the data, new infections as

determined by the presence of parasites were a poor predictor of the timing of boosts to antibody titres. New infections were often not followed by boosts to titres, and antibody titres were regularly boosted in the absence of detectable infection (Figure 6.4). Although the kinetics of antibodies to different antigens were often synchronous, with boosting and decay of titres happening simultaneously, there were many instances where the kinetics to different antigens appeared to act independently. For example in Figure 6.4d antibody titres to MSP-1 and MSP-2 are boosted without boosting of AMA-1. This poor agreement between times of antibody boost and infection could be due to poor sensitivity or specificity of the parasite detection methods, delayed antigen presentation by follicular dendritic cells [319], or bystander activation [67]. Due to this asynchronous boosting the kinetics of antibodies to each antigen were investigated independently of parasite prevalence data and the kinetics of antibodies to other antigens.

The three models were fitted to the Ghanaian and Gambian datasets (see Model fitting methods), with the parameters providing the best fit to both datasets shown in Tables 6.2 and 6.3. A sample of model fits to the antibody kinetics of two Ghanaian and two Gambian children is shown in Figure 6.5. For the Ghanaian cohort, Model 1 predicts that maternally acquired antibody persists with a half-life of 26 – 42 days, and that exposure-acquired antibody circulates with a half-life of 15 – 42 days. Model 2 predicts that maternally acquired antibody persists with a half-life of 27 – 55 days, and that ASCs generated upon exposure persist with a half-life of 13 – 16 days, producing IgG antibody that circulates in the blood-stream for 10 – 11 days. Model 3 predicts that maternal antibodies persist with a half-life of 20 – 41 days, 89% – 98% of ASCs are short-lived persisting with half-life 3.6 – 3.9 days, the rest being long-lived persisting with half-life 303 – 469 days. IgG antibody secreted by ASCs is estimated to persist in the blood stream with a half-life of 8 – 9 days.

For the Gambian cohort, Model 1 predicts that antibody circulates in the blood stream with a half-life of 33 – 98 days. Model 2 predicts that circulating antibodies in the blood-stream are due to ASCs with a half-life of 55 – 80 days, producing antibody that decays with a half-life 12 – 16 days. Model 3 predicts that circulating antibody is generated by short-lived ASCs with half-life 2.0 – 2.3 days, and long-lived ASCs with half-life 374 – 503 days producing IgG antibody with half-life 7 days. Following antigen exposure 74% – 87% of ASCs are estimated to be short-lived.

All three models were able to reliably reproduce the patterns observed in the data, with the more biologically realistic models resulting in improved fits (as judged by the model likelihood), although at the expense of a greater number of parameters. The fits of Model 3 to the Ghanaian data suggest that infants are only able to mount a transient antibody response to their first few infections, with only 2 – 11% of plasma cells estimated to be long-lived. In contrast, Model 3 predicted that the older Gambian children were able to mount normal antibody responses with 13 – 26% of the induced plasma cells being long-lived and contributing to the maintenance of humoral immunity.

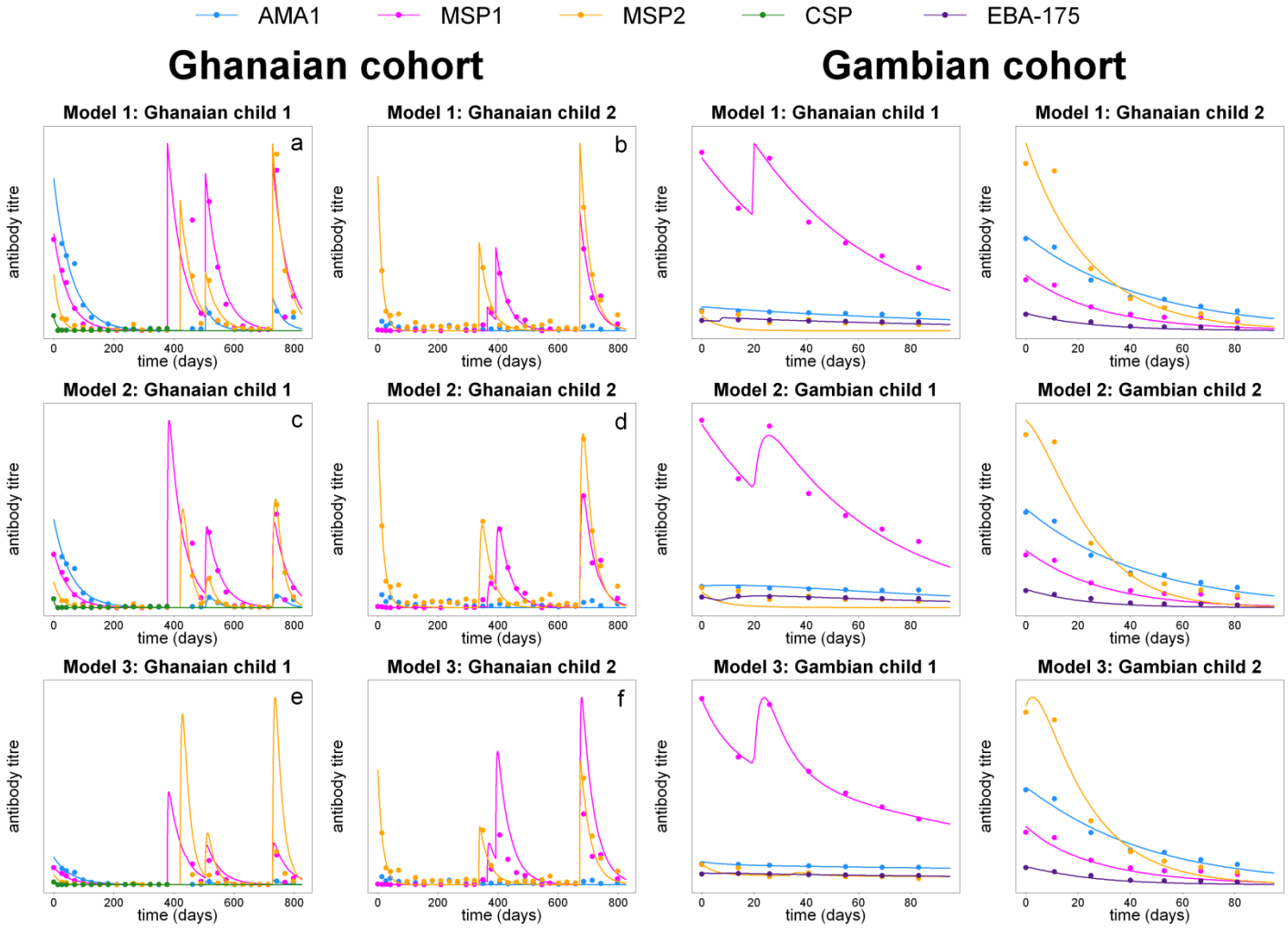


Figure 6.5: Model fits for a sample of two Ghanaian and two Gambian children. Estimated antibody kinetics for two Ghanaian children under Model 1 **(a-b)**, under Model 2 **(c-d)**, and under Model 3 **(e-f)**. Estimated antibody kinetics for two Gambian children under Model 1 **(g-h)**, under Model 2 **(i-j)**, and under Model 3 **(k-l)**.

Table 6.2: Median parameter estimates from the posterior distribution and 95% credible intervals for Models 1, 2 and 3 fitted to the longitudinal data from the Ghanaian cohort.

		AMA-1	MSP-1	MSP-2	CSP
Model 1					
maternal antibody ½-life (days)	d_m	42 (35 – 53)	26 (19 – 37)	37 (26 – 56)	27 (22 – 36)
maternal antibody ½-life st. dev. (days)	Σ_m	33 (24 – 51)	26 (17 – 46)	44 (25 – 80)	15 (10 – 30)
IgG antibody ½-life (days)	d_a	18 (13 – 28)	25 (17 – 40)	42 (30 – 66)	15 (12 – 24)
IgG antibody ½-life st. dev. (days)	Σ_a	22 (14 – 41)	35 (21 – 66)	56 (35 – 103)	11 (7 – 25)
observational variance	σ_{obs}	0.165 (0.160 – 0.171)	0.174 (0.168 – 0.180)	0.188 (0.182 – 0.194)	0.095 (0.091 – 0.100)
log-likelihood		1192	1397	1694	1185
AIC		-1560	-1986	-2560	-1992
Model 2					
maternal antibody ½-life (days)	d_m	48 (38 – 63)	55 (37 – 89)	27 (20 – 39)	55 (28 – 129)
maternal antibody ½-life st. dev. (days)	Σ_m	42 (27 – 66)	75 (42 – 147)	25 (14 – 46)	81 (32 – 300)
IgG antibody ½-life (days)	d_a	10 (9 – 11)	10 (9 – 12)	11 (9 – 12)	10 (8 – 11)
IgG antibody ½-life st. dev. (days)	Σ_a	11 (9 – 14)	11 (9 – 14)	11 (9 – 13)	10 (7 – 13)
plasma B cell ½-life (days)	d_b	16 (13 – 21)	16 (13 – 21)	16 (13 – 21)	13 (10 – 18)
plasma B cell ½-life st. dev. (days)	Σ_b	22 (16 – 32)	19 (13 – 28)	17 (13 – 24)	14 (9 – 23)
observational variance	σ_{obs}	0.164 (0.159 – 0.170)	0.128 (0.124 – 0.133)	0.129 (0.125 – 0.134)	0.055 (0.051 – 0.059)
log-likelihood		1045	1494	1818	1301
AIC		-1052	-1982	-2590	-2112
Model 3					
maternal antibody ½-life (days)	d_m	41 (35 – 49)	31 (24 – 42)	20 (17 – 24)	40 (26 – 72)
maternal antibody ½-life st. dev. (days)	Σ_m	29 (22 – 41)	29 (18 – 48)	13 (9 – 20)	46 (25 – 110)
IgG antibody ½-life (days)	d_a	8 (7 – 10)	8 (7 – 10)	8 (7 –10)	9 (7 – 10)
IgG antibody ½-life st. dev. (days)	Σ_a	11 (9 – 15)	12 (9 – 15)	12 (9 – 16)	13 (9 – 17)
short-lived B cell ½-life (days)	d_s	3.9 (3.3 – 4.5)	3.9 (3.3 – 4.5)	3.7 (3.2 – 4.3)	3.6 (3.1 – 4.2)
short-lived B cell ½-life st. dev. (days)	Σ_s	6.0 (4.9 – 7.4)	5.7 (4.6 – 7.0)	5.4 (4.4 – 6.7)	4.7 (3.7 – 5.9)
long-lived B cell ½-life (days)	d_l	371 (257– 548)	303 (197 – 477)	313 (207 – 482)	469 (309 – 711)
long-lived B cell ½-life st. dev. (days)	Σ_l	650 (381 – 1167)	644 (358 – 1236)	606 (341 – 1126)	801 (456 – 1531)
proportion short-lived	P	0.97 (0.92 – 0.99)	0.89 (0.80 – 0.95)	0.91 (0.84 – 0.95)	0.98 (0.92 – 0.99)
proportion short-lived st. dev.	Σ_p	0.09 (0.04 – 0.17)	0.21 (0.13 – 0.31)	0.19 (0.12 – 0.28)	0.06 (0.01 – 0.18)
observational variance	σ_{obs}	0.152 (0.147 – 0.158)	0.099 (0.095 – 0.103)	0.148 (0.141 – 0.151)	0.051 (0.047 – 0.055)
log-likelihood		1375	1856	2193	1414
AIC		-1284	-2310	-2904	-2114

Table 6.3: Median parameter estimates from the posterior distribution and 95% credible intervals for Models 1, 2 and 3 fitted to the longitudinal data from the Gambian cohort.

		AMA-1	MSP-1	MSP-2	EBA-175
Model 1					
IgG antibody ½-life (days)	d_a	64 (53 – 81)	69 (46 – 113)	33 (21 – 60)	98 (64 – 161)
IgG antibody ½-life st. dev. (days)	Σ_a	43 (30 – 68)	109 (64 – 221)	55 (30 – 124)	156 (88 – 319767)
observational variance	σ_{obs}	2.8 (2.7 – 3.0)	3.9 (3.8 – 4.1)	7.5 (7.2 – 7.8)	1.5 (1.4 – 1.6)
log-likelihood		-1428	-1913	-1797	-936
AIC		3358	4328	4096	2374
Model 2					
IgG antibody ½-life (days)	d_a	16 (14 – 18)	15 (13 – 18)	12 (11 – 14)	14 (12 – 16)
IgG antibody ½-life st. dev. (days)	Σ_a	20 (17 – 24)	20 (16 – 24)	15 (11 – 19)	19 (15 – 23)
plasma B cell ½-life (days)	d_b	77 (56 – 106)	78 (56 – 109)	55 (37 – 81)	80 (57 – 113)
plasma B cell ½-life st. dev. (days)	Σ_b	98 (63 – 153)	100 (63 – 159)	82 (47 – 139)	123 (77 – 192)
observational variance	σ_{obs}	2.8 (2.7 – 3.0)	4.1 (3.9 – 4.3)	7.4 (7.2 – 7.7)	1.6 (1.5 – 1.7)
log-likelihood		-1199	-1849	-1698	-942
AIC		3400	4700	4398	2886
Model 3					
IgG antibody ½-life (days)	d_a	7 (6 – 9)	7 (6 – 9)	7 (6 – 9)	7 (6 – 9)
IgG antibody ½-life st. dev. (days)	Σ_a	12 (9 – 16)	12 (9 – 16)	12 (9 – 16)	11 (8 – 15)
short-lived B cell ½-life (days)	d_s	2.1 (1.8 – 2.5)	2.0 (1.7 – 2.5)	2.3 (1.9 – 2.9)	2.3 (1.9 – 2.7)
short-lived B cell ½-life st. dev. (days)	Σ_s	0.9 (0.5 – 1.6)	0.9 (0.4 – 1.7)	1.2 (0.6 – 2.0)	1.2 (0.7 – 1.8)
long-lived B cell ½-life (days)	d_l	503 (328 – 787)	376 (233 – 600)	374 (235 – 606)	414 (251 – 658)
long-lived B cell ½-life st. dev. (days)	Σ_l	1564 (875 – 2902)	1257 (655 – 2429)	1195 (639 – 2349)	1680 (851 – 3250)
proportion short-lived	P	0.87 (0.77 – 0.93)	0.84 (0.73 – 0.91)	0.83 (0.73 – 0.90)	0.74 (0.60 –0.85)
proportion short-lived st. dev.	Σ_ρ	0.20 (0.11 – 0.30)	0.22 (0.13 – 0.32)	0.22 (0.13 – 0.31)	0.30 (0.19 – 0.39)
observational variance	σ_{obs}	2.6 (2.3 – 2.8)	4.4 (4.1 – 4.8)	2.4 (2.2 – 2.7)	1.3 (1.2 – 1.4)
log-likelihood		-1008	-1647	-1559	-838
AIC		3624	4932	4754	3248

6.4. Discussion

In humans exposed to malaria parasites, both short-lived [299, 303, 320] and long-lived [60, 309] anti-*Plasmodium* antibody responses have been observed in longitudinal studies. Underlying these antibody responses are antigen-specific memory B cells and plasma cells. Upon exposure to *P. falciparum* antigens activated B cells undergo rapid expansion and differentiation into ACSs [321]. Short-lived antibody responses suitable for protecting against current malaria infections are generated by short-lived plasma cells in the spleen, lymph nodes and other peripheral tissues. In the absence of essential survival signals only found in bone marrow, these cells die by apoptosis. We assumed that long-lived antibody responses are generated by long-lived plasma cells, which can continuously secrete IgG antibodies from survival niches in the bone marrow or spleen in the absence of detectable memory B cells [69]. An alternative explanation for the persistent secretion of antibodies is given by the continuous activation of antigen-specific MBCs and their differentiation into short-lived ASCs [67, 322].

The half-life of exposure-acquired IgG antibodies to *Plasmodium* antigens was estimated to be in the range of 7 – 9 days in both cohorts, in agreement with Kinyanjui *et al*'s estimate of 9.8 days [299]. In contrast the half-life of maternally acquired IgG antibodies from the Ghanaian cohort is estimated to be 26 – 42 days. Neither of these estimates match the 21 day half-life of naturally metabolised IgG1 in adults [323]. However the quantity estimated from the data is the half-life of detectable IgG circulating in the blood and not the half-life of naturally metabolised IgG. Differences between the half-lives of circulating IgG and naturally metabolised IgG could be due to depletion of circulating IgG by parasite opsonisation or penetration of IgG into tissue. Also, early studies have shown that half-life is inversely related to serum concentration and can vary from 11 – 70 days depending on IgG subclass [324]. An analysis of IgG kinetics split by subclass will be necessary to fully understand the observed differences in antibody half-life.

In our model of antibody kinetics, the long term maintenance of antibodies is assumed to be due to long-lived plasma cells, which are estimated to decay exponentially with a half-life of 303 – 503 days. The duration of long-lived ASCs will have implications for the detection of malaria transmission via serological methods [325]. In catalytic models for measuring malaria transmission, it is assumed that sero-positive individuals revert to sero-negative at a constant rate [310]. However the reality is more complex, as the time until sero-reversion will depend on the half-life of long-lived ASCs and the antibody titre threshold for sero-conversion.

The infection-blocking vaccine RTS,S induces high anti-CSP antibody titres and CSP-specific $CD4^+$ T cells, both of which have been shown to be associated with protection from infection [279, 291]. Vaccine-induced anti-CSP antibody titres exhibit a pattern of bi-phasic decay, with titres decaying rapidly immediately after vaccination and then decaying at a slower rate over a number of years [118, 129] – kinetics which can be reproduced by Model 3. Under conditions of natural exposure, we estimated that only 2% of anti-CSP antibody secreting cells are long-lived, but that these persist with a half-life of 469 days. Although no estimates of the half-life of anti-CSP antibody responses following RTS,S vaccination have been published to date, the observed patterns of antibody decay appear consistent with a 469 day half-life [139]. In addition, it is likely that the proportion of long-lived ASCs generated by vaccination will be different to the proportion generated during natural infection, possibly due to the effect of the adjuvant. Further analysis of immunological data from Phase II trials of RTS,S should allow a more detailed comparison of the kinetics of naturally acquired and vaccine-induced antibodies.

The models described in this study are necessary simplifications of the complex processes underlying the generation of antibody responses. ASCs are assumed to be generated in sudden bursts following antigen exposure (boosting), corresponding to the assumption of rapid proliferation and differentiation of MBCs. However blood-stage malaria infections can persist for weeks or months continuously exposing newly minted naïve B cells and MBCs to antigen. An alternative model formulation could include the underlying parasite dynamics and continuous stimulation of MBCs and differentiation into ASCs. Also, the simple models investigated here do not distinguish between infection and re-infection. It has been observed that functional MBCs generated in a primary infection give rise to a faster ASC and antibody response upon re-infection [318]. Alternative model formulations could consider the possibility of

faster and stronger secondary antibody responses and a greater likelihood of MBCs differentiating into long-lived ASC compared to naïve B cells. However, the data used here are unlikely to be sufficient to distinguish between these models of increased complexity.

The temporal resolution of the data presents a limitation to the model. Antibody titres are available every 4 weeks in the Ghanaian cohort and every 2 weeks in the Gambian cohort. With antibody titres sampled at a weekly frequency it is difficult to make inferences on the processes of MBC proliferation and decay of short-lived ASCs. The limitations encountered due to the temporal resolution of the data could be overcome by fitting the models to data from experimental malaria infection in mice where antibody responses can be measured more frequently. In addition, the kinetics of MBCs and plasma-cell responses can be directly measured as has been demonstrated by Langhorne and colleagues [318, 326] following blood-stage *P. chabaudi* infection in mice.

Although the cellular and molecular determinants of the duration of antibody response have been investigated in mouse models [318, 326] they remain poorly studied in humans. Memory B cells specific to *Plasmodium* antigens are detectable in human peripheral blood mononuclear cells (PBMC) and studies have reported their expansion and contraction [68], and their longevity [309, 327]. However there are no comparable studies of ASCs in humans exposed to malaria. Such studies would be very difficult to undertake, as ASCs are located primarily in bone marrow and lymphoid organs, and are detectable in PBMCs only in the short window between differentiation and migration to the bone marrow [326]. Data from mouse models demonstrate a strong correlation between numbers of ASCs in the bone marrow and liver, and concentrations of antibody in blood sera, suggesting that antibody titres are a good surrogate for the number of plasma cells [318]. In the absence of data on ASCs in humans, simple models of immunological processes can be used to infer the kinetics of plasma-cell responses from the observable antibody kinetics.

The simple models developed here assume that the immunological processes underlying antibody production remain constant with time and age. In reality we would expect development of the immune response with age. For example in young infants (less than 100 days old) from the Ghanaian cohort, antibody titres are rarely boosted, even though the infants are regularly exposed to *Plasmodium* parasites. This suggests that young infants may be unable to mount their own B cell response, perhaps because high avidity maternally transferred antibodies sequester the antigen and reduce its availability to prime the child's naïve B cells. Comparing the younger Ghanaian cohort with the older Gambian cohort, we see that older children generate a higher proportion of long-lived plasma cells with longer half-lives suggesting development of the immune response with age.

6.5. Conclusion

In summary, our findings demonstrate that the acquisition of immunity to *P. falciparum* is a gradual process with repeated infections inducing proliferation of short-lived plasma cells for generating antibody to combat ongoing infection and long-lived plasma cells for the maintenance of a sustained antibody response. Our results suggest that the antibody response in infants (< 2 years of age) is abnormal, waning over a period of days to weeks due to the absence of appreciable quantities of long-lived plasma cells. In contrast, we estimate that older children are able to generate a substantial proportion of long-lived plasma cells following *P. falciparum* infection giving rise to a long lasting antibody response.

Chapter 7. Simulation of the effectiveness and cost-effectiveness of RTS,S vaccination in combination with insecticide treated nets

In the earlier chapters of this thesis I discussed issues underlying the measurement of naturally acquired pre-erythrocytic immunity and vaccine efficacy in field trials, and immunological correlates of protection from *P. falciparum* infection. Drawing from insights on vaccine-induced immune responses on the individual level, I use mathematical models to simulate the impact of vaccination campaigns on the population level. Using the published summary results from the Phase II and Phase III RTS,S trials I estimate the impact on the clinical epidemiology of malaria of RTS,S/AS01 delivered through the Expanded Program on Immunization (EPI) or via mass vaccination campaigns . In addition, using data from a systematic review of the costs and cost-effectiveness of malaria control interventions [328] I estimate the cost-effectiveness of vaccination with RTS,S/AS01 in combination with long-lasting insecticide treated nets (LLINs).

7.1. Introduction

Should RTS,S be approved for licensure upon completion of Phase III trials, a number of questions will need to be addressed before vaccination of children in sub-Saharan Africa can commence [329]. The efficacy of RTS,S/AS01 against clinical and severe malaria has been well characterised on time scales of up to three years following vaccination [15, 136, 139, 203], but the duration of protection and the interaction with naturally acquired immunity remain poorly understood. Improved understanding of the biological properties of RTS,S will help to determine whether vaccination is likely to have a long term public health benefit. The decision to deploy the vaccine will then depend on economic factors, primarily the cost per dose of vaccine and the cost per case of malaria or disability adjusted life year (DALY) averted. Using published data from Phase II and Phase III trials of RTS,S and knowledge of malaria epidemiology encapsulated in models of malaria transmission we attempt to address these questions.

The efficacy of RTS,S/AS01 against first episodes of clinical malaria has been estimated as 55.8% (97.5% CI, 50.6% – 60.4%) in a cohort of 6000 children followed for 14 months in a Phase III trial [15]. There have been no direct estimates of the efficacy of RTS,S/AS01 against *P. falciparum* infection in African children, although Kester *et al* [129] have shown that RTS,S/AS01 has 50% (95% CI, 32.9% – 67.1%) infection-blocking efficacy in malaria-naïve adults when challenged with the bites of five *P. falciparum* infectious mosquitoes. Analysis of data on clinical episodes of malaria from extended follow-up of participants in Phase II trials shows that a reduction in estimates of vaccine efficacy can be detected for both RTS,S/AS01 and RTS,S/AS02 within three years [136, 139, 203], although these trials were not sufficiently powered to estimate the rate of waning of vaccine efficacy.

Vaccination with RTS,S may delay the acquisition of naturally acquired immunity to clinical malaria. Bejon *et al* [330] observed that young children vaccinated with RTS,S/AS01 had lower blood-stage antibody titres than unvaccinated

children, however they found that blood-stage antibodies were significantly associated with increased risk of clinical malaria – a finding attributable to heterogeneity in exposure causing blood-stage antibodies to act as a marker for exposure [204]. However, conditional upon becoming parasite positive, both LLIN use and vaccination with RTS,S/AS01 were significantly associated with increased odds of clinical malaria compared to asymptomatic infection. In contrast to Bejon's findings, Campo *et al* [331] found no significant differences in blood-stage antibody titres between children vaccinated with RTS,S/AS02 and unvaccinated children. However when stratified by age, the vaccine group had lower blood-stage antibody concentrations than the control group in younger children (< 2 years, comparable with the children in Bejon's study), but not in older children (≥ 2 years). The evidence to date is consistent with RTS,S delaying the acquisition of blood-stage immunity in younger but not in older children. Strong blood-stage immune responses induced following small infectious doses of merozoites [332] could provide a plausible biological mechanism for the similar levels of blood-stage antibodies in the vaccine and control cohorts despite the reduction in the force of infection in vaccinated children.

The long term public health benefit of vaccination will depend on the biological properties of the vaccine and the epidemiology of malaria transmission. Given an understanding of these biological properties, the cost-effectiveness of vaccination can be estimated using mathematical models. There have been several modelling studies of both the effectiveness [63, 64, 172] and cost-effectiveness [178, 179, 333] of RTS,S vaccination campaigns, most notably by a group at the Swiss Tropical and Public Health Institute who have estimated that RTS,S/AS02 administered through the EPI would have a substantial impact on malaria morbidity and mortality, but have negligible effects on malaria transmission at levels of endemicity typical of sub-Saharan Africa [169]. However, the cost-effectiveness of vaccines in the context of other ongoing interventions has not been considered, in particular the rapid scale-up of LLINs that has occurred in the last decade [334]. We add to the existing published literature by describing a model for estimation of the cost-effectiveness of RTS,S vaccination as part of a package of malaria control interventions across a range of epidemiological settings.

7.2. Methods

7.2.1. Transmission model

The impact of vaccination on the clinical epidemiology of malaria is investigated using a model of malaria transmission which captures key aspects of the *P. falciparum* lifecycle and its transmission between humans and mosquitoes [63]. The model has been validated by statistical fitting to data on parasite prevalence and clinical incidence from a variety of malaria endemic settings. Model details and validation have been described elsewhere [63], so only a brief summary is presented here and in Figure 7.1. Individuals begin life susceptible (*S*) to infection but with partial maternal immunity which decays in the first months of life. Individuals become infected at a rate determined by the force of infection (Λ) which depends on the number of mosquitoes per person, the proportion of infectious mosquitoes, and the person's level of pre-erythrocytic immunity. Once infected, a person passes through

the pre-patent (liver) stage of infection, and then progresses to develop clinical disease (*D*) or asymptomatic (*A*) infection, depending on their level of clinical immunity ϕ. A proportion f_T of those with clinical disease will be treated (*T*). Individuals with clinical disease recover to the asymptomatic stage at a rate r_D, and treated individuals recover at a rate r_T after which they are afforded a period of prophylactic protection (*P*) with average duration $1/r_P$. Asymptomatic individuals (patent infection – detectable by microscopy) will move to a sub-patent stage of infection (*U*) at a rate r_A, from which they eventually return to susceptible at a rate r_U. Clinical immunity is acquired at a rate *h* proportional to the force of infection. Increased clinical immunity will reduce ϕ, the probability of experiencing an episode of clinical malaria following infection.

Malaria is transmitted from human to human by *Anopheles* mosquitoes. Our model also incorporates the effects of insecticide treated nets on mosquitoes and malaria transmission [335]. We assume the vector population has the behavioural traits of the indoor and night-time biting *Anopheles gambiae* s. s. mosquito. LLINs are assumed to have three effects on the adult mosquito population: (i) directly killing mosquitoes that land on nets; (ii) repelling and possibly diverting mosquitoes to an animal blood host due to either insecticide irritation or the physical barrier of the net; and (iii) lengthening of the gonotrophic cycle leading to a reduced frequency of biting.

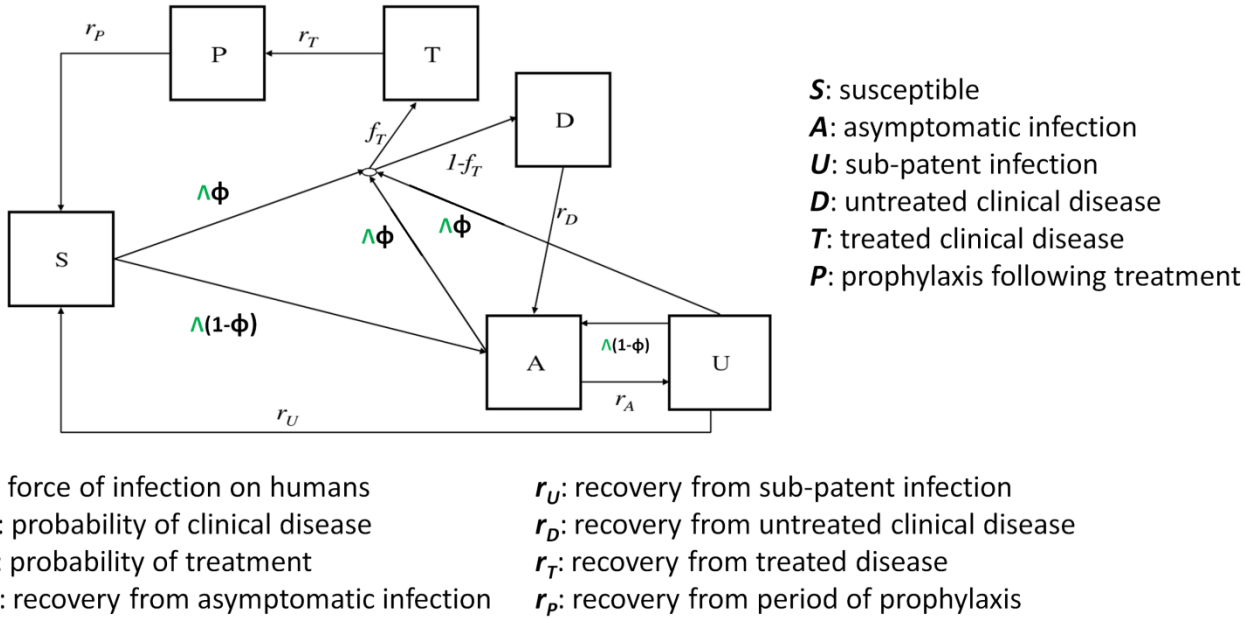


Figure 7.1: Flow diagram and parameters of a malaria transmission model used to investigate the effects of vaccination. Pre-erythrocytic vaccination reduces the force of infection Λ, highlighted in green. Full details are available in Griffin *et al* [63].

7.2.2. Pre-erythrocytic vaccination

Vaccination with the infection-blocking RTS,S/AS01 vaccine can be incorporated into models of malaria transmission [63, 169, 178]. In our model simulations we assume that RTS,S has infection-blocking efficacy $VE = 50\%$ waning exponentially with a half-life of 5 years. We assume that vaccination partially reduces the rate of acquisition of immunity. In the notation of Griffin *et al* [63] the rate of acquisition of clinical immunity h in unvaccinated children is proportional to the force of infection Λ, i.e. $h \propto \Lambda$. In vaccinated children, a proportion VE of infections are

prevented, and the force of infection is reduced to $(1-VE)\Lambda$. As vaccination was associated with lower blood-stage antibody titres in younger children [330] but not in older children [331], we assumed that the acquisition of immunity in vaccinated children was proportional to the force of infection partially reduced by vaccination, $h \propto (1-\frac{1}{2}VE)\Lambda$. The outcomes of vaccines causing a full reduction in the rate of acquisition of immunity, i.e. $h \propto (1-VE)\Lambda$, were also simulated for comparison. Unless otherwise stated, the model with partial reduction in acquisition of immunity is used in this analysis.

The characteristics of study sites for Phase II trials of RTS,S/AS01 in sub-Saharan African infants and children are summarised in Table 7.1. The transmission intensity at each site at the time of the trial isn't known precisely, but estimates of the EIR can be obtained from previous entomological studies at these sites [61, 336-339], although some of these measurements were made before the decline in malaria transmission due to expanded coverage of bed nets. These estimates of EIR span a wide range of transmission intensities, from 22 – 600 ibppy. The low levels of clinical incidence in children in the control group suggest that during the vaccine trials, transmission was lower than reported. For example, in studies of the relationship between EIR and clinical incidence in Senegalese villages [59], it was estimated that when EIR ≈ 200 ibppy the expected clinical incidence is 3 – 6 episodes per year in young children, and when EIR ≈ 20 ibppy the expected clinical incidence is 2 – 3 episodes per year. In contrast in the control groups of the vaccine trials we observe clinical incidence of 0.03 – 0.55 episodes per year suggesting much lower transmission intensity. Alternatively, the difference between these estimates of clinical incidence could be due to different definitions of an episode of clinical malaria or a reduction in transmission due to the improved access to healthcare associated with large scale clinical trials. Using our transmission model, we estimated the EIR that would result in the observed clinical incidence in children in the control group with LLIN coverage equal to that reported in trials. A comparison of the entomologically measured EIRs, and EIR estimated from clinical incidence are shown in Table 7.1.

Table 7.1: Characteristics of Phase II trial sites of RTS,S/AS01. *Incidence rates per person year of observation refer to first episodes of clinical malaria in the control group according to the surveillance method used in each study. **In the Bagamoyo Tanzania, Gabon and Ghana studies of RTS,S/AS01 no information on bed nets was available, so coverage was set to 75%, equal to that obtained in the Phase III trial of RTS,S/AS01.*Pf*PR is the parasite rate in children aged 2 – 10 years in 2007 as reported in the Malaria Atlas Project [340]. Measured EIR is derived from entomological studies, and estimated EIR is derived from the reported clinical incidence.

Site	N	Median age (IQR), months	Incidence person year*	LLIN use**	*Pf*PR	measured EIR (ibppy)	estimated EIR (ibppy)	Reference
Bagamoyo Tanzania	209	1.8m (1.7-1.9)	0.3	75%	0.35	80 – 600	5	[336, 341]
Gabon	215	1.5m (1.4-1.7)	0.032	75%	0.2	23 – 61	1	[337, 341]
Ghana	81	1.6m (1.5-1.8)	0.55	75%	0.7	269	10	[61, 341]
Kenya Kilifi	447	11m (8-14)	0.29	59%	0.25	22 – 53	2	[338, 342, 343]
Korogwe Tanzania	447	12m (9-15)	0.15	52%	0.15	90	1	[339, 342]

7.2.3. Simulation of extended follow-up of vaccine trials

Extended follow up of Phase II trials of RTS,S has shown sustained protection from clinical malaria for up to three years, although efficacy appears to decline over time, for example Guinovart *et al* [136] observed that children vaccinated with RTS,S/AS02 had 35.4% (95% CI, 4.5 – 56.3%) efficacy against clinical malaria in the first 6 months, and 9% (95% CI, -30.6% – 36.6%) in the following 12 months. Apparent reductions in vaccine efficacy over time could be due to biologically mediated waning in vaccine-induced antibody titres [342] and a corresponding reduction in the vaccine's infection-blocking efficacy; heterogeneity in exposure [344]; or faster acquisition of immunity in the control cohort. To compare these hypotheses for the reduction of efficacy the outcome of vaccine trials with extended follow up are simulated. Trials are simulated for a sample of the study sites described in Table 7.1, namely vaccination of infants with RTS,S/AS01 in Gabon and Ghana [130], at both the lower estimates of EIR corresponding to the observed clinical incidence in the control groups, and the higher estimates of EIR from entomological studies [61, 337].

7.2.4. Cost-effectiveness

The cost-effectiveness of a package of malaria control interventions can be estimated using model predictions of the number of episodes of clinical malaria averted, the cost per unit intervention and the savings on treatment. Using transmission model derived estimates of the number of episodes of clinical malaria averted by packages of malaria control interventions, a decision tree similar to that described by Shillcutt *et al* [345] and Coleman *et al* [346] can be used to estimate the number of DALYs and deaths averted. The decision tree, shown in Figure 7.2, follows the possible outcomes of a patient experiencing an episode of clinical malaria. We assume that a fixed proportion of patients are treated with artemisinin combination therapy (ACT) and fully recover unless they fail to adhere to the treatment regimen [347, 348] or treatment fails [349]. If treatment fails, patients will progress to either uncomplicated or severe malaria. A fixed proportion of patients with uncomplicated malaria will seek outpatient treatment in health centres or hospitals, and the rest will fully recover naturally. A fixed proportion of patients with severe malaria will require and be able to access inpatient care in hospitals. There are assumed to be three possible outcomes following an episode of severe malaria: full recovery, neurological sequelae, and death [350]. The parameter values for the decision tree and their uncertainty distributions are given in Table 7.2.

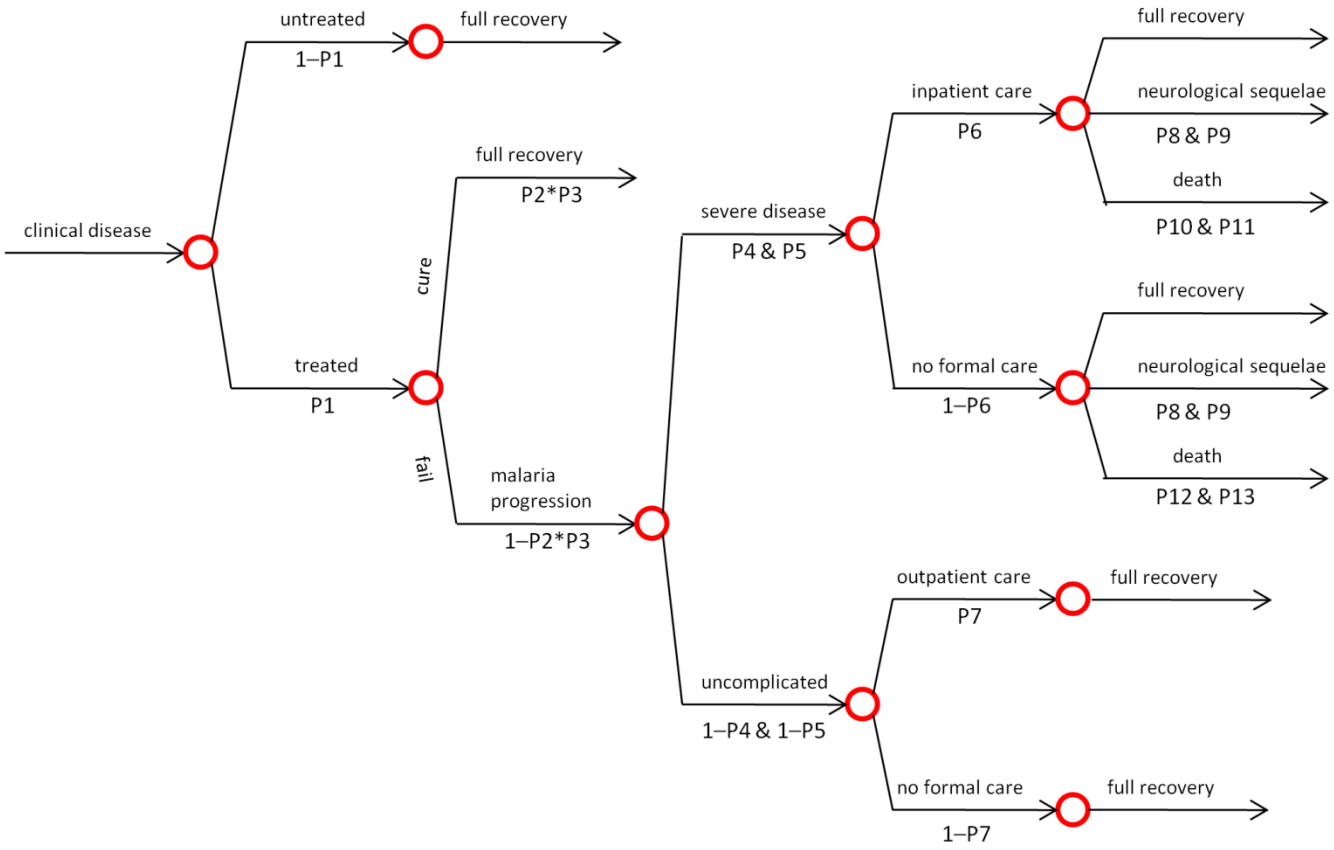


Figure 7.2: Decision tree for estimation of the outcome of an episode of clinical malaria. Progression through the decision tree is determined by the probabilities *P* given in Table 7.2.

First-line treatment was assumed to be with an ACT paid for at the patient's expense. The costs of LLIN distribution and treatment, either of uncomplicated malaria as an outpatient or severe malaria as a hospital inpatient were taken from a systematic review of the costs of malaria control interventions [328]. All costs are in 2009 US dollars (USD). The costs of vaccine delivery are based on data collected by Hutton and Tediosi [351] for EPI vaccination in a Tanzanian setting. The cost of vaccination with RTS,S/AS01 was assumed to be USD 5.00 per dose. Prices in the range USD 2.00 – 10.00 were also considered as part of the sensitivity analysis.

Discounted Years of Life Lost (YLL) due to death are estimated as 27.47 years for children (age < 5 years) and 24.83 years for adults (age ≥ 5 years) [346]. Disability weights used for estimation of Years Lost due to Disability (YLD) are taken from Murray *et al* [352]. Disability Adjusted Life Years are calculated with and without discounting at a rate of 3% per year [353]. All parameters and their distributions are shown in Table 7.2. Incremental Cost-Effectiveness Ratios (ICER) per DALY averted are calculated from a societal perspective. Costs incurred by patients self-treating for an episode of clinical malaria are included. We do not account for loss of earnings due to illness or time taken off work to care for sick children [354]. A ten year time frame was used for analysis – costs incurred and cases averted after ten years were not included.

Parameters for the decision tree and costs of treatment and preventive interventions were sampled from the distributions in Table 7.2 using 10,000 Monte Carlo simulations. Estimates of ICER per DALY averted were calculated for each sample, and the median and 95% sampling interval (SI) presented. ICERs per DALY averted were compared against several ceiling ratios [355]: USD 100.00, USD 260.00 (corresponding to the estimate of 150.00 in 1990 USD at which an intervention is considered cost-effective in low-income countries [352]), USD 500.00, and USD 2191 (equal to the mean gross domestic product per capita for sub-Saharan Africa based on estimates from the WHO CHOICE project [356]).

Table 7.2: Decision tree parameters, costs of interventions and treatment and disability weights. All costs are in 2009 USD.

Parameter	Description	Value	Lower limit	Upper limit	Distribution	Source
Decision tree parameters						
P1	Proportion self-treated	0.60	0.07	0.94	triangular	[357]
P2	Adherence	0.75	0.59	0.90	triangular	[347, 348]
P3	ACT cure rate	0.85	0.78	0.95	triangular	[349]
P4	Probability of severe disease after treatment failure– under 5	0.05	0.03	0.07	triangular	[346, 358]
P5	Probability of severe disease after treatment failure – over 5	0.01	0.005	0.015	triangular	[346, 358]
P6	Proportion seeking inpatient care for severe malaria	0.48	0.19	0.88	triangular	[346, 358]
P7	Proportion seeking outpatient care for uncomplicated malaria	0.48	0.19	0.88	triangular	[346, 358]
P8	Severe malaria leads to neurological sequelae – under 5	0.0132	0.0041	0.0224	triangular	[350, 358]
P9	Severe malaria leads to neurological sequelae – over 5	0.005	0.0025	0.0075	triangular	[350, 358]
P10	Severe malaria leads to death in inpatients – under 5	0.192	0.1	0.3	triangular	[346, 358]
P11	Severe malaria leads to death in inpatients – over 5	0.10	0.075	0.125	triangular	[346, 358]
P12	Severe malaria leads to death with no formal care – under 5	0.50	0.40	0.60	triangular	[346, 358]
P13	Severe malaria leads to death with no formal care – over 5	0.25	0.20	0.30	triangular	[346, 358]
Costs of treatment and preventive interventions						
C1	Cost of ACT self-treatment (USD)	0.14	0.13	0.19	triangular	[359]
C2	Cost of outpatient treatment for uncomplicated malaria (USD)	5.84	2.36	23.65	triangular	[328]
C3	Cost of inpatient treatment for severe malaria (USD)	30.26	15.64	137.87	triangular	[328]
C4	Vaccine price per dose (USD)	5.00	2.00	10.00	triangular	–
C5	Vaccine delivery cost per course (USD)	1.02	0.81	1.23	triangular	[178, 351]
C6	LLIN cost (USD)	7.03	2.97	10.00	triangular	[328]

Table 7.2 (contd.): Decision tree parameters, costs of interventions and treatment and disability weights. All costs are in 2009 USD.

Parameter	Description	Value	Lower limit	Upper limit	Distribution	Source
Disability weights						
D1	Disability weight: uncomplicated malaria	0.21	–	–	fixed	[352]
D2	Disability weight: severe malaria	0.25	–	–	fixed	[352]
D3	Disability weight: neurological sequelae	0.47	–	–	fixed	[352]
D4	Duration of uncomplicated untreated malaria (days)	4	–	–	fixed	[346]
D5	Duration of uncomplicated treated malaria (days)	2	–	–	fixed	[346]
D6	Duration of severe malaria for inpatients (days)	4.5	2	10	triangular	[358]
D7	Duration of neurological sequelae (years)	35.4	–	–	fixed	[352]
D8	Discount rate (per year)	3%	–	–	fixed	[353]
D9	Discounted YLL due to child death (years)	27.47	–	–	fixed	[346]
D10	Discounted YLL due to adult death (years)	24.83	–	–	fixed	[346]

7.3. Results

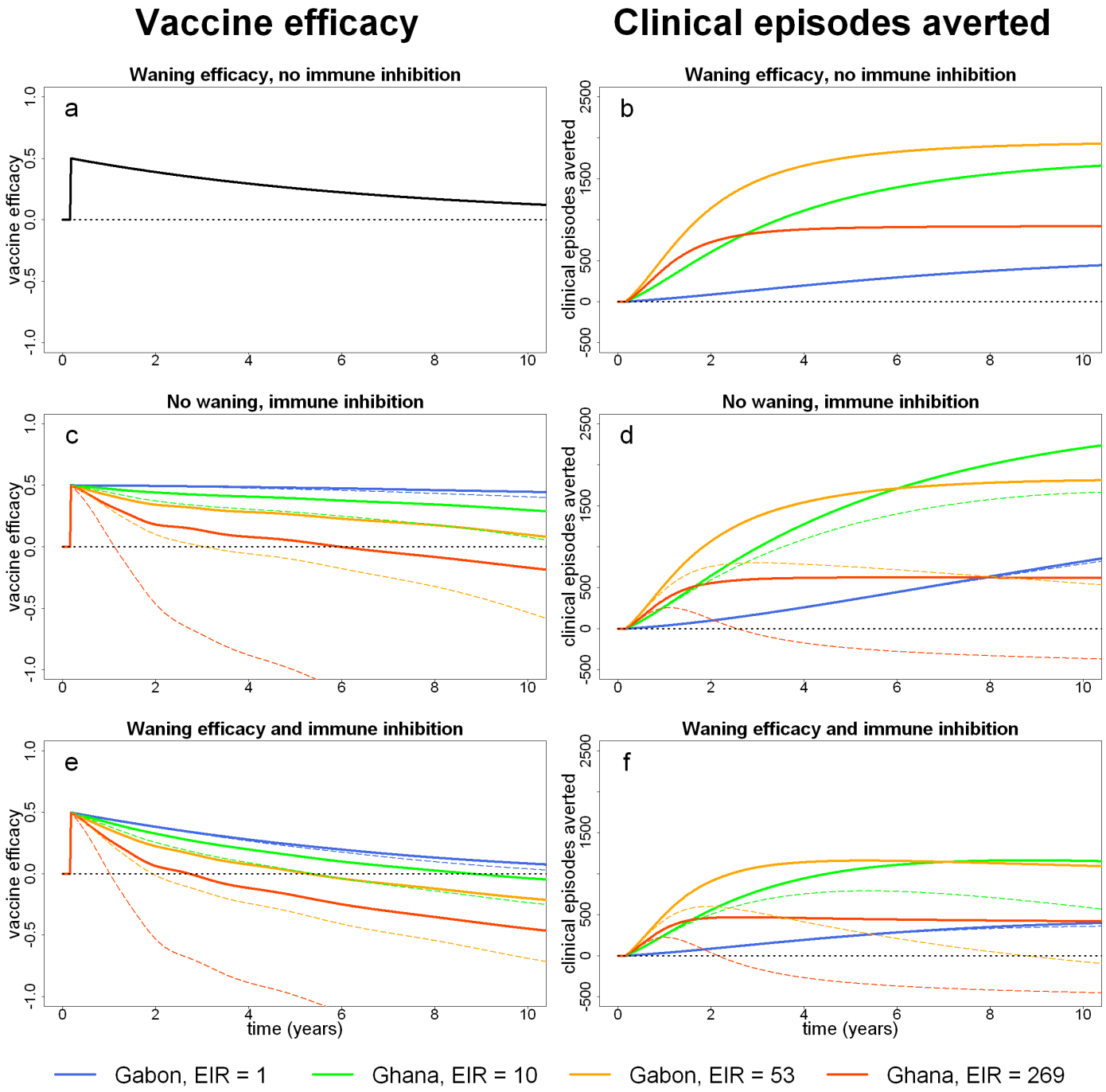


Figure 7.3: Simulated outcome of a trial comparing a cohort of 1,000 children vaccinated with RTS,S/AS01 with a control cohort. For the Ghanaian and Gabonese sites, simulations are presented at the lower estimates of EIR derived from clinical incidence observed in the control groups, and the higher estimates of EIR from entomological studies. Vaccine efficacy against clinical malaria and number of episodes averted for **(a – b)** a vaccine with exponentially waning efficacy and no reduction in immune acquisition; **(c – d)** a non-waning vaccine that reduces the rate of acquisition of immunity; and **(e – f)** a waning vaccine that reduces the rate of acquisition of immunity. In panels c – f the solid lines represent the outcomes of simulations with partial reduction in the rate of acquisition of immunity $h \propto (1-\frac{1}{2}VE)\Lambda$, and the dashed lines the outcome with full reduction in the rate of acquisition of immunity $h \propto (1-VE)\Lambda$.

Figure 7.3 shows estimates of vaccine efficacy against clinical malaria (in the left-hand column) and the number of episodes of clinical malaria averted (in the right-hand column). Vaccine efficacy is estimated from the reduction in the incidence of clinical malaria in the vaccine group compared to the control group at a fixed point in time – this is the vaccine efficacy that would be measured in a field trial if there was no bias due to heterogeneity in exposure [344]. If vaccine efficacy falls below zero, it indicates a rebound effect where the incidence of clinical malaria is higher in the vaccinated group than in the control group [360, 361]. A more serious prospect than a rebound effect is the possibility of the cumulative number of cases averted becoming negative, i.e. if vaccination causes a long-term increase in cases of malaria. Vaccine efficacy against clinical malaria was simulated under three scenarios for waning of efficacy against infection and reduction in the rate of acquisition of immunity:

- Figure 7.3 a – b: if efficacy against infection wanes exponentially with a 5 year half-life, but vaccination doesn't reduce the rate of acquisition of immunity, then a rebound isn't predicted and a positive number of cases will be averted.
- Figure 7.3 c – d: if efficacy against infection doesn't wane, but vaccination reduces the rate of acquisition of immunity, then the measured efficacy against clinical malaria will wane over time as the control group acquire clinical immunity at a faster rate than the vaccinated group. The reduction in the rate of acquisition of immunity was assumed to be partial (solid lines) or full (dashed lines).The model predicts a rebound effect at high transmission settings, but not at the low transmission settings. A net increase in episodes of clinical malaria is predicted only at high transmission settings if there is full reduction in immune acquisition.
- Figure 7.3 e – f: if efficacy against infection wanes exponentially and vaccination reduces the rate of acquisition of immunity then vaccine efficacy against clinical malaria decreases to near zero, possibly even causing a small rebound in sites with low transmission.

Under our model for the acquisition of naturally acquired immunity [63], the waning of efficacy observed in Phase II trials [136, 203] cannot be explained by the differential acquisition of immunity in the vaccine and control cohorts alone. Thus some biological waning of efficacy appears to be necessary to reproduce the patterns observed in these trials. The model simulations suggest that rebound effects may occur in high transmission settings, although RTS,S has yet to be tested in very high transmission settings in the absence of other interventions. Even if there is a rebound effect the cumulative number of episodes of malaria averted is still estimated to be positive, because when infants are vaccinated the period at which the vaccine is most efficacious coincides with the period when infants are most at risk of clinical malaria. If there is a rebound effect it will be experienced in older children who have acquired some degree of partial immunity despite being vaccinated. Thus a rebound effect may be acceptable if it occurs in older children subjected to lower incidence. However a potential cause for concern is the possibility of a rebound effect causing a net increase in the number of episodes of malaria – an outcome observed in Figure 7.3f when there is full reduction in the rate of acquisition of immunity (dashed lines) in high transmission sites (red and orange). This possibility, however slight, will need to be ruled out by analysis of extended follow up data from Phase II and Phase III trials, and a greater understanding of the interaction between vaccination and naturally acquired immunity.

7.3.1. Epidemiological impact of vaccination campaigns

Simulation of the impact of vaccination in transmission settings similar to the trial sites described in Table 7.1 suggests that vaccination of young children through EPI campaigns will cause a negligible reduction in malaria transmission (Figure 7.4a). Although EPI vaccination will only cause minor reductions in clinical incidence at the population level (Figure 7.4b), there will be a substantial reduction in episodes of malaria amongst vaccinated children, in agreement with Maire *et al*'s predictions [169].

Simulation of mass vaccination campaigns suggests that short term reductions in transmission intensity and incidence of clinical malaria can be obtained (Figure 7.4 c,d), although transmission intensity will return to initial levels after vaccine efficacy has waned, and there may even be an increase in clinical incidence as a consequence of the reduced immunity in the population.

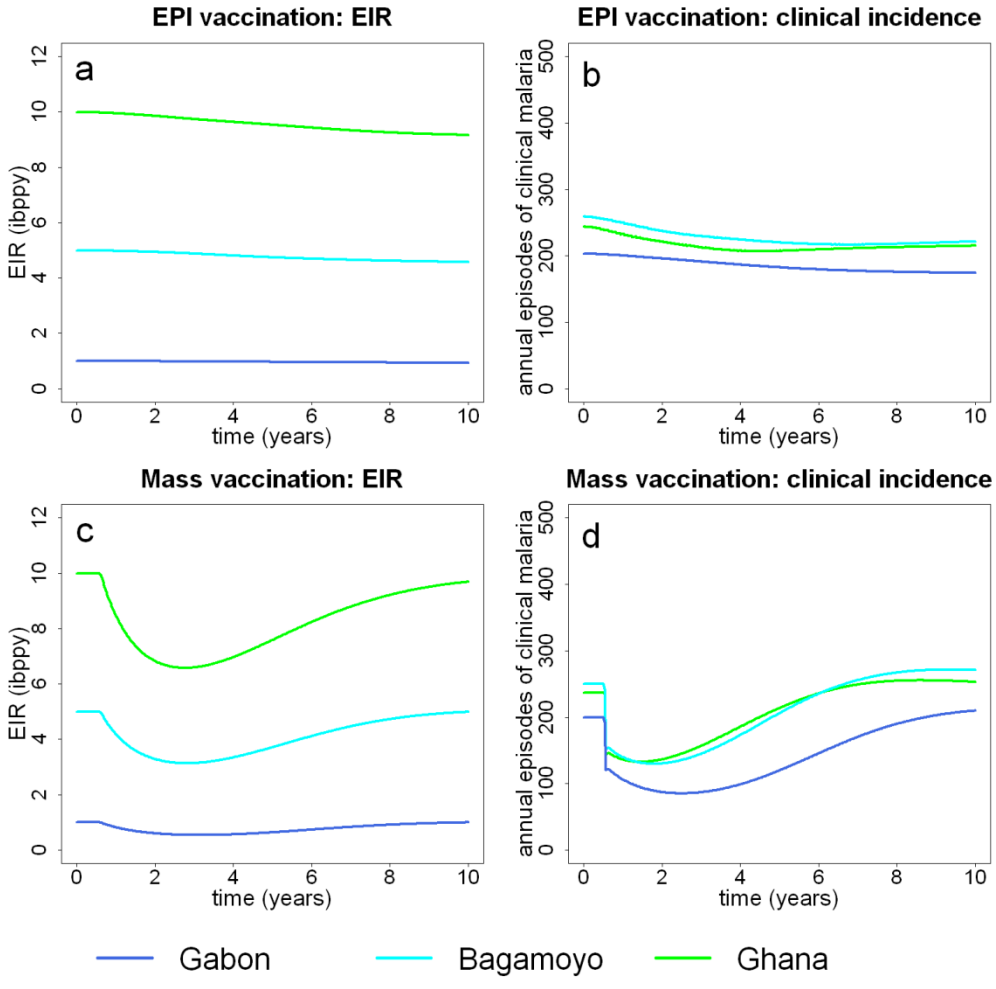


Figure 7.4: Simulation of the effect of EPI vaccination at 90% coverage in a population of size 1,000 on EIR and total clinical incidence **(a,b)**. Simulation of the effect of mass vaccination at 80% coverage in a population of size 1,000 on EIR and clinical incidence **(c,d)**.

7.3.2. The mass effect of vaccination and interaction with insecticide treated nets

Vaccination campaigns can reduce incidence of malaria by preventing infection in vaccinated individuals (the direct effect) and by reducing malaria transmission in the population (the mass effect). A comparison of the number of episodes of malaria averted due to the direct effect of vaccination alone and the combined direct and mass effect is shown in Figure 7.5 a-b for mass vaccination and EPI vaccination campaigns. At low transmission intensities mass vaccination can reduce transmission to an extent that a substantial additional benefit is obtained from the mass effect. However at high transmission intensities, the mass effect can actually be detrimental to the success of the vaccination campaign as vaccine-induced reductions in transmission can lead to lower population level immunity. We estimate that above EIR ≈ 50 ibppy mass vaccination may lead to an increase in the incidence of clinical malaria.

Vaccination via EPI results in very limited reductions to transmission intensity, and as such the mass effect will be beneficial only at low to moderate transmission intensities (EIR < 20 ibppy). Model simulations suggest that EPI vaccination will be most effective in areas of moderate transmission (EIR ≈ 10 ibppy). EPI vaccination becomes less effective in high transmission settings, possibly even causing an increase in clinical incidence at very high transmission settings (EIR > 250 ibppy).

Although the decrease in effectiveness of vaccination at high transmission intensities may not agree with our intuition, it can be explained by considering the number of *P. falciparum* infectious bites. For example, in a setting with EIR = 100 ibppy, an unvaccinated child may become infected 20 times over a transmission season. A child receiving a 50% efficacious vaccine may still be infected 10 times, more than enough to experience incidence of clinical malaria comparable to an unvaccinated child.

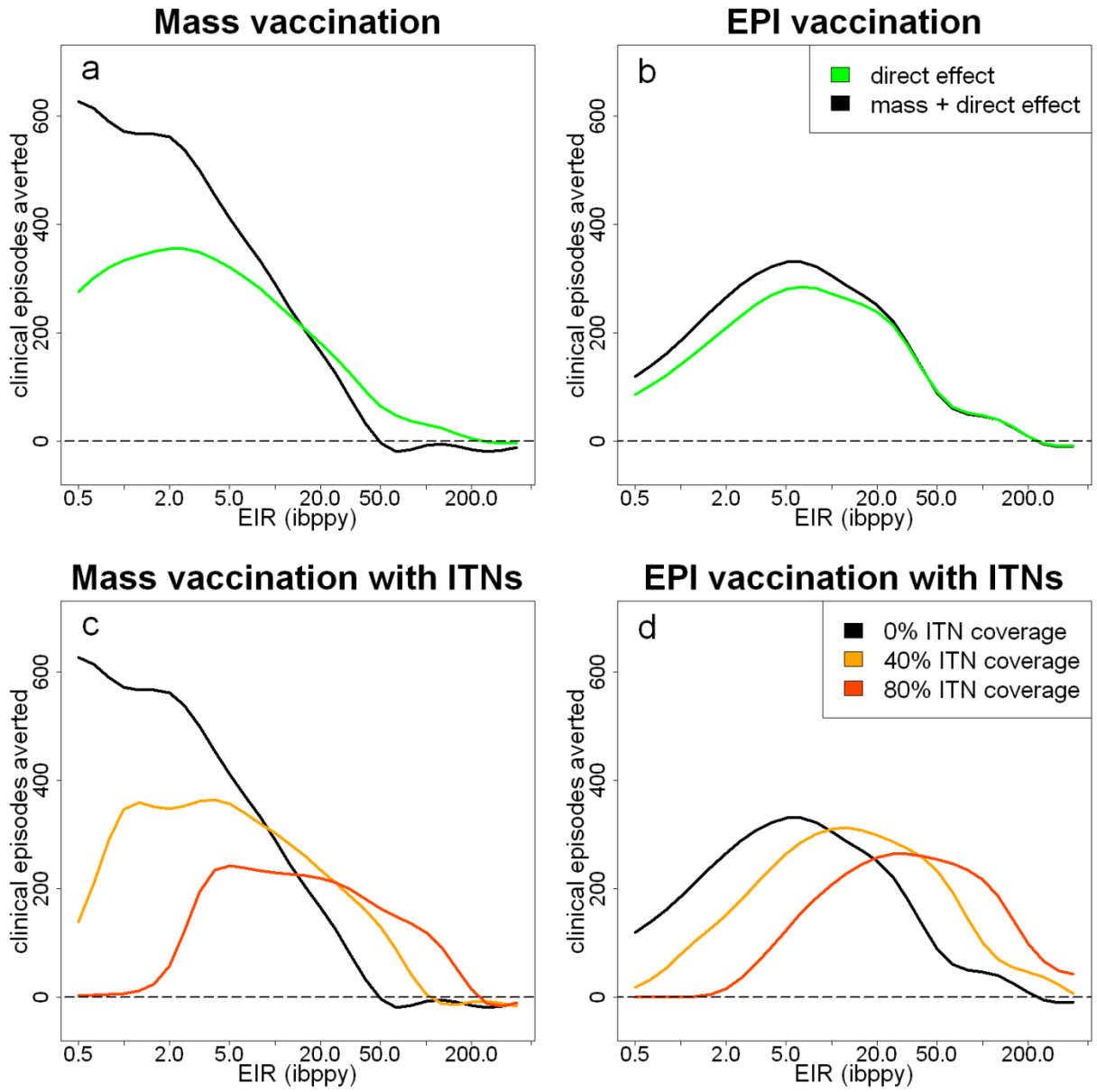


Figure 7.5: Estimated number of episodes of malaria averted in a population of size 1,000 by the direct effect alone and the combined direct and mass effect for a mass vaccination campaign **(a)** and an EPI vaccination campaign **(b)**. The estimated impact of mass vaccination **(c)** and EPI vaccination campaigns **(d)** combined with ITNs at different levels of coverage. The reported EIR is the baseline value before introduction of vaccines or bed nets. (c) and (d) show only the number of clinical episodes averted due to vaccination.

Partially efficacious pre-erythrocytic vaccines are unlikely to be deployed in isolation; in the recent Phase III trials of RTS,S, approximately 75% of vaccinated children were reported as sleeping under bed nets [15]. The impact of vaccination must therefore be considered as part of a package of malaria control interventions – in particular ITNs. Figure 7.5 c-d shows the expected number of clinical episodes of malaria averted in a population of size 1,000 due to EPI vaccination in combination with ITNs – only the cases averted by vaccines over and above those averted by ITNs are shown. In low transmission settings increased ITN coverage will result in fewer episodes of malaria being averted by mass vaccination campaigns as malaria transmission will have been suppressed by ITNs. In higher transmission settings, increasing ITN coverage will reduce transmission to levels where mass vaccination can become more effective. Combining EPI vaccination with ITN distribution will make vaccination less effective in low transmission

settings (because bed nets alone will be so effective) and more effective in high transmission settings because bed nets will reduce transmission to a level where vaccination can become effective.

7.3.3. Cost-effectiveness of RTS,S/AS01 delivered through the Expanded Program on Immunisation

Estimates of the ICER per discounted DALY averted by EPI vaccination in the absence and presence of LLINs are shown in Figure 7.6. In the absence of LLINs, vaccination is estimated to be most cost-effective in moderate to low transmission settings with an ICER of USD 253 (95% sampling interval (SI), 93 – 696) when EIR = 10 ibppy. In comparison the ICER per DALY averted by LLINs at 80% coverage is estimated to be USD 33 (95% SI, 5.50 – 140) when EIR = 10 ibppy (this estimate is in good agreement with the median value of USD 27 per DALY averted by bed nets derived from a systematic literature review [328]). Vaccination will cease to be cost effective above an EIR ≈ 250 ibppy as vaccination is predicted to cause a net increase in the incidence of clinical malaria over a ten year time frame. The distribution of LLINs in low transmission settings will cause malaria vaccination to be less cost-effective as malaria transmission will have been reduced by LLINs. Against a baseline of 80% LLIN coverage in a setting with pre-intervention EIR = 10 ibppy, the cost-effectiveness of vaccines will be reduced with an ICER per DALY averted of USD 787 (95% SI, 289 – 2312).

In contrast, at high transmission settings vaccines will become more cost-effective when combined with LLINs. When EIR = 50 ibppy, the ICER per DALY averted by EPI vaccination alone is estimated to be USD 793 (95% SI, 312 – 2095), but when vaccination is administered against a baseline of 80% LLIN coverage the ICER per DALY averted drops to USD 391 (95% SI, 145 – 1081). For comparison, the ICER per DALY averted in the absence of vaccines by LLINs at 80% coverage is estimated to be USD 250 (95% SI, 102 – 676).

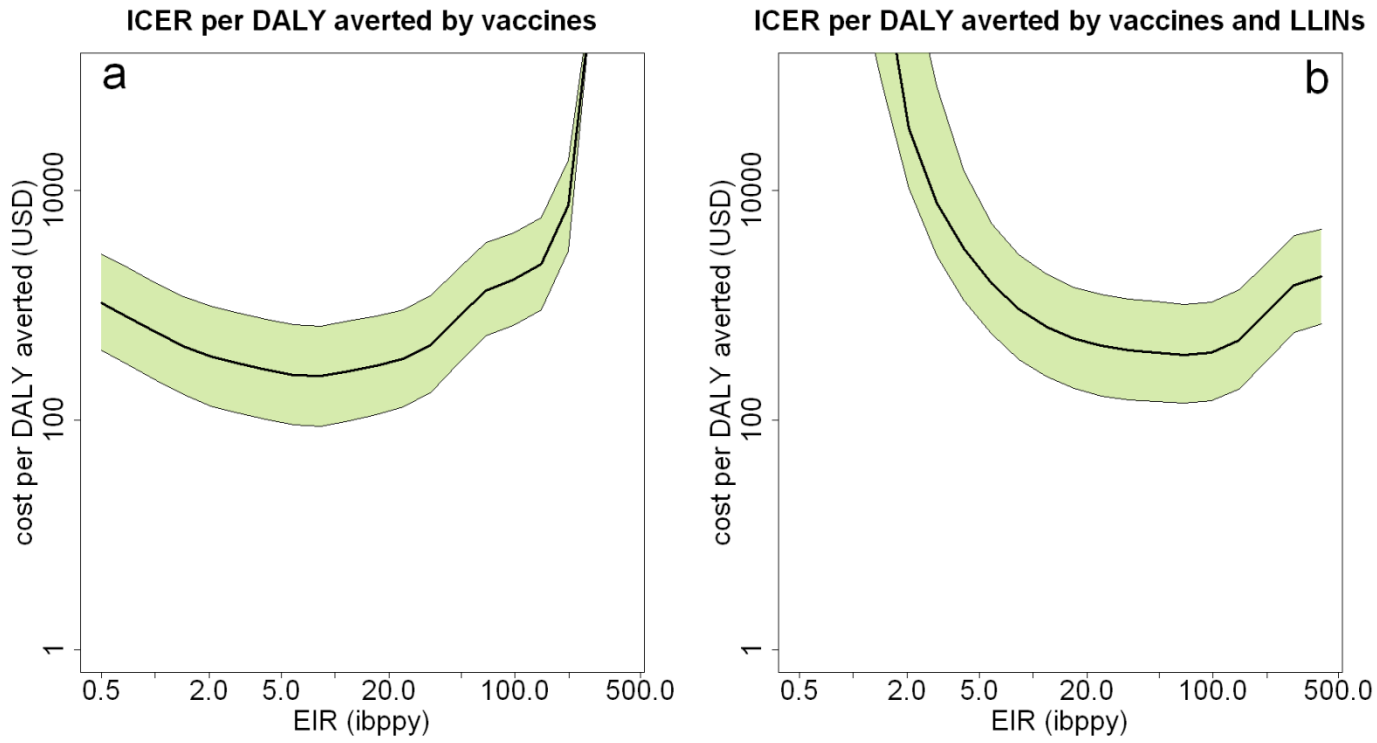


Figure 7.6: Incremental cost-effective ratio per discounted DALY averted by EPI administered RTS,S/AS01 in a setting with no LLINS **(a)** and in a setting with 80% LLIN coverage **(b)**. The shaded regions represent 95% sampling intervals estimated from Monte Carlo sampling of the cost-effectiveness parameters.

The estimates of cost-effectiveness presented in Figure 7.6 only consider costs incurred and episodes averted in a ten year window. However an infant vaccinated in the ninth year of the vaccination campaign will continue to be partially protected after the ten year window for analysis even though the entire cost of vaccination will be captured in the analysis. EPI vaccination is therefore likely to be more cost-effective in the long term than the estimates presented in Figure 7.6. The ICERs presented in Figure 7.6 are for discounted DALYs, undiscounted DALYs will result in lower ICER estimates. For example, presenting undiscounted instead of discounted ICERs per DALY averted will cause an approximately two-fold improvement in cost-effectiveness, with the cost per ICER by vaccination when EIR = 10 ibppy dropping from USD 253 (95% SI, 93 – 696) to USD 114 (95% SI, 42 – 319).

Upon evaluation of the cost-effectiveness of an intervention, the decision to deploy it can be informed by comparing the ICER per DALY averted with a ceiling ratio [355] – if the ICER per DALY averted is less than the ceiling ratio then the intervention is declared to be cost-effective. Although the choice of ceiling ratio is somewhat arbitrary, it is still a useful tool for assessing cost-effectiveness. The probability that vaccination is cost-effective when compared to a number of ceiling ratios of incremental cost per DALY averted is shown in Figure 7.7. In the absence of LLINs, RTS,S is most likely to be cost-effective at low transmission intensities (EIR ≈ 10 ibppy).In the presence of LLINs at 80% coverage, vaccination is likely to be most cost-effective in higher transmission settings around EIR ≈ 50 ibppy.

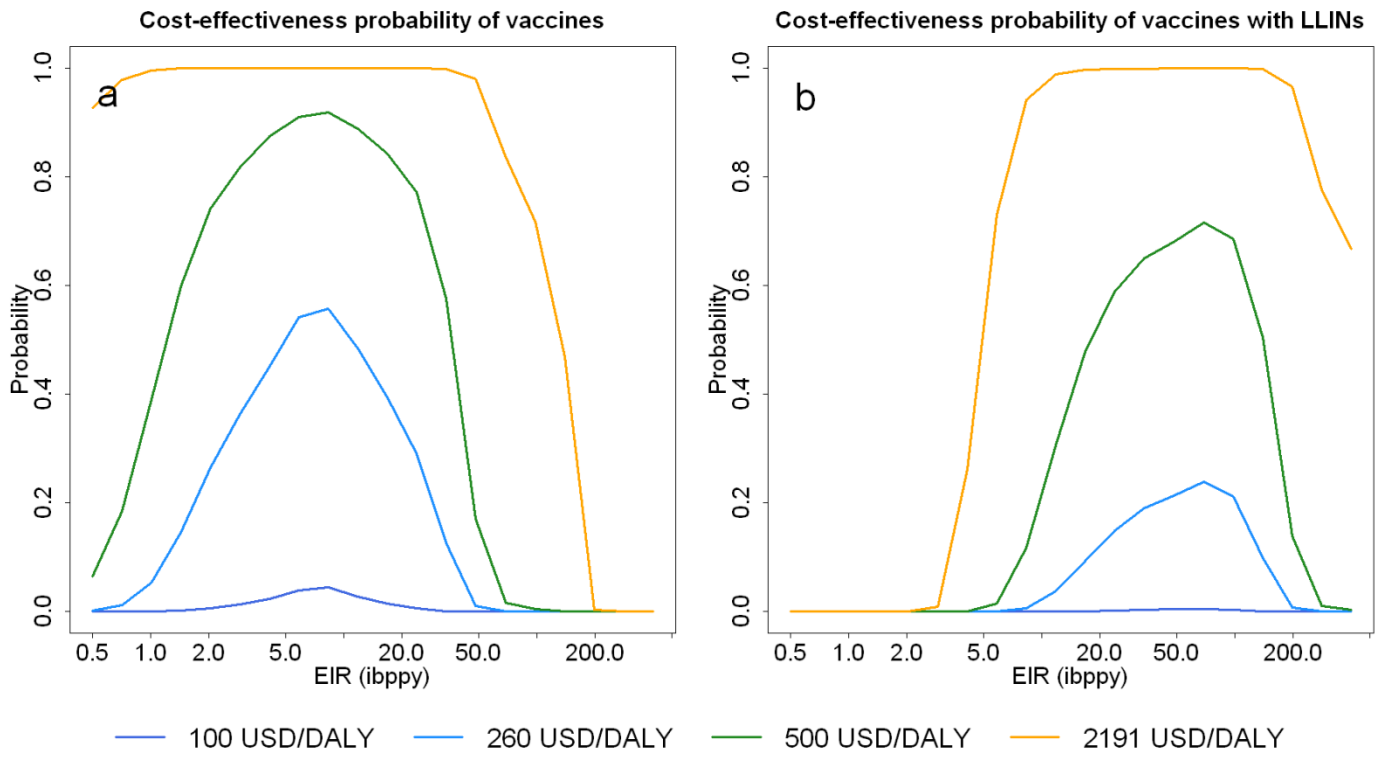


Figure 7.7: Probability that an EPI vaccination campaign is cost-effective at different thresholds for the cost per DALY averted in the absence of ITNs **(a)** and in the presence of LLINs at 80% coverage **(b)**.

7.4. Discussion

In this analysis we have validated the findings of other modelling groups that vaccination with RTS,S/AS01 can cause substantial reductions in morbidity and mortality in young African children, although it is unlikely to cause major reductions in malaria transmission. In the absence of other interventions, vaccination is estimated to be most effective at low transmission intensities. Combining vaccination with other malaria control interventions may change the settings at which vaccines are most effective. For example in low transmission settings LLINs will suppress malaria transmission, reducing the number of cases of malaria that can be averted by vaccination. In contrast, in regions of high transmission (EIR ≈ 50 ibppy) vaccines will become more effective when combined with LLINs.

RTS,S/AS01 delivered through the EPI is estimated to be most cost-effective in low transmission settings (EIR ≈ 10 ibppy) with an estimated ICER per DALY averted of USD 253 (95% SI, 93 – 696). This is significantly more expensive than the estimated ICER per DALY averted by LLINs of USD 33 (95% SI, 5.50 – 140). Thus LLINs should remain the first choice preventive intervention, although vaccination may still be useful after LLIN distribution but with an increased ICER per DALY averted of USD 787 (95% SI, 289 – 2312). Although LLINs reduce the cost-effectiveness of vaccines in low transmission settings, they actually improve it in high transmission settings as LLINs can reduce malaria transmission to the optimal range for vaccines. For example at an EIR = 50 ibppy, the ICER per DALY averted by vaccines alone is USD 793 (95% SI, 312 – 2095), but when administered against a background of LLINs at 80% coverage, the ICER per DALY averted drops to USD 391 (95% SI, 145 – 1081).

The simulations presented in this analysis are dependent on two key assumptions of the biological properties of the RTS,S vaccine: the duration of efficacy and the interaction with naturally acquired immunity. Extended follow up of Phase II trials over a three year time scale indicates that there is reduction in vaccine efficacy against clinical malaria over time, although these trials do not have sufficient statistical power to estimate rates of waning. More accurate estimates of the duration of protection can be obtained from pooled analyses of data from Phase II trials (P. Bejon, M. White *et al* – in preparation) or extended follow up of Phase III trials. Alternatively, if an accurate immunological correlate of vaccine-induced protection from infection such as anti-CSP antibody titre or number of CSP-specific T cells can be established [232, 279] then the rate of waning of efficacy could be estimated in terms of the rate of change of immune correlates.

Estimates of effectiveness and cost-effectiveness are particularly sensitive to assumptions regarding the interaction between vaccination and naturally acquired immunity – an area that remains poorly understood. The consequences of delaying the acquisition of immunity could be most severe in high transmission settings, with reduced levels of clinical immunity in vaccinated children leading to a rebound effect with increased incidence of clinical malaria after vaccine efficacy has waned. The possibility of a rebound effect in vaccinated children has been suggested before [361, 362], although fortunately no such effect has been observed so far, despite some evidence for a reduced blood-stage antibody response in young children [330]. However an increase in incidence of clinical malaria has been detected in a trial of seasonal malaria chemoprophylaxis in young Ghanaian children in the post intervention period [363]. Trials of RTS,S have been undertaken across sites of varying transmission intensities, but not in areas of very high transmission in the absence of other malaria control interventions. Although model simulations can provide estimates of the outcome of vaccination in high transmission settings, they are no substitute for results from well conducted trials.

The model of cost-effectiveness utilised in this analysis has a number of limitations. Incidence of severe malaria and malaria associated deaths are assumed to be proportional to incidence of clinical malaria, however it has long been recognised that immunity to severe malaria is acquired at a faster rate than immunity to clinical malaria [55]. Future iterations of the transmission model will incorporate the acquisition of immunity to severe malaria [57, 63], allowing more accurate estimation of the incidence of severe malaria and its associated costs.

At present model estimates of the cost-effectiveness of vaccination are dependent on the assumed cost per dose of vaccine. In this analysis we assumed a cost of USD 5.00 (range 2.00 – 10.00) per dose, the same as Maire *et al*'s [178] estimate, although we have chosen more conservative values for the biological parameters describing efficacy and duration of protection. To date there have been no indications of the cost per dose of RTS,S, although GlaxoSmithKline have declared that the vaccine will be sold at a price of 5% above the cost of manufacture [364]. Until the time when the cost per dose of RTS,S is known, cost-effectiveness models will not be able to estimate the cost-effectiveness of vaccines, but they will be able to identify transmission settings and age groups where vaccination has the highest probability of being cost-effective.

When evaluating the cost-effectiveness of vaccination with RTS,S it is important to consider the interaction with other malaria control interventions, in particular bed nets. We found that vaccination combined with LLINs was less cost-effective per DALY averted than vaccination alone, except in regions of high transmission intensity. Our approach to estimating the cost-effectiveness of vaccines combined with LLINs was conservative, as only the episodes of malaria averted by vaccination in addition to those averted by LLINs were included in the DALY calculations, that is, we compared the combined package of vaccines and LLINs to the baseline of just LLINs. An alternative approach would be to compare the combined package of vaccines and LLINs to a baseline of no intervention, and then split the benefits of the intervention package according to the effectiveness of each individual intervention. This would lead to a higher estimate of the cost-effectiveness of vaccines as some of the averted DALYs attributed to LLINs would be attributed to vaccines. In addition, our analysis may underestimate cost-effectiveness as the simple decision tree model does not consider potential economies of scope achievable through delivery of LLINs through the EPI system [365].

The combination of a model of the dynamics of malaria transmission and a decision tree for estimating costs has a number of advantages over models of cost-effectiveness using decision tress alone such as those described by Goodman *et al* [358], Morel *et al* [366], and the Lives Saved Tool (LiST model) [367, 368]. Transmission models can account for changes in transmission intensity and population-level immunity due to the effects of the intervention under evaluation, indirect benefits to those not receiving the intervention due to the mass effect, and the interaction between multiple interventions.

7.5. Conclusion

The effectiveness and cost-effectiveness of vaccination with RTS,S/AS01 will depend on the transmission intensity of *P. falciparum* malaria and the coverage of other malaria control interventions, most notably insecticide treated nets. Model estimates of cost-effectiveness will be dependent on assumptions regarding key knowledge gaps, in particular the duration of vaccine-induced protection, the interaction with naturally acquired immunity, and the cost per dose of vaccine. Despite these limitations, we estimate that RTS,S/AS01 delivered through the Expanded Program on Immunisation, across a range of transmission settings, can be cost-effective when compared to a range of ceiling ratios.

Chapter 8. Discussion

Malaria vaccines: where malaria meets vaccines

The much-welcomed progress in the malaria vaccine development effort has thrust a number of challenging problems onto the research agendas of such diverse fields as immunology, clinical trials, malaria epidemiology, vaccine logistics and health economics. Addressing these challenges requires bringing together the previously disjoint spheres of malariology and vaccinology. Throughout this thesis I have used mathematical models as tools to investigate a number of the problems that need to be tackled as part of the wider malaria vaccine development effort.

8.1. Summary of findings

In Chapter 2, I investigated the effects of unaccounted for heterogeneity in exposure and vaccine response on the interpretation of results from clinical trials of malaria vaccines. I demonstrated that heterogeneity in exposure, when some people are bitten more frequently than others, can cause vaccine efficacy to be underestimated in clinical field trials. In contrast, heterogeneity in vaccine response, where some vaccinees receive a higher level of protection than others, can cause vaccine efficacy to be overestimated. The possibility of waning vaccine efficacy poses an additional problem when analysing results from clinical trials. A reduction in observed vaccine efficacy over time could be due to decay of vaccine-induced immune responses, differential acquisition of immunity in the vaccine and control cohorts, or a consequence of the effects of heterogeneity in exposure.

Most of the malaria vaccines currently undergoing clinical development rely on boosting some element of the naturally acquired pre-erythrocytic immune response [104]. However, the association between markers of pre-erythrocytic immunity and protection from infection remains poorly understood. To address this knowledge gap, I undertook a systematic review of the published literature which is described in Chapter 3. A potential reason for the lack of consensus on the effect of the pre-erythrocytic immune response is that trials to evaluate the association between markers of the pre-erythrocytic immunity and protection from infection have been underpowered to detect statistically significant effects. By analysing existing published studies, I identified a number of factors which can improve the power of a study, namely, increasing the size of the study, performing longitudinal follow-up, analysing immune response data as a continuous as opposed to a binary variable, and using Cox proportional hazards for statistical analysis.

Conventional statistical tests such as Cox proportional hazards or logistic regression are somewhat limited tools for investigating the association between markers of pre-erythrocytic immunity and protection from infection as they do not naturally account for non-linear effects such as thresholds for protection [231]. In Chapter 4 I used a

mathematical model based on dose-response curves to estimate the association between antibodies to the pre-erythrocytic antigens CSP, TRAP and LSA-1 and protection from infection in a cohort Kenyan adults under natural exposure to *P. falciparum* infection [237]. Using the dose-response curves for naturally acquired pre-erythrocytic antibodies I estimated that a vaccine that boosts anti-CSP antibodies in adults is likely to prevent approximately 50% of infections. The addition of a TRAP component to a CSP-based vaccine is estimated to increase infection-blocking vaccine efficacy to approximately 75%.

In Chapter 5, using data from an artificial challenge study of RTS,S in malaria-naïve adults [129], I investigated the association between vaccine-induced anti-CSP antibodies and CSP-specific $CD4^+$ T cells and protection from infection. In the cohort under investigation, 50% (95% CI, 33% - 67%) and 32% (95% CI, 18% - 48%) of volunteers vaccinated with RTS,S/AS01 and RTS,S/AS02, respectively, were protected from infection. Mathematical models based on dose-response curves suggest that approximately 2/3 of this protection is attributable to vaccine-induced anti-CSP antibodies and 1/3 to CSP-specific T cells. Using a within-host model of the pre-erythrocytic stages of *P. falciparum* infection I estimated that vaccination with RTS,S causes a 97.7% (95% CI, 96.3% – 98/7%) reduction in the number of parasites entering the blood stream from the liver.

In Chapter 6, I extended my focus beyond static measures of immunity to consider the dynamics of antibody responses under natural exposure to malaria. Simple models of antibody kinetics and the underlying cellular processes responsible for antibody generation were developed and fitted to longitudinal data from cohorts of young Ghanaian and Gambian children [303, 311]. In the models of antibody kinetics it was assumed that antibody is generated by populations of short-lived and long-lived antibody secreting cells (ASC). In the younger Ghanaian cohort, 2% – 11% of ASCs generated following exposure to *P. falciparum* antigens were estimated to be long-lived, with a half-life of 303 – 469 days. In contrast, the older Gambian children were estimated to produce a larger proportion of long-lived cells (13% – 26%) with a half-life of 374 – 503 days, suggesting age dependent development of the antibody response to malaria infection.

In Chapter 7, I used a previously published mathematical model of malaria transmission [63] to estimate the effectiveness and cost-effectiveness of vaccination with RTS,S/AS01. I demonstrated that model predictions of the long term impact of vaccination are dependent on assumptions regarding the interaction between vaccination and naturally acquired immunity. In particular, if vaccination reduces the rate of acquisition of immunity there is the potential of a rebound effect with vaccinated children experiencing a higher incidence of clinical malaria than unvaccinated children. However, if a rebound effect does occur it is likely to be in older children experiencing a lower incidence of clinical malaria. We estimated that delivery of RTS,S/AS01 through the expanded program on immunisation will be most effective at moderate transmission settings (EIR ≈ 10 ibppy). When combined with widespread distribution of LLINs, vaccination is estimated to be most effective in regions of higher baseline transmission intensity (EIR ≈ 50 ibppy).

Although estimates of the cost-effectiveness of vaccination are entirely dependent on the assumed cost per dose of vaccine, it is still useful to identify the scenarios where vaccination has the highest probability of being cost-effective.

With an average cost per dose of USD 5.00, RTS,S/AS01 delivered through the EPI was estimated to be significantly less cost-effective than mass distribution of LLINs with an estimated ICER per DALY averted of USD 253 (95% sampling interval (SI), 93 – 696) for vaccines compared to USD 33 (95% SI, 5.50 – 140) for LLINs at moderate transmission intensities.

8.2. Limitations of methods and results

The results presented throughout this thesis must be interpreted in terms of the underlying data and the methods used for data analysis. A lack of critical assessment of the limitations of the data and models can lead to results being over-interpreted. A discussion of some of the limitations of the data and models used to produce the results in each of the chapters of this thesis is presented below.

8.2.1. Limitations of Chapter 2

The findings in Chapter 2 that heterogeneity in malaria exposure and vaccine response can cause estimates of vaccine efficacy from Phase II/III field trials to be underestimated or overestimated, respectively, is not based on trial data but rather on a theoretical model of a vaccine trial carried out with a treatment re-infection study design. This model makes a number of assumptions: each individual is subjected to a constant, non-seasonal force of infection, and that all infections are detected immediately with perfect sensitivity and specificity.

The simulated examples in Chapter 2 consider the effects of heterogeneity in exposure and vaccine response separately. In reality, we would expect both of these sources of heterogeneity to act at the same time as well as waning of efficacy. Finally, the findings of Chapter 2 are most robust when infection with malaria is the endpoint of interest, and not episodes of clinical malaria which is the primary endpoint in the ongoing Phase III trial of RTS,S/AS01 [15].

8.2.2. Limitations of Chapter 3

The review in Chapter 3 of the association between markers of pre-erythrocytic immunity and protection from infection or clinical malaria was undertaken using a systematic search of the published literature. Using the full methodology of a systematic review would no doubt improve the quality of the gathered evidence. Although based on metrics used with success in clinical medicine [229], the Power Score devised for assessing quality of evidence from a study is somewhat arbitrary, as its components were manually selected. Despite this drawback, the Power Score still succeeds in giving an approximate measure of a study's power to detect an effect of a pre-erythrocytic immune response.

Simulations of treatment re-infection trials were undertaken using a model where probability of infection was dependent on pre-erythrocytic antibody titre according to an exponential dose-response curve. Although the exact outcome of these simulations is dependent on model assumptions for the dose-response relationship between antibody titre and protection from infection, they still give qualitative insights for the interpretation of the results of studies for evaluating the effects of pre-erythrocytic immune responses. In the model simulations we assumed that there was a true association between antibodies and protection from infection. We did not consider the 'inverse' problem whereby an antibody with no true association with protection from infection may appear significantly correlated with protection. Furthermore, we did not explicitly consider the role that antibodies may play as markers for exposure.

8.2.3. Limitations of Chapter 4

In Chapter 4, data on antibody titres and time to detection of *P. falciparum* parasites from a study of Kenyan adults [237] was combined with a model of the relationship between antibody titres and probability of infection to estimate the efficacy of antibody-mediated malaria vaccines. Firstly, the results are dependent on the data used. Antibodies to the pre-erythrocytic antigens CSP, TRAP and LSA-1 were measured before follow-up of volunteers, thus in this dataset it is only possible to measure associations between these three antibodies and protection. If there is significant correlation between different arms of the immune response, and if protection from infection is really mediated by cellular or early blood-stage immune responses, then this combination of data and model could result in misleading conclusions.

Secondly, the results are dependent on the choice of model. The model for estimating vaccine efficacy as a function of antibody titre makes the assumption that the dose-response relationship between naturally-acquired antibodies and protection from infection is the same as that between vaccine-induced antibodies and protection from infection. Furthermore, the prediction of vaccine efficacy at high antibody titres assumes that the dose-response curves estimated at low naturally-acquired antibody titres can be extrapolated to higher vaccine-induced antibody titres.

8.2.4. Limitations of Chapter 5

In Chapter 5, a mechanistic model of sporozoite infection was combined with data from a Phase II challenge trial of the RTS,S/AS01 and RTS,S/AS02 malaria vaccines [129] to investigate the association between vaccine-induced immune responses and protection from infection. The model assumes that each sporozoite successfully developing within the liver releases an average number of 20,000 merozoites into the blood stream, an estimate derived from observations of cross-sections of the livers of chimpanzees infected with *P. falciparum* [28]. Revised model formulations will allow the number of merozoites per sporozoite to be fitted to the data as there is likely to be correlation between the mean number of inoculated sporozoites and the number of merozoites per sporozoite.

In addition, infection is assumed to be detected only once blood-stage parasites increase to densities where they can be detected by slide microscopy. This density was assumed to be 50 million parasites/mL based on studies by Bejon *et al* [295]. Variation in either threshold for detection of parasites by microscopy or the parasite multiplication rate per generation [369] will lead to variation in the estimated number of parasites emerging from the liver. More frequent measurements of parasite density using quantitative PCR (which has a sensitivity of 20 parasites/mL) would provide valuable additional data.

A number of markers for the vaccine-induced cell-mediated immune response were available in the dataset: CSP-specific $CD4^+$ T cells producing the cytokines TNF-α, IL-2, IFN-γ and the co-stimulatory molecule CD40L, or CSP-specific $CD4^+$ T cells producing at least two cytokines. A more detailed analysis would include combinations of multiple markers of cell-mediated immunity, vaccine-induced anti-CSP antibody titres and protection from infection.

8.2.5. Limitations of Chapter 6

In Chapter 6 mathematical models of the immunological process underlying the generation of IgG antibody were developed and fitted to data from two longitudinal cohorts of African children exposed to malaria. The models were fitted using mixed effects methods within a Bayesian framework with Markov Chain Monte Carlo sampling of parameter space. The advantage of this method is that it uses all of the data to produce estimates of the mean values of parameters determining the duration of antibody response across the cohort of children, and the naturally occurring variation between children. Alternative approaches to model fitting would be to fit one set of global parameters to the data from all children together, or to fit a set of local parameters to each child separately.

There are a number of drawbacks to using mixed effects methods for model fitting, the first being the computational expense as large numbers of parameter updates are needed to explore the full parameter space. Secondly, mixed effects models are composed of two components: the model of antibody kinetics for each child, and the model for the distribution of the mixed effects parameters. For quantities such as half-lives which must take on positive values, we assume a Log-Normal distribution, and for the proportion of short-lived ASCs we assume a Logit-Normal distribution. A poor choice of distribution for the mixed effects parameters will result in a low likelihood.

As the likelihood of a mixed effects model is comprised of two components, direct model comparison with likelihoods is difficult, as we will be comparing the combined likelihood of antibody kinetics model plus mixed effects model, and not just the likelihood of the antibody kinetics model. Furthermore, as the number of parameter to be estimated in mixed effects models is poorly defined [315], estimates of the Akaike Information Criterion must be interpreted with caution. An alternative approach to model comparison would be to take advantage of the fact that the models are nested and calculate the probability that a model reduces to a sub-model.

8.2.6. Limitations of Chapter 7

In Chapter 7 the effectiveness and cost-effectiveness of vaccination with RTS,S/AS01 in combination with ITNs was investigated using a previously published model of malaria transmission [63]. There are three key knowledge gaps which will contribute to the uncertainty of model estimates of the impact of vaccination programmes. The first is the cost per dose of vaccine. Until GlaxoSmithKline make a public statement of the likely cost per dose there is very little we can do to capture the uncertainty in price other than assume a wide range for the cost per dose of vaccine in the sensitivity analysis. Secondly, there is uncertainty in the duration of protection provided by RTS,S/AS01 with no estimates of half-life published to date. We assume vaccine efficacy decays with a half-life of 5 years. A more detailed sensitivity analysis could explore the effect of varying half-life in the range 1 – 10 years. Another major source of uncertainty, and perhaps the hardest to quantify, is the interaction between vaccination and naturally-acquired immunity. A particular concern has been the potential for a rebound effect whereby vaccinated children experience a higher incidence of clinical malaria after vaccine efficacy has waned. In Chapter 7 we considered a number of potential scenarios for the interaction between vaccination and the acquisition of immunity. An alternative approach would be to use an ensemble of models with different assumptions about the effect of vaccination on immunity [171].

Although a sensitivity analysis was performed, a potential drawback is that only the uncertainties appearing in the decision tree in Figure 7.1. are accounted for, and not the uncertainties in the underlying transmission model. In the current model formulation, episodes of severe malaria and death are estimated from the decision tree. The parameters for probability of severe malaria are taken from a small number of studies [350, 358]. A more detailed sensitivity analysis would consider a wider range of probabilities of progression to severe malaria as provided by surveys of expert opinion [370]. Future formulations of the malaria transmission model under development at Imperial College will include acquisition of immunity to severe malaria, thus partially bypassing the need to depend on expert opinion or estimates from a single study site.

8.3. Implications of research

Many of the methods outlined and results presented in this thesis may have implications for the analysis of the large amount of epidemiological, clinical and immunological data being generated by Phase II and Phase III trials of the RTS,S malaria vaccine. Given the importance and cost of such large scale clinical trials, it is vital that data is analysed so as to produce useful, accurate and unbiased results. A number of steps can be taken to address the possibility of heterogeneity in exposure introducing bias to estimates of vaccine efficacy identified in Chapter 2 and the associated publication [344]. In a pooled analysis of extended follow-up data from seven Phase II field trials of RTS,S (P. Bejon, M. White *et al* – in preparation), time to event data was analysed using Cox proportional hazards analysis with a fitted gamma frailty to account for heterogeneity in exposure.

The results of Chapter 5 indicate that RTS,S has a protection profile intermediate between that of a leaky and an all-or-nothing vaccine, in agreement with the results of extended follow up of Phase II field trials [118, 134]. Both vaccine-induced anti-CSP antibodies and CSP-specific $CD4^+$ T cells are strongly associated with protection from infection, however, the factors determining the magnitude of vaccine-induced immune responses remain poorly understood. The choice of adjuvant has a well established relationship with vaccine immunogenicity [287], but other factors determining immunogenicity such as age, prior exposure and boosting regimen remain poorly understood.

In the Phase II artificial challenge trial of RTS,S a highly protected subset of volunteers with high levels of anti-CSP antibodies and CSP-specific T cells was identified. This suggests that if the immunogenicity of adjuvanted RTS,S can be improved, then efficacy against infection can be substantially increased. A collaborative project between GlaxoSmithKline and Crucell Holland BV is currently underway for evaluation of the safety, immunogenicity and efficacy of a prime-boost combination vaccine with priming with an adenovirus 35-CSP vaccine followed by boosting with RTS,S/AS01 [371]. This combination vaccine has been demonstrated to be significantly more immunogenic than RTS,S/AS01 alone in rhesus macaques, inducing high levels of CSP-specific T cells [372]. If this finding can be replicated in humans, such that the vaccine-induced immune responses in people receiving the combination vaccine are comparable to those on the higher end of the spectrum of RTS,S/AS01 induced responses, then vaccine efficacy against infection in excess of 70% may be achievable.

An alternative route to increasing the efficacy of RTS,S-based vaccines could be the addition of other pre-erythrocytic or blood-stage antigens to form a multi-component vaccine [284]. Our analysis of the relationship between naturally acquired anti-CSP and anti-TRAP antibodies and protection from infection in Chapter 4 suggests that the addition of a TRAP component to RTS,S/AS01 may be a worthwhile approach. A combination TRAP/RTS,S/AS02 vaccine has already been trialled in a small number of volunteers but failed to provide any protection from infection, possibly due to poor presentation of the TRAP antigen [280]. Combining RTS,S with blood-stage antigens is unlikely to lead to improvements in infection-blocking efficacy but may cause substantial increases in efficacy against clinical malaria. A recent trial of FMP2.1/AS02, a vaccine comprised of a recombinant protein based on AMA1 and the AS02 adjuvant demonstrated 17.4% efficacy against episodes of clinical malaria, and 64.3% efficacy against clinical malaria caused by parasites with AMA1 corresponding to that of the vaccine strain [107]. This result suggests that the combination of RTS,S with blood-stage antigens may be a fruitful approach.

In many studies of the relationship between vaccine-induced immune responses and protection from infection, immune responses are assumed to be constant over time [118]. In reality vaccine-induced immune responses will decay over time [279], possibly with an associated change in protection. Mathematical models can be used to help understand the kinetics of vaccine-induced immune responses. This could be especially useful when evaluating changes in immune responses following the booster dose of RTS,S/AS01 that is to be administered to participants in Phase III trials 18 months after the third dose [15].

Mathematical models provide a convenient framework within which to evaluate the effectiveness of vaccination campaigns, however they can't work magic as there are some knowledge gaps which models just can't bridge

without making strong assumptions. Two key knowledge gaps encountered by models of the effects of malaria vaccination are the duration of vaccine-induced protection, and the interaction with naturally acquired immunity. However models can be used to test various assumptions, and hence aid in the design of experiments or statistical tests.

8.4. Conclusion

The long wait for an effective malaria vaccine is almost over, and although many problems in the intersection of malariology and vaccinology have been solved by groups from across the world, many problems and knowledge gaps remain. Throughout this thesis, I have attempted to contribute to the understanding of several of these problems ranging from the interpretation of clinical trial results, to vaccine immunology to the effectiveness of vaccination campaigns. There are however many remaining questions which have no easy answers. Will a vaccine that prevents approximately one half of clinical episodes malaria be good enough, and will its effects last for long enough? How much will it cost and who should pay? Will it even matter against the rapidly changing landscape of malaria control where we have seen massive reductions in morbidity and mortality across the globe? Despite these reservations, the RTS,S malaria vaccine is set to have completed Phase III clinical trials by 2015, after which important decisions on vaccine deployment will need to be made by both scientists and policy makers.

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