

Malaria elimination modelling in the context of antimalarial drug resistance

Richard James Maude

Christ Church

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Mahidol-Oxford Tropical Medicine Research Unit
Nuffield Department of Clinical Medicine
University of Oxford

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RESPONSIBILITIES

I was responsible for overall study design, collation and analysis of data from the various sources and design and development of the mathematical models. From 2008-2011, I was coordinator of the MORU field studies in Chittagong, Bangladesh where I recruited patients and collected the clinical data used in this thesis. I also organized data entry and collation of historical data in Chittagong and Khagrachari.

Cambodia malaria data were collected for the National Malaria Control Program by Po Ly and Tol Bunkea. Data on parasite clearance rates in Cambodia and Thailand were collected as part of other studies organized by colleagues in MORU, particularly Arjen M Dondorp and Francois Nosten.

ABSTRACT

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Richard James Maude, Christ Church, Clinical Medicine DPhil

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Introduction

Antimalarial resistance, particularly artemisinin resistance, is a major threat to *P. falciparum* malaria elimination efforts worldwide. Urgent intervention is required to tackle artemisinin resistance but field data on which to base planning of strategies are limited. The aims were to collect available field data and develop population level mathematical models of *P. falciparum* malaria treatment and artemisinin resistance in order to determine the optimal strategies for elimination of artemisinin resistant malaria in Cambodia and treatment of pre-hospital and severe malaria in Cambodia and Bangladesh.

Methods

Malaria incidence and parasite clearance data from Cambodia and Bangladesh were collected and analysed and modelling parameters derived. Population dynamic mathematical models of *P. falciparum* malaria were produced.

Results

The modelling demonstrated that elimination of artemisinin resistant *P. falciparum* malaria would be achievable in Cambodia in the context of artemisinin resistance using high coverages with ACT treatment, ideally combined with LLITNs and adjunctive single dose primaquine. Sustained efforts would be necessary to achieve elimination and effective surveillance is essential, both to identify the baseline malaria burden and to monitor parasite prevalence as interventions are implemented. A modelled policy change

to rectal and intravenous artesunate in the context of pre-existing artemisinin resistance would not compromise the efficacy of ACT for malaria elimination.

Conclusions

By being developed rapidly in response to specific questions the models presented here are helping to inform planning efforts to combat artemisinin resistance. As further field data become available, their planned on-going development will produce increasingly realistic and informative models which can be expected to play a central role in planning efforts for years to come.

PUBLICATIONS ARISING FROM THIS THESIS

Publication of material included in this thesis

- **Maude RJ**, Hasan MU, Hossain MA, Abu Sayeed A, Paul SK, Rahman W, Maude RR, Vaid N, Ghose A, Amin R, Samad R, Bin Yunus E, Rahman MR, Bengali AM, Hoque MG, Day NPJ, White NJ, White LJ, Dondorp AM, Faiz AM. Temporal trends in severe malaria in Chittagong, Bangladesh. *Malaria Journal*. 2012;11:323.
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GLOSSARY

95% CI, 95% confidence interval

ACT, artemisinin combination therapy

AP, atovaquone-proguanil

API, Annual Parasite Index

AUC, area under the curve

CI, confidence interval

CNM, Cambodia National Malaria Control Programme

FEMSE, Fast Elimination of Malaria by Source Eradication

GNI, Gross National Income

HIS, Healthcare Information System

HRP2, histidine rich protein 2

IQR, interquartile range

ITN, insecticide treated bed net

IRS, indoor residual spraying of insecticide

LLITN, long-lasting insecticide treated bed net

MDA, mass drug administration

MIS, malaria information system

MRI, magnetic resonance imaging

MSAT, mass screening and treatment

NMCP, National Malaria Control Programme of Bangladesh

OD, operational district

P. falciparum, *Plasmodium falciparum*

P. malariae, *Plasmodium malariae*

GLOSSARY

P. vivax, *Plasmodium vivax*

Pf, *Plasmodium falciparum*

pLDH, parasite lactate dehydrogenase

PQ, piperaquine

Pv, *Plasmodium vivax*

RDT, Rapid Diagnostic Test

Rx, treatment

SD, standard deviation

SEM, standard error of the mean

VMW, Village Malaria Worker

WHO, World Health Organisation

OUTLINE

This thesis comprises three main components:

1. Collection and analysis of epidemiological data on malaria in Cambodia and Bangladesh to derive parameter values and summary statistics for fitting and validation of mathematical models.
2. Collection and analysis of parasite clearance data from Cambodia and Bangladesh from clinical studies to derive increased understanding of artemisinin resistance and parameter values for artemisinin sensitivity in Cambodia and Bangladesh for mathematical modelling.
3. Three population dynamic mathematical models of *P. falciparum* malaria focussing on antimalarial treatment and artemisinin resistance in Cambodia and Bangladesh.

Summary of Chapters

Chapter 1 is the Introduction. It gives a background to the thesis introducing malaria, mathematical modelling, modelling of malaria elimination, the artemisinin antimalarials, and malaria in Cambodia and Bangladesh. It introduces each of the three components: malaria epidemiology, parasite clearance and the mathematical models and presents the aims.

Chapter 2 describes the Methods for analyses of the epidemiological and parasite clearance data. It describes in detail the structure of the mathematical models and summarises the scientific questions they were designed to answer.

Chapter 3 presents the Results comprising epidemiological and parasite clearance data analyses and the results of the mathematical modelling.

Chapter 4 is the Discussion which puts the main findings of the thesis into context and examines their implications.

Chapter 5 is the Conclusion. This summarises the findings, describes the impact that the work comprising this thesis has had on malaria control and elimination policy and outlines the future directions for this work.

Chapter 6 is the Appendix. This contains a detailed description of hypotheses for parasite recrudescence following artemisinin treatment. This is followed by further details of the mathematical models: summary equations, model assumptions, parameter values, additional detail of the model fitting and validation and the code for **Model 1**.

Chapter 1 : Introduction

1.1 Background

1.1.1 Malaria biology and clinical aspects

Malaria is a potentially life threatening disease caused by infection of red blood cells by parasites of the genus *Plasmodium*. It is transmitted to people by the bite of female *Anopheles* mosquitoes. There are 5 species of *Plasmodium* which affect humans, *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*. *P. falciparum* and *P. vivax* are by far the commonest. *P. falciparum* is the main cause of severe and fatal malaria worldwide, the main target for elimination, and is thus the primary focus of this thesis. It is estimated that there are approximately 200 million cases and half a million deaths from malaria annually.¹

The life cycle of the *Plasmodium falciparum* malaria parasite is shown in **figure 1.1-1**. Infection occurs by injection of sporozoites from an infected mosquito to a human host. These migrate to the liver where they invade hepatocytes and multiply forming schizonts. The hepatic schizonts rupture, releasing thousands of merozoites into the blood. The merozoites invade red blood cells forming trophozoites. Multiplication of these intraerythrocytic forms produces circulating schizonts which upon rupture release further merozoites which reinvade red blood cells. These parasites then either go on to multiply again forming more schizonts or develop into a male or female gametocyte, the sexual forms of the parasite. When a mosquito bites the infected person, gametocytes are taken up with the blood meal. Male and female gametocytes combine in the mosquito stomach to form a zygote. These invade the wall of the mosquito midgut and form an oocyst. Multiplication in the oocyst then forms sporozoites which migrate to invade the mosquito

salivary gland ready for injection when the mosquito next bites a human, thus completing the cycle.

Only the sexual blood stage parasites, i.e. gametocytes, are infectious to mosquitoes. Trophozoites, schizonts and merozoites in the blood and schizonts in the liver are all asexual stage parasites and are not infectious to mosquitoes. This distinction is important for the modelling which is presented later in this thesis.

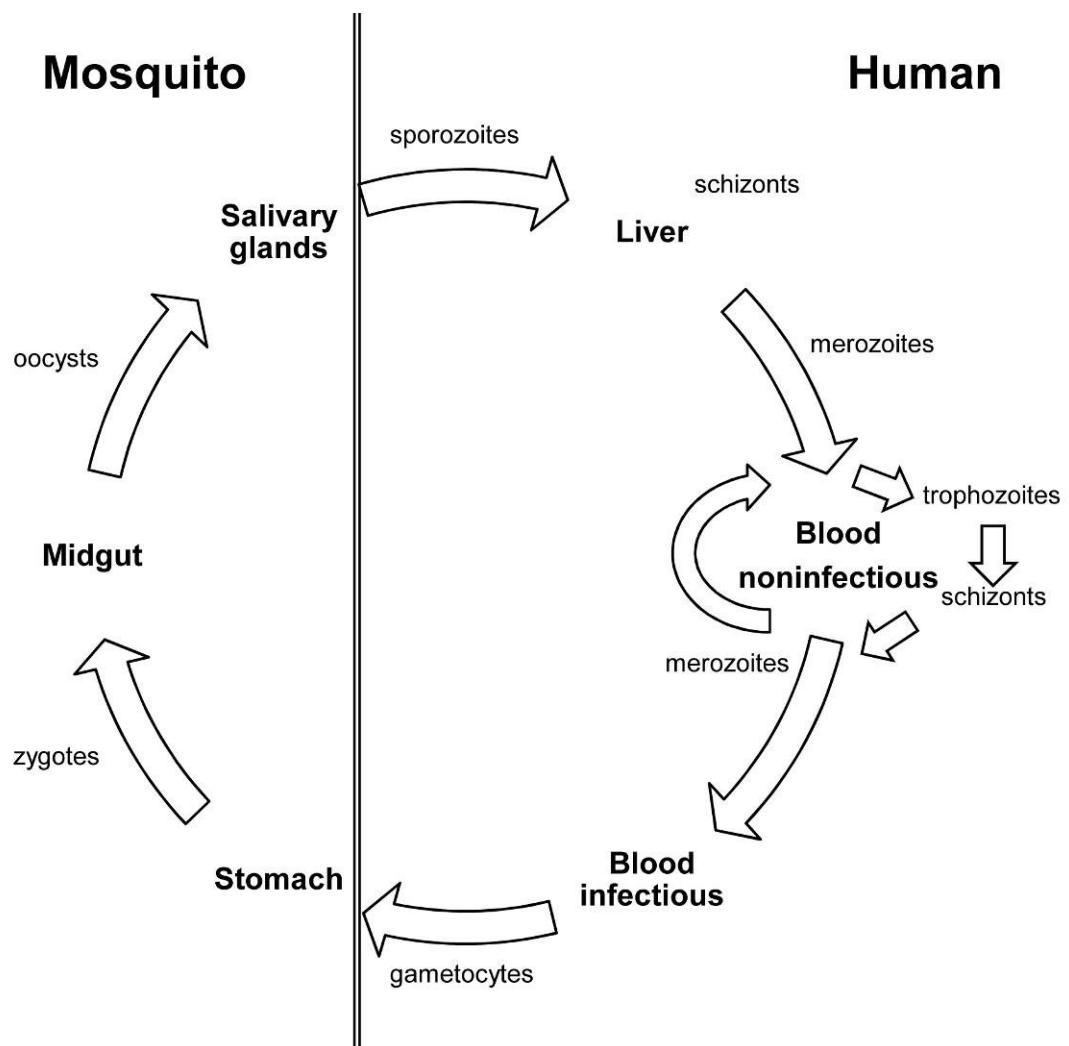


Figure 1.1-1. The *Plasmodium falciparum* malaria parasite life cycle.

The initial clinical presentation of malaria is nonspecific consisting of headache, lassitude, fever and malaise. Early treatment at this stage with effective antimalarials results in a prompt and rapid recovery. Delayed initiation of treatment or ineffective

antimalarials allow a higher parasite burden to develop and this may result in severe malaria. Severe malaria has a mortality of around 10-20% in those receiving treatment and close to 100% in those who don't.² Protective immunity in malaria is poorly understood but is known to depend on the intensity of transmission in an area. Those with more protective immunity have fewer or no symptoms and are less likely to develop severe disease. In high transmission areas, high constant rates of infection lead to gradual acquisition of partial immunity in childhood. This results in higher parasite densities and most acute clinical and severe cases occurring in children and low parasite densities and less clinical and severe disease in adults. In low transmission areas, acquisition of immunity is low and all age groups suffer similar rates of infection. There is also a higher risk of progression to severe disease. In low transmission areas, there is often marked seasonal variation in numbers of cases and epidemics can occur.

Malaria is diagnosed by detection of *Plasmodium* parasites in the peripheral blood by light microscopy or detection of malaria antigen in a blood spot by rapid diagnostic test (RDT). The most widely available RDTs detect *P. falciparum* histidine rich protein II (PfHRP2) (**figure 1.1-2**).



Figure 1.1-2. Rapid Diagnostic Test for *P. falciparum* using HRP2.

Treatment of malaria aims to rapidly eliminate malaria parasites from the body. This has three main benefits. The first is benefit to the infected individual by aborting their symptoms and preventing the progression to severe disease and death. The other two benefits are to the population in that it minimises transmission of infection to others and prevents the acquisition and spread of antimalarial drug resistance.

First-line recommended treatment for *P. falciparum* malaria worldwide is currently artemisinin combination therapy (ACT).² These consist of an artemisinin antimalarial plus a second class e.g. piperazine, lumefantrine or mefloquine. The two combined medications have different mechanisms of action and this further reduces the risk of acquisition and spread of antimalarial resistance.³

Where resistance does occur in a population it can be difficult to detect in the early stages. Low levels of resistance may slow parasite clearance but the parasite burden in an individual can still be reduced sufficiently for their symptoms to resolve. Their infection may then return weeks later but be assumed to be due to a newly acquired infection. With higher levels of resistance, treatment failures occur. In low transmission settings this can lead to increased malaria transmission and morbidity. In all settings, it can result in an increase in deaths from malaria.

Widespread availability of effective antimalarial treatment forms the cornerstone of malaria control activities worldwide. Other important strategies are distribution of long-lasting insecticide treated bed nets (LLITN) and indoor residual spraying of insecticide (IRS). These are used at the household level and by acting against mosquitoes are able to

directly reduce transmission. IRS is less effective and thus not used in large parts of Asia, including Cambodia and Bangladesh, as much of the malaria transmission occurs in forests and forest fringes. The modelling in this thesis thus concentrates on antimalarials and LLITN. Several malaria vaccine candidates are under development but are yet to reach sufficient efficacy to be of use in the field.⁴

ACT treatment, LLITN and IRS can be highly effective control measures but are frequently compromised by problems with their implementation. These include poor access to health services and inadequate distribution networks. There is also a lack of reliable surveillance systems with which to monitor malaria control activities and their impact.

In recent years there has been a renewed effort to improve malaria control worldwide with the aim of eliminating malaria wherever possible. These efforts and the role of mathematical modelling to help plan them are discussed further in **Section 1.1.3**.

1.1.2 Mathematical modelling

Opening the ‘black box’: an introduction to mathematical modelling for non-mathematicians

What is mathematical modelling?

According to the Oxford English Dictionary, *mathematical modelling* is “the process of devising...a simplified mathematical description of a system or process, used to assist calculations and predictions”.⁵ It can be wrongly confused with statistical modelling, the major difference being that the latter is only able to organize, summarize, and interpret known data, whereas with mathematical modelling, predictions can be made for scenarios for which no data yet exist.

For infectious diseases, mathematical modelling can therefore be a valuable tool; for example, to investigate the likely evolution of an epidemic and explore the possible effects of control measures such as vaccines or new drugs; e.g. Smith and Blower 2004.⁶ However, modelling is not limited to epidemics and, in theory, any scenario for any infectious disease could be modelled: the only limitations being the degree of understanding of the biological system involved and the availability and quality of data on which to base predictions.

A brief history of mathematical modelling

The origins of mathematical modelling may be found in the early application of empirical statistical methods by Daniel Bernoulli who, in the 1760s, used mathematical methods to

evaluate the effectiveness of the technique of variolation to protect against smallpox.⁷ In 1840, William Farr analysed data on deaths from smallpox in England and Wales, including fitting a smoothed curve to mortality rates over time, to quantify and predict the likelihood of recovery or death.⁸ The concept of R_0 (defined below) has been in use for well over a century, perhaps the earliest being that by Böckh in 1886.⁹ In 1906, John Brownlee extended Farr's work and fitted Pearsonian frequency distribution curves to a large series of epidemics.¹⁰

The first attempts at mathematical modelling in its modern sense came in 1906, when William Hamer applied post-germ theory thinking to formulate specific hypotheses about the transmission of infectious disease in simple but precise mathematical statements and investigated the properties of the resulting models. He examined the regular recurrences of measles epidemics and postulated that the course of an epidemic depends on the rate of contact between susceptible and infectious individuals, the so-called 'mass-action principle'.¹¹ In 1911–1917, Ronald Ross (who won the Nobel Prize for proving the transmission of malaria by mosquitoes) used the mass action principle to develop the first host-vector-host model as a set of simple mathematical formulae and demonstrated the relationship between numbers of mosquitoes and the incidence of malaria.¹² This model was refined by George MacDonald in the 1950s and the resulting Ross-MacDonald model has contributed greatly to the understanding of malaria epidemiology.

MacDonald applied the concept of the basic reproductive number, R_0 , in 1957.¹³ Giesecke defined it as “the average number of persons directly infected by an infectious case during

its entire infectious period, after entering a totally susceptible population.” Put simply, an infection will generally only spread only if $R_0 > 1$.¹⁴

Soper (1929) extended the ideas of Hamer and Ross, and deduced the underlying mechanisms responsible for the often-observed periodicity of epidemics.¹⁵ Kermack and McKendrick developed the ‘Susceptible-Infectious-Removed’ (SIR) (the general epidemic) model in 1927, variants of which are still widely used today.¹⁶ From 1955 to 1960, Bartlett developed probabilistic models which better represented the randomness inherent in biological systems.¹² Much of the literature following this up until the early 1990s was concerned with further development of probabilistic modelling.

Before the advent of personal computers in the 1970s, a major limitation to progress was the sheer impracticality of manipulating by hand, multiple interdependent complex mathematical formulae and large datasets. The last twenty years has seen the construction of increasingly complicated models made possible only by the exponential rise in computer processing power and new technologies such as satellite imagery and GIS mapping.¹⁷ This has been accompanied by a rapid increase in the number of modelling papers published (**figure 1.1-3**).

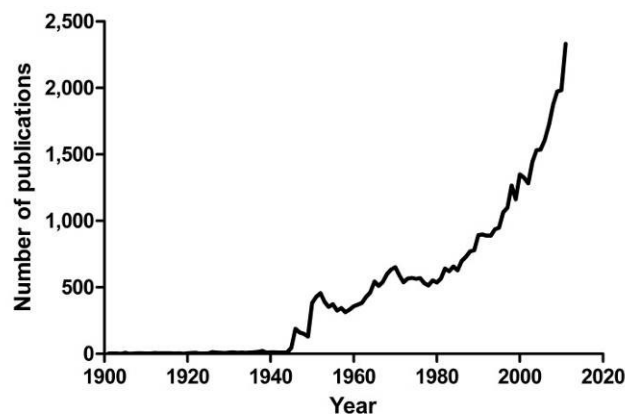


Figure 1.1-3. Published scientific papers on mathematical modelling of infectious disease per year since 1900.

This was generated by searching PubMed with the string (mathematical "modelling" or "model" and "infection"[TI] or "infectious"[TI] or "communicable"[TI]).

The problem of complexity

It does not follow that the more precise and complex a model, the better it is.

Mathematical models are devised to answer specific questions; different types of models and different levels of complexities are required for different questions. The art lies in choosing the appropriate type of model and level of complexity. The most complex model of all is, in fact, reality, whereas the purpose of modelling is to simplify a process so it may be better understood and can be used to make predictions. On the other hand, an over-simplified model is likely to be a poor representation of reality and thus uninformative. It still needs to capture the essential behaviours of interest and incorporate essential processes. To understand a modelled process, one needs to be able to understand what is happening in the model. This is particularly important when an outcome differs from what is intuitively expected. The relationship between reality and models is nicely illustrated by words attributed to John Maynard Smith that “to take a complex, poorly

understood reality and explain it by a complicated, poorly understood model, is not progress.”

The ‘black box’ of modelling

A key to a successful model is a close interaction between modellers and those who stand to benefit the most from modelling outputs: i.e. policy makers, public health professionals, and clinicians. Modelling has traditionally been the domain of theoreticians who have naturally, at times, tended to concentrate on the abstract development of modelling techniques, rather than answering the important public-health questions of the day.¹² Over the last decades, mathematical modelling has grown into a highly specialized discipline, the techniques of which have evolved way beyond the level of understanding of the average ‘lay’ person. Unfortunately, this has had a negative influence on the general perception of mathematical modelling as a discipline.

Many scientific papers that use mathematical modelling are quite inaccessible to the lay reader. They frequently, and necessarily, contain large amounts of mathematical notation which may be unintelligible to those without formal training in advanced mathematics. In contrast with reporting laboratory science, there is also no standard format for modelling papers, so that important details are often omitted, preventing the possibility for full recreation of the model and assessment of its limitations.

Compounding these problems, the average reader has next to no knowledge of modelling techniques. This has led to the perception that modelling is a ‘black box’, that at one extreme may be thought to be little better than educated guesswork, and is thus ignored,

and at the other extreme, may be trusted blindly and then depended on as the sole justification for important public-health interventions. Both these approaches are unhelpful at best and dangerous at worst. An increase in basic knowledge of modelling techniques by the end users, as well as a more accessible format of modelling publications can both contribute to a more effective communication and facilitate the critical appraisal of modelling work. A core set of information that should be in any modelling paper in order to allow adequate assessment of its quality is given in **table 1.1-1**.

Table 1.1-1. Core set of information that should be provided in a modelling paper.

Requirements
1. Precisely specified scientific question 2. List of modelling assumptions 3. Discussion of the limitations of the model 4. Definition of every term used in equations or diagrams 5. Full list of parameter values with a reference or justification for each 6. Presentation of the model structure adequate to reconstruct the model 7. A sensitivity analysis in which the sensitivity of modelling results to different values for uncertain parameters is assessed 8. Answer to the question

The ideal modelling project is one where modellers and non-modellers work closely together in an on-going collaboration.¹⁸ The ideal model is one that is developed to answer a specific and relevant scientific question. Non-modellers can provide and clearly

define the important questions for a modelling project to answer, contribute data to parameterize the model and understanding of the fundamental processes necessary to structure the model, aid with the interpretation of model outputs, and provide further data with which to refine the model.¹⁸ Modellers contribute their specialist mathematical and programming skills, interpret and communicate the results of model simulations and analyses and help the non-modellers to convert frequently imprecise conceptual models into precise mathematical frameworks. Modellers also interpret the results of model simulations and analyses in collaboration with non-modellers to provide a biologically meaningful answer to the original question.

What is inside the ‘black box’?

Types of mathematical models

There are two main types of method used in mathematical modelling of the transmission of infectious diseases: deterministic and stochastic. Deterministic models deal with populations of individuals; stochastic models can be either population-based or individual-based.

In a deterministic model, it is assumed that processes occur continuously at an ‘average’ rate. This has the effect that every time a model is run with a particular set of input data (‘parameters’) and initial conditions, it will always produce the same result (**figure 1.1-4a**, model shown in **figure 1.1-6**). A drawback of such modelling is that there is no random variation, a fundamental feature of biological systems, and deterministic models deal with fractions of individuals and can thus produce unrealistic results. This causes problems particularly when modelling low numbers of individuals, for example when

modelling the eradication of an infection. In this situation, a deterministic model would never achieve eradication because the number of infected individuals never reaches zero. The model instead reports ever decreasing fractions of one person. The advantage of deterministic models is that they are relatively fast to develop and run and are therefore useful for understanding the main trends and dynamic features of modelled systems.¹⁹

In comparison, stochastic models incorporate the effects of random chance and probability. There are many methods for doing this and the modeller must select both the method and the degree of random variation required. Such models always produce different results for a given set of parameters. Thus, a single run of a stochastic model cannot be used to draw conclusions. Instead, usual practice is to calculate the mean results from a large number of stochastic runs using the same input variables (**figure 1.1-4b**). This can require a lot of computing power and may be very time-consuming. Stochastic models deal with discrete events occurring to integer (whole) numbers of individuals and thus are much more appropriate for dealing with low numbers of infections, for example in elimination modelling.¹⁹ Stochastic effects are always more influential in small populations so, when dealing with a large population, a large number of runs of a stochastic model will produce very similar results to a single run of an equivalent deterministic model (**figure 1.1-4a and 1.1-4b**).

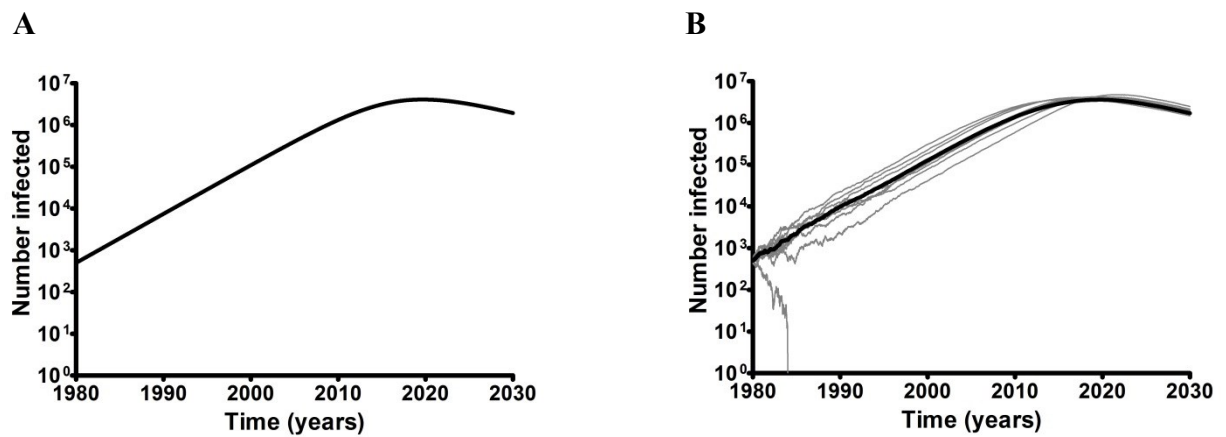


Figure 1.1-4. Results of a population dynamic model (details given below in ‘*Example of a simple model*’) of HIV spread in a population showing the number of people with HIV over time.

A is from a deterministic model and **B** shows ten runs of a stochastic model with the same structure and parameters. In one of these runs, the infection becomes extinct in the population by random chance – so called ‘stochastic fade-out’. The black line in **B** represents the mean of ten runs and this can be seen to be almost identical to the deterministic result **A**.

Individual-based (or agent-based) modelling is an increasingly popular type of stochastic modelling. It uses the large population approximation, where it is assumed that the probability of an event occurring per unit time is derived from the per capita rate of that event. For example, each individual makes a certain number of contacts per day. If that contact is infectious, there is a certain probability that transmission will occur. This type of modelling is becoming increasingly popular with the increase in computing power available and can include large numbers of interacting individuals, each with different characteristics.¹⁷

How to make a mathematical model

All types of mathematical models are developed in essentially the same way. This can be thought of as a recipe that requires certain essential ingredients. These ‘ingredients’ are:

1. an understanding of the fundamental processes in the biological system, 2. input data (‘parameters’ and ‘initial conditions’), 3. a set of assumptions (or simplifications), 4. a set of mathematical equations (‘code’) and means of solving the equations (either analytically or using a computer). The ‘recipe’, or general process of developing a mathematical model, is summarized in **figure 1.1-5** as the modelling cycle. This cycle is an on-going iterative process whereby modelling outputs are compared to known data and the model is continuously improved and refined to make its predictions as useful and accurate as possible.

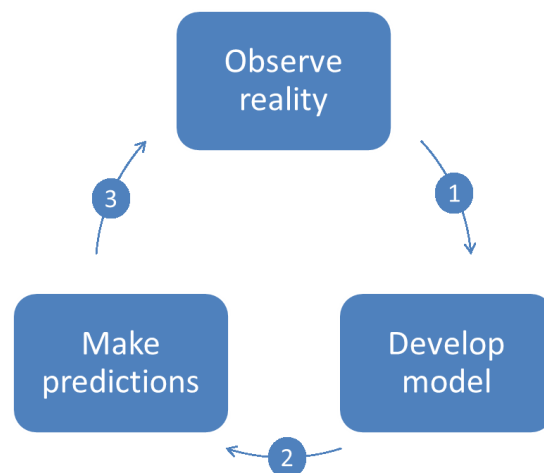


Figure 1.1-5. The modelling cycle.

The modelling cycle can be summarized as follows:

First cycle:

1. Observe reality. The impetus to develop a biological model is the identification of a problem or question concerning a biological process or system in the real world.

Close real-world observations are needed to understand the fundamental biological processes necessary to build a realistic model and will identify specific questions that need to be answered.

2. Develop model. It must first be decided which parameters are important and data must then be collected which will generate these parameters. The model structure itself is now developed i.e. the system is described by a set of mathematical equations. This is normally entered into a computer as a set of code or may be analysed theoretically. The assumptions inherent in the model structure must be listed in order to make the modelling process as transparent as possible. This also gives an indication of the limitations of the model.
3. Make predictions. The model can now be run, which means instructing the computer to solve the mathematical equations. At this stage, problems with the model code are identified and an often painful process of error correction may be required for the model to run satisfactorily. The model output (or 'solution') is then obtained and interpreted.

Second and subsequent cycles: follow the same three steps, except, rather than start from scratch, the model thus far developed is continuously refined and further developed in light of newly collected data, improved insights into the biological relationships between model parameters, and comparison of output data with real directly observed data. The quality and utility of predictions made by the model should thus improve with each cycle and the process is repeated until there is a good enough agreement with reality for the model to be useful.

Since modelling infectious diseases requires an accurate description of fundamental processes of biology or epidemiology, the very process of developing these mathematical

models can be highly informative, and will often reveal areas where fundamental knowledge is still lacking.

The equations required to adequately describe a biological system have become too complicated to solve by hand. As a result, most modelling is done nowadays by computer. Indeed, one of the most commonly used techniques, that of solving ordinary differential equations that describe the transition of populations between disease states, e.g. susceptible, infected, and recovered, can only be done in a timely fashion using a computer. A number of commercial modelling software packages have been developed to facilitate this type of calculation, e.g. Berkeley MadonnaTM,¹⁹ Matlab and XPPAUTO, although most modellers prefer to use a computer programming language, such as R or C++, as this gives greater flexibility.

An example of a simple model

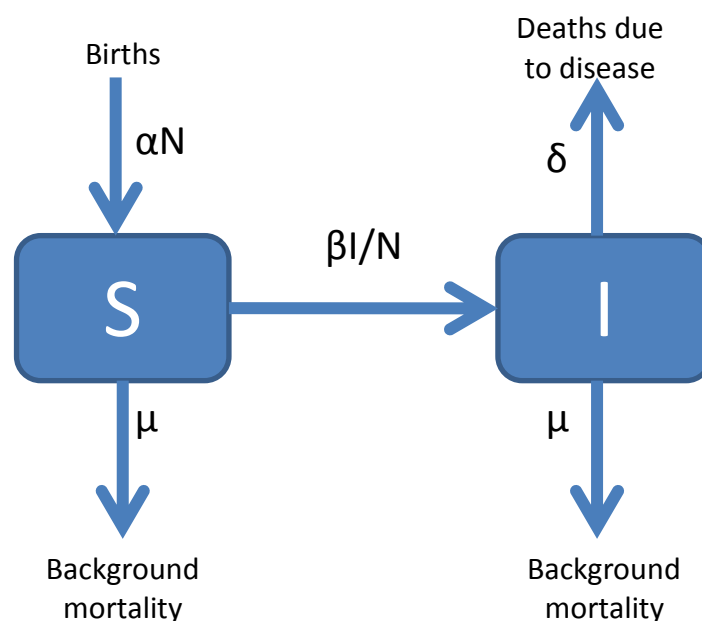


Figure 1.1-6. A simple, two-compartmental model of the transmission of HIV.

S is the susceptible population, I is the infected population, N is the total population at risk (i.e. $N = S + I$), α is the birth rate, β is the HIV transmission rate, μ is the background mortality (1/average life expectancy) rate and δ is the additional mortality due to HIV (1/average time from infection to death).

An example of a simple mathematical model is shown in **figure 1.1-6**. This is a simple population dynamic deterministic model of HIV infection in a population of size N . It was developed to examine the rate of spread of HIV infection in a susceptible population. In this model, the focus is on how I (the number of people infected with HIV) varies over time. There are two compartments: Susceptible (S) and Infected (I); hence this type of model is referred to as an ‘SI model’. The model is best understood by focusing on each of the two compartments in turn: the population of susceptible people (compartment S) only increases as children are born, but there are two ways of leaving S : either by dying (all causes other than HIV) or by becoming infected with HIV. People only enter the compartment I from S by becoming infected with HIV; they can leave compartment I by two routes: dying of HIV or dying of other causes.

The model has some inherent simplifying assumptions. These include that the population is well-mixed, everybody in I is transmissible for their entire life (e.g. no leaving the sexually active pool), everybody in S is susceptible for their entire life (no leaving the sexually active pool), children born of infected are themselves susceptible (i.e. ignores vertical transmission from mother to child), everybody has the same number of sexual contacts (very unlikely in reality) and that the transmission probability is constant over the entire infectious period (not true for primary infection, treatment, AIDS, etc.).

Patients are born at rate αN and die at rate μ , with a separate mortality rate due to HIV of δ . People become infected at a rate dependent on the proportion of infected (I/N) individuals in the population. The transmission parameter, β , represents the likelihood of transmission between an infected and an uninfected individual per unit time. β is a product of c , the rate of a susceptible contacting infected individuals, and p , the probability of transmission when an infectious individual contacts a susceptible individual. Thus:

$$\text{Per capita rate of becoming infected} = \frac{cpI}{N} = \frac{\beta I}{N} \quad (\text{a})$$

As susceptible individuals become infected, the number of people in state S decreases with time (t) and those in box I increase over time (the rate of change over time is written as $\frac{d}{dt}$):

$$\text{Rate of change of } S = \frac{dS}{dt} = -\frac{\beta I}{N} * S \quad \text{and} \quad \text{Rate of change of } I = \frac{dI}{dt} = \frac{\beta I}{N} * S \quad (\text{b})$$

But some patients are born and some die (ignoring those who are born with infection to preserve simplicity), so the full equations for S and I should be:

$$\frac{dS}{dt} = -\frac{\beta I}{N} * S + \alpha N - \mu S \quad \text{and} \quad \frac{dI}{dt} = \frac{\beta I}{N} * S - \mu I - \delta I \quad (\text{c})$$

We have now constructed a deterministic mathematical model for the spread of HIV in a population.

A stochastic variant of this model may also be generated from this deterministic model by introducing a degree of randomness into each of the two final equations (c) above (for example, by using a Poisson distribution). The last step is to assign values to each of the parameters in equations (c) (called ‘parameterising’ the model). The results of the

deterministic and stochastic variants of this model can be seen side-by-side for comparison in **figure 1.1-4**. In **figure 1.1-7**, it is shown that the model replicates real HIV prevalence data from Mozambique (**figure 1.1-7a**).²⁰ It is important to realise that statistical modelling could produce a similar result to the model output in **figure 1.1-7b**, but mathematical modelling has the advantage that it can predict what is likely to happen in the future and can therefore be used to examine the impact and likely timescale of proposed interventions (**figure 1.1-7c**).

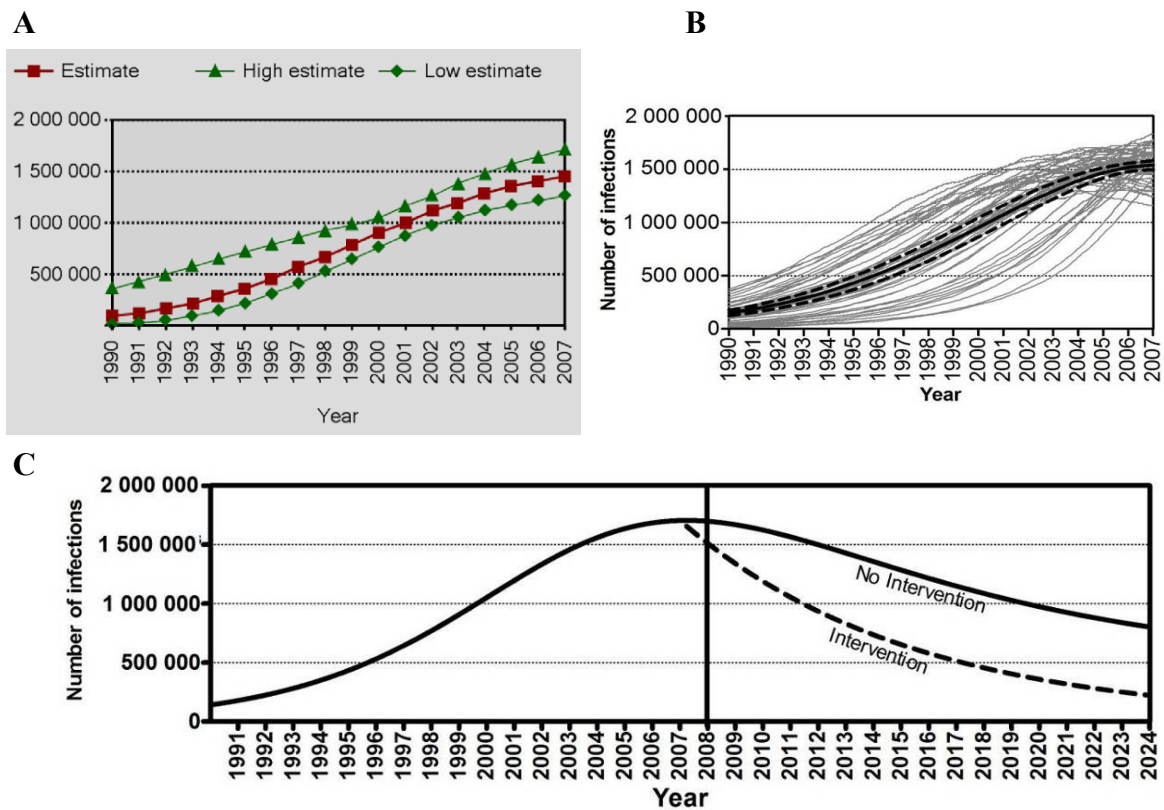


Figure 1.1-7. Number of people living with HIV in Mozambique over time.

A Real epidemiological data from WHO (reproduced with permission);²⁰ **B** fifty runs of a stochastic population dynamic mathematical model (based on that shown in **figure 1.1-6**) with mean and 95% confidence intervals shown as black lines. Parameter values were as follows: $\delta=1/10y^{-1}$, $\alpha=\mu=1/50y^{-1}$, $N=4 \times 10^6$ and $\beta=0.37$. The model was run from 1970

with 1000 as the initial value of I . In **C**, the deterministic model was used to predict what might happen to the epidemic after 2007. It can be seen that the epidemic was predicted to reach a peak before the number of infected individuals falls because of on-going mortality. A transmission-blocking intervention in 2007, e.g. the introduction of condoms, was predicted to reduce the number of infected individuals.

Future directions and conclusions

Mathematical modelling will play an increasingly prominent role in the battle against infectious diseases. There has been an enormous increase in the quantity of scientific data and in understanding of the fundamental processes of biological systems. Technology, particularly computer hardware and modelling software, is progressing rapidly. New techniques such as satellite imagery and GIS mapping are only now beginning to be used to study infectious diseases. Along with this rapid progress of science comes an even more rapid increase in the number of scientific questions to be answered. Ronald Ross in 1911 could not possibly have envisioned the sophistication of the models used today, just as it cannot be conceived of what might be achievable in this most fruitful area of research in the future. The modelling seam of research gold has in many ways only just been uncovered. Transparent reporting, close collaboration between modeller and end-users, and an increase among the latter of the appreciation of the strengths and limitations of mathematical models will increase the potential impact of this important tool.

1.1.3 Modelling malaria elimination

The role of mathematical modelling in guiding the science and economics of malaria elimination

Elimination of malaria is defined as the interruption of local mosquito-borne malaria transmission in a defined geographic area, i.e. incidence of zero contracted cases.²¹ This is distinct from eradication (worldwide removal of a malaria parasite species) and control (reducing the malaria burden so that it is no longer a public health problem).²¹ The Global Malaria Eradication Programme (GMEP) attempted to eliminate malaria from low transmission areas of the world in the 1950s and 1960s and, despite success in 37 of 143 endemic countries and large reductions in many others, it ultimately failed. The goal of malaria control was then adopted in its place.²¹ Over the past 5 years, funding and political commitment for malaria control have again increased markedly. Consequent reductions in malarial morbidity and mortality in several endemic countries as well as strong global advocacy have resulted in global eradication being considered as the new target.²¹ The WHO now aims to reduce greatly the malaria burden in high transmission areas and to eliminate malaria from low transmission areas with the ultimate goal of global malaria eradication as more robust tools become available.²¹

Of all the infectious diseases of man, only smallpox has been eradicated. This disease had the advantage of a single potent control measure, i.e. a highly effective vaccine with a long duration of protection. No intervention of comparable efficacy is available for malaria and the transmission dynamics of malaria are more complex than smallpox,

making it potentially more difficult to eliminate. Many malaria control tools are now available, the most potent of which are ACT, insecticide-treated bed nets and indoor residual spraying with insecticide. However, it is likely that none of these are sufficiently effective on their own to achieve elimination, even with the possible future addition of vaccination, and various combinations of targeted control strategies are probably required.²²⁻²⁶ Widely varying malaria epidemiology and availability of resources mean that the ideal combination of strategies will vary between locations.²⁶

It is not possible to trial all malaria control interventions in all settings, and few studies have aimed for elimination.²⁷ The data on which countries can base malaria elimination policy decisions are thus usually limited and frequently non-existent.²⁴ Somehow these limited data must be used to predict the effect of scale-up of interventions beyond the areas and populations in which they were originally studied. By combining these limited data with detailed mechanistic understanding, mathematical modelling is able to do this and can evaluate different strategies and the effect of confounders much more rapidly than is possible through trial and error in the field. It also allows exploration of why particular control measures may be more effective in a particular setting, thus providing the opportunity to optimise these and devise new strategies.²⁶ During the GMEP, mathematical modelling assisted by clarifying the primacy of interruption of transmission by vector control, supporting wide-scale use of the insecticide dichlorodiphenyltrichloroethane (DDT). In the current era, there are more powerful means of manipulating data using computers. Mathematical modelling of malaria transmission and methods of economic evaluation are consequently much more developed. The combination of these two, together with high-quality surveillance data,

has great potential as a pragmatic tool to help guide malaria elimination efforts and in particular to predict which are likely to be the most efficient and cost-effective strategies in different epidemiological settings. To date, however, they have remained largely separate disciplines.

Guiding the science

Modelling and malaria control policy

Application of mathematics to aid logical reasoning around interventions to reduce malaria transmission was pioneered by Ronald Ross in the early 1900s.^{22, 28} He argued that mathematics is a way to apply careful reasoning to a problem and from his calculations deduced that, for example, to eliminate malaria one merely had to reduce transmission below a certain threshold level and that combinations of interventions are generally more effective than single interventions.^{28, 29} Unfortunately at the time his work was largely ignored by those who were planning such interventions, although later his conclusions were proven to be correct.²⁸⁻³⁰

In the mid-1900s, George MacDonald further developed Ross's model and used it to demonstrate the importance of vector control and interruption of transmission in malaria elimination.¹³ MacDonald concluded that this would be much easier to achieve in low transmission settings. MacDonald's model did not include many features important in malaria transmission, e.g. human population dynamics, seasonal vector dynamics, superinfection and elements of the malaria life cycle.²⁸ DDT was then widely used to kill mosquitoes and, together with the then still highly effective antimalarial chloroquine, had great success in reducing the malaria burden. Dwindling investment in malaria control as

well as DDT resistance and concerns about its ecological impact resulted in it being generally withdrawn from use in the 1970s.³⁰

In 1966, a computer simulation based on MacDonald's work was used to plan a malaria control field research trial using DDT spraying and mass drug administration (MDA) in Kankiya, Nigeria. The results differed greatly from the model predictions, with the intervention being far less effective than predicted, and it was thought this was due both to difficulty obtaining accurate parameter estimates for key epidemiological indices and an inability of these indices and the model structure to describe adequately the epidemiology of malaria in that area.³¹ Models are always simpler than reality and thus often fail to capture the degree of heterogeneity present in reality. This homogeneity usually leads to overestimation of the impact of a control measure and thus 'exaggerated optimism'.²⁹ MacDonald's model makes a number of simplifying assumptions, including homogeneous transmission in an area, no acquired immunity and mosquitoes biting randomly.²⁹ It is not sufficiently realistic to be suitable for detailed design of control strategies at the implementation level, although its conclusions have proven very useful conceptually.³²

A few years later, another model was developed as an integral part of the Garki Project in Nigeria (1969–1976). This model included the addition of human immunity to a more sophisticated derivative of MacDonald's model. It was used to explore the impact of vectorial capacity on the incidence and prevalence of malaria infection in humans and aimed to predict the effects of specific control measures (larvicide, adulticide and MDA), alone and in combination.³³ Although it achieved its first aim reasonably well, it was

unable to reproduce accurately the effect of control measures. Although a significant advance from previous models, the Garki model suffered from oversimplifying assumptions and difficulties with quantifying accurately many of the input parameters. On-going model development was limited to testing the baseline model against new data and subsequent adjustment of only the vectorial capacity, without changing much of the model structure and most of the parameters. The Garki model helped increase the rigour of study design but, owing to its limitations, remained primarily a teaching tool.

Policy-makers have tended not to include mathematical modelling in their planning of national and regional malaria control strategies.²² In its place there has been a reliance on malaria surveillance to estimate the impact of control programmes. As illustrated in **figure 1.1-8**, this is dangerous when the target is elimination as reliance on surveillance data alone can result in false reassurance that elimination has been successful.

Surveillance data can also often be very unreliable and poor data quality can give misleading results. This diminishes its value for policy-makers and in this context modelling can be particularly helpful as an additional source of guidance. The potential contribution of modelling to predict timelines for malaria elimination has been presented elsewhere.³⁴

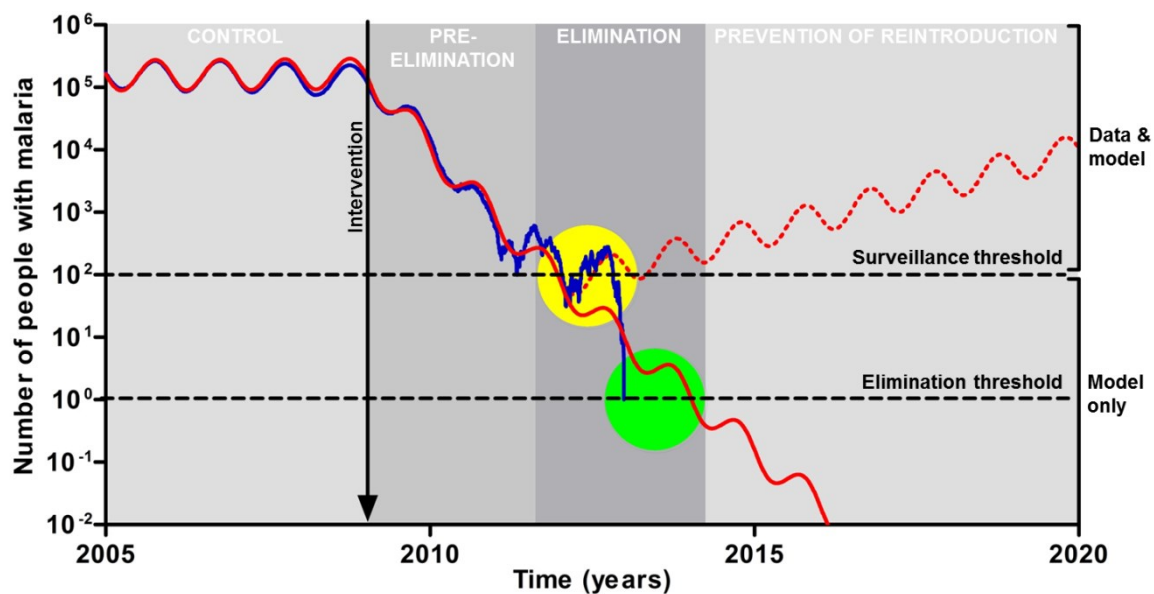


Figure 1.1-8. Deterministic versus stochastic modelling of malaria elimination and the importance of effective surveillance.

The deterministic output is shown in red and stochastic in blue. The model used is presented in Chapter 2.2.²³ Only one run of the stochastic model is shown for clarity. The phases of the WHO malaria control-to-elimination continuum²¹ are indicated by the shaded background. The ‘limit of detection by surveillance’ indicates the number of cases below which a malaria surveillance programme is unlikely to detect any malaria, thus suggesting ‘apparent elimination’ (yellow circle). An arbitrary example is shown in the figure. Because of this detection limit, only the upper portion of the figure can be represented by surveillance data (‘data & model’), whereas the lower portion can only be represented by modelling predictions (‘model only’). Improving the sensitivity of surveillance would lower this detection limit. For ‘true elimination’ to occur (green circle), the number of malaria cases must fall below the ‘elimination threshold’ (<1 case). Only a perfect surveillance system detecting every case would have a limit of detection by surveillance equal to the elimination threshold. This is not generally the case in the

field where surveillance systems are far from perfect and can miss many cases. Thus, the limit of detection by surveillance is generally above the elimination threshold. If malaria control interventions are stopped inappropriately early when apparent elimination occurs (black dotted line), numbers of cases would begin to increase again. Modelling gives an indication of how long control measures would need to be continued to achieve true elimination.

To overcome policy-makers' distrust of modelling, there is a need for it to be more pragmatic and intervention-focused.^{22, 28} Country- or region-specific modelling is more practically useful to control programmes than general conclusions as it takes account of factors that vary between areas, e.g. bed nets are much less effective where mosquitoes bite during the day and patterns of drug and insecticide resistance are highly variable. Modelling can also prevent unnecessary interventions that would waste money and can help determine the best use of resources. Ideally, to optimise their impact on policy, malaria elimination models need to be developed alongside control programmes. They can be used in the initial stages to help plan which interventions to employ and then refined as surveillance data are collected. More realistic, useful and situation-specific recommendations can then be made. A major limiting factor preventing full integration of modelling in malaria control activities has been the lack of availability of location-specific surveillance data of sufficient quality and timeliness to inform on-going model development in real-time.

Integration of modelling into control programmes has occurred for other diseases where the interplay between modelling and policy is far more developed, e.g. onchocerciasis²²

and vaccination programmes.³⁵ The UK Health Protection Agency regularly employs modelling to help plan major infectious disease control programmes, e.g. influenza.^{36, 37} Although there continue to be great strides in the mathematical modelling of malaria with a number of theoretical models providing useful insights,^{38, 39} it is only very recently that the role of pragmatic, prospective, intervention-focused modelling to answer specific disease control questions²² has begun to be recognised as important in malaria.^{18, 23, 40-43} There remains a notable lack of models that specifically compare different modalities for malaria control or that examine the impact of combinations of interventions.^{23, 24, 31, 44, 45} Perhaps most surprising of all is that despite the recent upsurge in interest in eradication and elimination there are few models that specifically consider these.^{18, 23, 24, 38, 41, 44, 45} Many models of malaria control are not appropriate for examining elimination and some of the reasons for this will be discussed in the next section.

Modelling methods for elimination

Infectious disease elimination is a unique situation that requires specific considerations in modelling. The most frequently employed modelling technique to investigate infectious disease dynamics is deterministic modelling. This is the most efficient method for considering multiple scenarios and producing initial results and recommendations quickly, as the model development and run times are very short.²⁴ It is also most appropriate when data are sparse. An example of such a deterministic model developed for malaria elimination is shown in **figure 1.1-9**.²⁴ However, this style of modelling is not appropriate for the end stages of elimination strategies where numbers of infected individuals become very low and individual variation in risk, movement and treatment-seeking behaviour become more significant. Deterministic modelling relies on the

assumption that the population under consideration is large. As illustrated in **figure 1.1-8**, when numbers of infected cases are small, this can lead to unrealistic persistence of infection in a population. In reality, random variation in a biological system can result in elimination earlier than that predicted by a deterministic model. A stochastic model includes a degree of random variation that more accurately reproduces behaviours in nature. They are run multiple times, each giving slightly different outputs, and can thus give probabilities of particular outcomes as results rather than the exact numbers that come from deterministic models. This can give an indication of the degree of risk of a particular strategy not succeeding. However, stochastic models are more complex and time consuming to develop, require more data and take much more time to run.

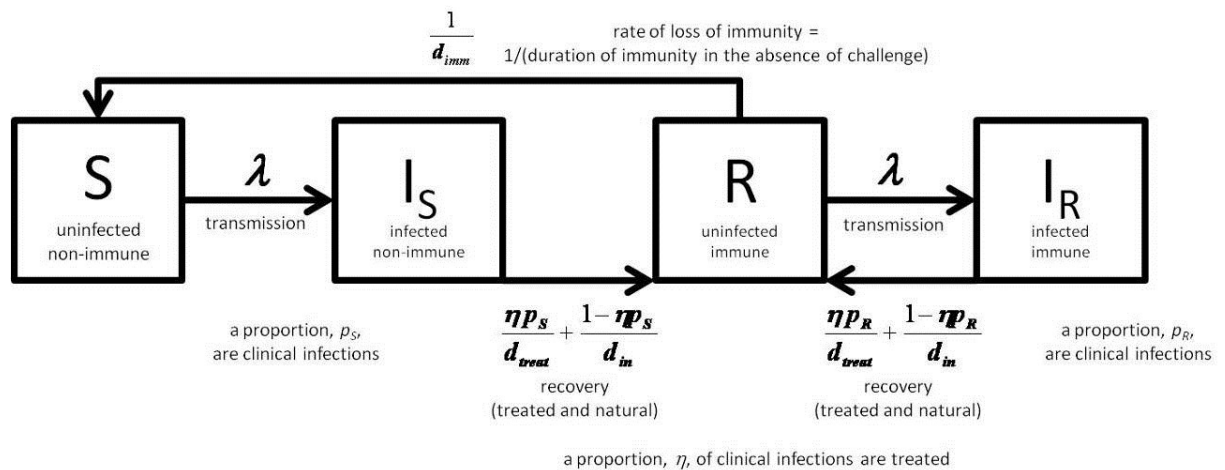


Figure 1.1-9. A simple deterministic mathematical model of malaria transmission.

The diagram shows the compartmental structure of the model with time-dependent variables: S (uninfected and non-immune); I_S (infected with no prior immunity); R (uninfected with immunity); I_R (infected with prior immunity). d_{treat} is average duration of treated infection and d_{in} is average duration of untreated infection. The model represents a situation where disease is being controlled using treatment of symptomatic/clinical malaria. Uninfected individuals become infected at a rate proportional to the overall

prevalence of malaria infection. Recovery takes place either as treatment of clinical malaria at a given coverage or as natural clearance of the parasites. Immunity is assumed to be lost if immune uninfected individuals are not challenged for a given time period. The model equations and a detailed description are found elsewhere.⁴ The model was used to demonstrate the potential for combinations of interventions for elimination programmes. This model does not include explicit vector population and transmission dynamics, multiple levels of immunity, or the liver or asexual stages of infection. It is a simple representation of an extremely complex biological system and is for the purposes of understanding the more general behaviour of malaria transmission specifically during elimination and could be used as a first step by policy-makers for strategy planning for a few years ahead.

Another important consideration when modelling elimination is the common simplifying assumption of homogeneous transmission for the entire population. Although this allows for simpler models (e.g. **figure 1.1-9**), it can lead to an overestimation of the impact of control measures and unrealistic optimism about the ease of elimination.³⁴ In reality, malaria transmission can be highly heterogeneous. Among other factors, failure of an elimination attempt may result from on-going transmission in spatial reservoirs. To capture this heterogeneity, a transmission dynamic spatial model is required, that is a model that includes multiple interacting populations (or individuals) in geographically defined areas, each with their own characteristics. This type of model also allows the investigation of spatially explicit control measures, e.g. selectively distributing bed nets to high transmission foci. Such strategies can greatly increase the impact and cost effectiveness of control measures.⁴⁶ Spatial models allow inclusion of population

migration between and within countries, which can be an important contributor to the spread of malaria, and allows for exploration of country versus regional approaches to elimination. To parameterise realistic spatial models correctly requires much more data than their non-spatial equivalent. In data-poor situations, the extra effort required to produce a spatial model may give little useful extra information. They also require more computing power and this increases as the number of metapopulations increases. Partly because of these difficulties, little transmission dynamic spatial modelling of malaria has been attempted previously and there have been no substantial attempts to model migration patterns of malaria infections.⁴⁷ Rather, there is a substantial body of empirical spatial statistical models that incorporate spatial autocorrelation structure into regression models to produce static geographical patterns of infection, e.g. spatial mapping of malaria prevalence from the Malaria Atlas Project, which is being used to help with planning spatially explicit control measures in Kenya.⁴⁸ As such statistical models ignore transmission dynamics; they cannot be used to predict the impact of malaria elimination measures.

There are several models of malaria elimination in development as part of control programmes, although few details of these have yet been published. A prominent consortium of modellers have agreed that multiple models with different approaches and assumptions to answer specific questions are necessary for malaria elimination.²⁶ A combination of simple and complex models is probably ideal.²⁴ Following this paradigm, the initial approach for the malaria elimination effort in western Cambodia included the deterministic and stochastic versions of **Model 1** presented in this thesis (**figures 3.3-2 and 3.3-3**).^{23, 49} A spatial framework is also currently under development. The clinical

and epidemiological data analyses also presented in this thesis (**Sections 3.1 and 3.2**) will be used to help parameterize this.

The importance of economics

No matter how informative a model is about the relative impact of different strategies, ultimately the choice of strategy for use on the ground will likely be heavily influenced by cost and affordability. Highly effective strategies for malaria elimination may be economically inefficient. Economics therefore must be included in the modelling to identify the most efficient mix of interventions. The models presented in this thesis are intended to form the basis of collaborative economic models which are now under development. These economic models fall outside the scope of this thesis. The potential role of modelling in guiding the economics of malaria elimination and what will be required for this to provide useful results has been explored in detail in a publication based on this section.⁵⁰

Conclusion

If the aim is for elimination and eradication as goals for malaria control worldwide^{21, 51} then it is necessary to learn from the mistakes and successes of the mid-20th century GMEP. There are many more powerful strategies available today and it is imperative that there is some way of identifying which are most likely to be successful in different epidemiological settings. Transmission dynamic mathematical modelling is essential to this. Whichever interventions are chosen need to be the most cost efficient possible. Enormous financial resources are being devoted to malaria control, vastly more than ever before, but funding is not unlimited. Successful elimination in some areas should help

reassure donors that investing in malaria is worthwhile and ensure that funding is sustained. Economic modelling will be an invaluable tool to aid in this prioritising process. The combined tool of transmission dynamic mathematical modelling plus sophisticated economic analysis will only be as good as the data on which it is based. As interventions are rolled out it is imperative that high-quality surveillance data are collected, collated and made widely available. The models will improve and increase in precision as a result. If malaria elimination and eradication can be shown to be feasible at a reasonable cost, this would be powerful motivation for policy-makers to remove the barriers to their implementation.⁵²

1.1.4 Artemisinins and artemisinin resistance

Artemisinin antimalarials: preserving the ‘magic bullet’

Development inside China

Discovery and Isolation

Qinghao (‘blue-green herb’) is the Chinese name for a relatively common plant otherwise known as *Artemisia annua* or sweet wormwood. It has been used as a remedy by Chinese herbalists for over 2000 years. The earliest known record was in the book “52 Prescriptions”, discovered in the Mawangdui tomb of the Han Dynasty in 168 B.C., where it was first described for the treatment of haemorrhoids.^{53, 54} The first treatment with qinghao of disease resembling malaria was described around the second century AD when Zhang Ji, in his text “On Cold Damage”, recommended treating ‘fevers with sweating and jaundice’ with a mixture containing boiled qinghao.⁵⁵ Around 340AD, in Hong Ge’s Handbook of Prescriptions for Emergency Treatment, a cold extraction method of qinghao was described for the treatment of intermittent fevers.⁵⁴ Qinghao has also been mentioned in several later standard Chinese materia medica texts as treatment for fever,^{53, 56} and has also been used for lice, wounds, boils, sores and convulsions.⁵⁷ It has remained in common use in Chinese herbal medicine⁵⁸ up to the present day.

Formal investigations of the antimalarial properties of qinghao came as a direct result of the Vietnam War. In May 1967, Ho Chi Minh made a request to Zhou Enlai, the then Prime Minister of China, to help provide new treatments for malaria to reduce its already high mortality among North Vietnamese soldiers.⁵⁸ In response, the Chinese government

initiated a search for new antimalarial compounds in an initiative called “Office (or Project) 523”⁵⁸ involving groups of scientists throughout China. This involved a systematic examination of a large number of indigenous plants used in traditional Chinese remedies.⁵⁴

The breakthrough came in 1971 when a low temperature method, similar to Hong Ge’s but with diethyl ether in place of water, was used to produce a crude extract from qinghao.⁵⁷ This extract was subsequently shown to possess antimalarial activity in mice infected with *Plasmodium berghei*.⁵⁶ This was in contrast to the earlier finding of lack of efficacy of boiled qinghao. These results were first officially reported in March 1972.⁵⁷ Animal studies showed no evidence of toxicity in rats, cats or dogs when administered in high daily doses for up to 7 days.⁵³ The active ingredient of qinghao, was isolated in 1972,⁵⁶ as a colourless, needle-shaped crystal. In 1975, the name ‘qinghaosu’ (‘active principle of qinghao’) was chosen for this new compound along with the more Western sounding ‘artemisinin’, later changed to ‘artemisinin’.⁵⁴ The molecular formula for artemisinin ($C_{15}H_{22}O_5$), was derived from the results of high resolution mass spectrometry and elemental analysis, following which the structure was determined by spectral analysis, chemical reactions, and X-ray diffraction. It was shown to be a new type of sesquiterpene lactone with a peroxy-group.⁵³ By 1973, a method had been found to convert artemisinin to its more active metabolite, dihydroartemisinin (DHA),⁵⁷ and subsequently from DHA to a large number of other derivatives.⁵⁹ The endoperoxide moiety was found to be essential for antimalarial activity and substitutions at the lactone carbonyl group were found to increase potency.⁵⁶ Industrial replication of these processes, including reduction with sodium borohydride to convert artemisinin to DHA, provided

the first opportunity for the large scale production of artemisinin derivatives from plant materials. Two derivatives in particular attracted interest as they were more soluble and much more active than artemisinin; the oil soluble ether derivative artemether produced in Kunming and the water soluble hemiester derivative artesunate, produced in Guilin.⁵⁹ Complex methods have been described for the total synthesis of artemisinin and its derivatives⁶⁰ but these are not cost effective for large scale production⁵⁴ thus the artemisinins continue to be of plant origin.

Development as monotherapy

In 1972, 21 patients with *P. falciparum* and *vivax* malaria were successfully treated using the diethyl ether extract of qinghao in Beijing.⁵⁷ Different extractions of qinghao were used successfully from 1974-8 in a large multicentre trial of 2099 patients with malaria (1511 of these had *P. vivax* and 558 *P. falciparum*, including 143 with chloroquine resistant, and 141 with cerebral, malaria).⁵³ Artemisinin produced a rapid clinical cure (clearance of fever and asexual parasites in the blood) in almost all patients without obvious toxicity, and a faster parasite clearance than quinine in cerebral malaria. However, there were high recrudescence rates of around 10-25%.

It was not until 1979 that Chinese scientists first published clinical findings for the artemisinin antimalarials in an international scientific journal, the Chinese Medical Journal, in a paper entitled “antimalarial studies on qinghaosu”.⁵³ This paper summarized much of what was known about the chemistry, pharmacology and clinical efficacy of the artemisinins including the results of in vitro studies, animal studies and human trials, and was the first time this new group of antimalarials came to the attention of the West. At

that time, however, China was a relatively closed country and there was great scepticism in the West that a previously unknown natural remedy could be so effective.⁵⁸

Furthermore, manufacturers of artemisinin drugs in China did not meet international standards for Good Manufacturing Practice (GMP).⁶¹ Bodies such as the World Health Organization (WHO) were not sufficiently convinced by the available research results to recommend the artemisinins at that time for treatment of malaria.

Since 1979, a number of other Chinese clinical trials investigated the efficacy of oral, intramuscular and intravenous artesunate, intramuscular artemether and oral dihydroartemisinin. Very little of this trial data has been published outside of China, one exception being studies by a group in Guangzhou. In total, artemisinins were administered to 2150 cases with *P. falciparum* (including 91 with severe disease), and 105 cases of *P. vivax* malaria. These compounds were found to be rapidly effective with recrudescence rates of 5-10% following 5 to 7 day treatment courses and there was little evidence of toxicity.⁶²

In October 1981, at The Fourth Meeting of the Scientific Working Group on the Chemotherapy of Malaria of the WHO, in Beijing, Chinese researchers summarized the research results to date on the antimalarial qinghaosu and its derivatives. The WHO representatives were very impressed by what they heard and approached the Chinese government for samples of the plant and details of the extraction techniques to verify the Chinese findings. However the Chinese representatives were reluctant to share these details and further collaboration did not occur at that time.⁶³

Development outside China

Confirmation and toxicity studies

As a result of the caution expressed by the Chinese, a group of scientists from the U.S. army's Division of Experimental Therapeutics, Walter Reed Army Institute of Research (WRAIR), began a search for the plant in the USA. They attempted to replicate the extraction of artemisinin from *Artemisia annua* serendipitously discovered growing in Virginia and near Washington DC, supplemented by material from the US National Arboretum. In 1984 they finally succeeded and published their findings, including its extraction with petroleum ether, chromatographic separation and potent antimalarial activity against chloroquine sensitive and resistant strains of *P. falciparum in vitro*.⁶⁴ In the following years, much of the earlier Chinese work was replicated and the remarkably high efficacy of the artemisinins against malaria *in vivo* was confirmed. In Thailand and Vietnam, between 1987 and 1994, a number of studies also confirmed the Chinese findings of high efficacy and low toxicity.^{65, 66} However, a series of animal experiments by another group at the WRAIR, using artemotil (arteether) and artemether and published in 1994, showed that these drugs could cause fatal neurotoxicity in high dose, causing brain damage in rats and dogs.⁶⁷ This finding caused some organizations to lose interest in these compounds. However, this neurotoxicity has since been shown not to occur with the water soluble derivatives, such as artesunate, and no correlate of the animal pathology has been reliably demonstrated in humans.⁶⁸

Treatment of severe disease

Parenteral artemether has to be given by intramuscular injection as it is soluble in oil but not in water. Its bioavailability is thus more variable than intravenous artesunate due to its

slow and erratic absorption and it is perhaps not surprising that it is a less effective antimalarial, particularly in severely ill patients. However, several randomized trials in the 1990s and early 2000s showed it to be equally efficacious to intravenous quinine,⁶⁹ although a lack of superiority over quinine hindered its further development.

Due to its solubility in water, artesunate is the only compound that is suitable for intravenous injection for use in patients with severe disease. In 2005, the results of a large multinational antimalarial treatment trial of severe malaria were published, the SEAQUAMAT study.⁷⁰ This was a randomized trial in predominantly Asian adults of intravenous quinine versus intravenous artesunate. This trial found a 34.7% lower mortality in patients who received artesunate rather than quinine. As a result, the 2006 WHO Guidelines⁷¹ were changed to include, for the first time, intravenous artesunate as recommended first line treatment for severe malaria. This replaced quinine for low transmission settings and was recommended as an alternative to quinine for children in high transmission settings. Initially, due to concerns about possible toxicity in humans, based on the previous animal studies, the dose of intravenous artesunate was kept low but in this guideline it was doubled to 2.4mg/kg. There was no evidence of neurotoxicity in the SEAQUAMAT study and a subsequent detailed study showed no effect of intravenous artesunate on the heart.⁷² AQUAMAT, a subsequent multicentre trial based on the SEAQUAMAT design in African children found a 22% reduction in mortality with artesunate versus quinine.⁷³ This led to intravenous artesunate being recommended first line for severe malaria worldwide in the 2010 WHO malaria treatment guidelines.²

Rectal preparations of artemisinins are also available for situations where parenteral therapy is not immediately available; a single dose of prereferral rectal artesunate has recently been shown to reduce mortality when access to injections will take several hours.⁷⁴

Combination therapy

Two disadvantages of the artemisinins when used as monotherapy are the relatively high recrudescence rate of around 10% and the need for a 7 day course (with associated poor compliance) in order to achieve radical cure.⁶⁸ One solution to these problems is to use the drug in combination with a second antimalarial, the so-called artemisinin combination therapies (ACT). With ACT, the duration of treatment is only 3 days and the exposure of parasites to artemisinin monotherapy is minimized, reducing the likelihood of artemisinin resistance arising.

Because of spreading mefloquine resistance around the Thai-Burmese border area, artesunate-mefloquine ACT was introduced as a replacement for mefloquine monotherapy in Thailand in the early 1990s.⁷⁵ This was the first large scale use of an ACT in the field. A series of clinical studies showed this new regime to have extremely high cure rates, to have successfully halted the spread of mefloquine resistance and reduced the incidence of malaria.⁷⁶ It continues to be used there today as two separately formulated medications and a fixed dose combination has recently become available.

Chinese researchers at the Beijing Academy of Military Medical Sciences in the 1980s combined oral artemether with another drug they had developed, benflumetol

(lumefantrine), as a treatment for non-severe malaria. In 1990, Chinese officials came to an agreement with Swiss pharmaceutical company Novartis (then Ciba-Geigy) to work together to develop, test and manufacture this artemisinin combination therapy, later called Coartem. Novartis assisted their Chinese partners to redesign local production facilities, upgrade their quality assurance systems and construct new factories to ensure compliance with GMP standards. Novartis acquired the rights to market the therapy outside China in 1994 although Chinese companies continue to supply the raw material for the drug to Novartis, and China continues to hold a domestic patent for the therapy. Today, Coartem is produced by Novartis in China and the US and is the most widely used artemisinin combination worldwide. FDA approval was given for use of artemether-lumefantrine in the USA in April 2009. This ACT has the advantage of being one of few coformulated ACTs to be produced to international GMP standards and it is therefore on the WHO list of prequalified medications. Coformulation means both drugs are in the same tablet and this prevents patients from taking only the artemisinin component to avoid side effects from the partner drug, a particular problem with artesunate-mefloquine. The other major coformulated ACT is dihydroartemisin-piperaquine (DHA-PQP) and this has been shown to be a safe, well tolerated and highly effective treatment of *P.*

falciparum malaria in Asia and Africa.⁷⁷ DHA-PQP has not yet been confirmed to be manufactured in accordance with GMP standards, although prequalification is currently underway. Hence, artemether-lumefantrine remains the drug of choice recommended by the WHO for uncomplicated malaria.

Together with their partners, the Drugs for Neglected Diseases initiative have recently developed two other coformulated ACTs, artesunate-amodiaquine (ASAQ/Coarsucam)

and artesunate-mefloquine (ASMQ). ASAQ was introduced in 2007 and joined artemether-lumefantrine as the only other WHO prequalified ACT. It has the advantage of once a day administration and is now available in 24 countries in sub-Saharan Africa. ASMQ was first used in Brazil in 2008 and it is being rolled out to other countries in South America and Southeast Asia over the next few years.

Oral ACTs have been shown in a number of clinical trials over the past decade to be the most effective antimalarials for uncomplicated disease.⁷⁸⁻⁸⁵ A large body of clinical and trial experience has also reassured the international community of the very low toxicity of the artemisinins. Because of concerns about resistance having arisen to all major classes of antimalarials other than the artemisinins and a wish to preserve the effectiveness of the artemisinins, the WHO chose to switch policy in 2004 to recommend ACTs as treatment for malaria in areas where resistance to antimalarial monotherapies had arisen (chloroquine, sulfadoxine/pyrimethamine and amodiaquine).⁸⁶ In 2006, they became recommended first-line treatment for uncomplicated *P. falciparum* malaria worldwide.⁷¹

Availability of artemisinins

By May 2008 only 11 countries had not yet adopted ACT for first-line treatment of uncomplicated disease due to *P. falciparum*.⁸⁷ Unfortunately, there are still few factories approved to international GMP standards which can manufacture ACTs so that demand continues to exceed supply. There have also been problems with falsely inflated purchase costs, fake artemisinins in Asia^{88, 89} and Africa and poor quality control within certain batches of drugs. In addition, available supplies of these drugs have been insufficient to meet demand in the developing world thus market prices of ACTs are many times higher

than the now almost useless chloroquine and sulfadoxine-pyrimethamine.⁹⁰ New initiatives by the Global Fund, WHO, Gates Foundation and Roll Back Malaria, among others, to encourage the increase in production and subsidise costs are helping to remedy this situation. There are also a number of new synthetic artemisinin derivatives and ACTs currently in development.⁹¹

The vast majority of intravenous artesunate is still produced in China and Vietnam and it is not widely available elsewhere.⁹² The major hurdle has been that none of the factories in these countries were GMP-certified and thus could not be licensed by many national bodies.⁹² It has also been difficult to secure sufficient quantity of supply. Only Brazil, China, Iran, Lao, Myanmar, Papua New Guinea, Solomon Islands, Thailand and Vietnam have so far adopted artesunate as national treatment policy for severe malaria.⁸⁷ In 2006, intravenous artesunate replaced quinine as first line treatment for severe disease in the national Therapeutic Guidelines of Australia, where it remains unlicensed but is available under a Special Access Scheme.⁹² It is also available on a named patient basis in the UK and USA. In November 2010, intravenous artesunate from the Guilin Pharmaceutical Co. Ltd. in China became fully GMP certified⁹³ and production is rapidly being scaled up.

Artemisinin resistance

Antimalarial resistance can be detected as reduced *in vivo* or *in vitro* responses, or via use of molecular markers proven to mediate drug resistance. Because artemisinins are used predominantly in combination with other drugs (as ACTs), measures of parasite clearance are more sensitive markers of reduced susceptibility than recrudescence rates (which are influenced strongly by resistance to the partner drug). Until recently, there has been no

evidence for a significant reduction in artemisinin efficacy at either clinical or *in vitro* levels. In broad terms, the rapid antimalarial action of artemisinins, combined with their pharmacokinetic properties (with terminal elimination half-lives in the order a few hours) led to the belief that resistance to artemisinins would be very slow to develop since exposure of parasites to sub therapeutic levels of drug would be very brief. However, in 2006, the WHO issued an ultimatum to the pharmaceutical industry to stop selling artemisinins as monotherapy to reduce the risk of resistance arising and increase the useful lifespan of this class of antimalarials.⁹⁴ In fact artemisinins had already been widely available as monotherapy for many years in a few countries. In Cambodia, artemisinin monotherapies were first introduced from China over 30 years ago.⁶⁸ For most of the period since then, they have been available through the private sector in sub therapeutic doses due to inappropriately abbreviated treatment courses,⁹⁵ poor quality manufacture or combination with fake drugs.⁸⁹ Thus many malaria sufferers in Cambodia received artemisinin monotherapies that failed to cure their infection. This combination of continuously multiplying parasites in the presence of non-lethal drug levels produces ideal conditions for the generation of artemisinin resistance.^{23, 96, 97} Cambodia was one of the first countries to switch first line treatment to ACT in national antimalarial drug policy (artesunate-mefloquine in 2000)⁹⁵, and a blanket ban on the use of artemisinin monotherapies has recently been introduced there.

Since 2003, there have been isolated reports of high failure rates and reduced *in vitro* responses to artemisinins in parts of Asia.⁹⁸⁻¹⁰² In December 2008, it was reported that there were prolonged parasite clearance times, despite adequate drug levels, in 2 out of 60 cases of falciparum malaria in a study in Cambodia.¹⁰³ This was followed in mid-2009 by

a detailed report describing markedly prolonged time to parasite clearance in patients treated with artesunate in Pailin, Cambodia.¹⁰⁴ The reduced parasitological response could not be explained by pharmacokinetic or other host factors. The most likely explanation for this very concerning finding is infection with *P. falciparum* that is to some degree resistant to artesunate. Inappropriate use of artemisinin monotherapies is probably at least partly responsible for this resistance having arisen first in Cambodia, particularly as they have been available for so long there. This practice is not unique to Cambodia,¹⁰⁵ however, and the lack of any robust signs of resistance elsewhere suggests that there are likely to be other contributory factors. If no action is taken, it is likely that this mildly resistant phenotype will become increasingly resistant and ultimately spread elsewhere. Previously, chloroquine and sulfadoxine pyrimethamine resistant malaria first arose in Cambodia and subsequently spread across the world.^{106, 107} With the on-going increase in ease of travelling between distant places, the potential for rapid spread of such phenotypes is greater now than it has ever been. By negating the hugely beneficial contribution of artemisinin combination therapies, artemisinin resistant malaria, wherever it appears, would be a disaster for malaria control and elimination efforts. In 2012, it was shown that sensitivity to artemisinins in northwest Thailand is gradually falling,¹⁰⁸ although far less than has occurred in western Cambodia. Genetic analysis of the resistant parasites in Thailand and Cambodia indicate that the Thai resistance may have emerged independently and is not as a result of spread.¹⁰⁸ Other countries using artemisinins for malaria therapy are also at potential risk of spread and acquisition of resistance, particularly those which receive migrants from at risk populations in western Cambodia and northwest Thailand including Myanmar and Bangladesh. A large multinational study

is underway across Asia and Africa to determine the current extent of artemisinin resistance.¹⁰⁹

Molecular detection of artemisinin resistance has remained compromised by the fact that the mechanism of action of this group of compounds is still not well understood. The organellar location of their antimalarial action, and the physical nature of their target and how they interact with it, represent some of the greatest controversies in current malaria biology.¹¹⁰ Amplification of the *P. falciparum* multidrug resistance gene *Pfmdr1* is associated with increased IC₅₀ values for artemisinins¹¹¹ but the effect is small and is clearly not associated with reduced *in vivo* susceptibility; parasite clearance rates remain unchanged in parasites with multiple copies of *Pfmdr1*.

Genetic studies in Cambodia, Thailand and Laos have identified genome regions associated with artemisinin resistance¹¹² but a specific molecular marker remains elusive. In the absence of robust molecular markers of artemisinin resistance, parasite clearance half-lives are being used as a marker of sensitivity to screen populations for early signs of developing resistance.¹¹³ One such analysis for Bangladesh is presented in **Chapters 2.2.2 and 3.2.2**.

Preserving the magic bullet

In order to avoid the doomsday scenario of global spread of artemisinin resistance there must be urgent intervention in western Cambodia, northwest Thailand and western Myanmar. In response to the preliminary results from Pailin and elsewhere, the WHO convened a series of meetings to discuss how to proceed. It was agreed that containment

of artemisinin resistance in Cambodia would involve removing selection pressure and reducing and ultimately eliminating falciparum malaria in western Cambodia. Strategies being considered include prohibiting the use of artemisinin monotherapy and introducing an alternative, such as dihydroartemisinin-piperaquine or atovaquone-proguanil, as treatment or in a mass screen and treat campaign, in combination with insecticide treated bed nets.^{87, 114, 115} In the absence of sufficient data and time in which to collect it mathematical modelling is being employed to help inform these decisions.^{18, 23} The initial models for this effort and the required data collection and analyses for model development for Cambodia and Bangladesh form the basis of this thesis.

Conclusion

Currently there is a paucity of promising novel antimalarial drugs under development and a loss of the artemisinins to resistance would be a disaster for international malaria control. With the most effective and least toxic antimalarial known still in the arsenal, in combination with far more effective vector control measures than ever before, there is currently the best opportunity in history to eliminate, and hopefully eradicate, malaria. It would be a tragedy if this opportunity were squandered by failure to preserve the artemisinins as a 'magic bullet' against malaria.

1.1.5 Malaria in Cambodia

Cambodia is a tropical country of 14,310,000 people in Southeast Asia.¹¹⁶ It shares borders with Thailand, Laos and Vietnam. It consists of 24 Provinces, each divided into municipalities and districts, each further divided into communes. The capital city is Phnom Penh. 19.5% of the population is urban.¹¹⁷ The Gross National Income (GNI) per capita is \$830.¹¹⁶

It has an area of 69,898 square miles and a tropical monsoon climate. There are two seasons, the wet (May to October) and the dry (November to April). Central Cambodia is a densely populated low-lying plain which contains a large lake, Tonle Sap, and part of the Mekong River delta. Much of the rest of the country consists of thinly forested upland plains with high plateaus and mountains in the southwest and east (**figure 1.1-10**).



Figure 1.1-10. Typical rural dwelling in Cambodia.

Healthcare in Cambodia is provided through government health centres (**figure 1.1-11**) and hospitals. There is also a large private sector including informal vendors, pharmacies, clinics and hospitals.



Figure 1.1-11. Government Health Centre in Siem Reap Province, Cambodia.

Malaria in Cambodia is widespread across the country, mostly in and around forests. The principal vectors being *Anopheles dirus*, *minimus* and *maculatus*. These species prefer hilly or forested areas and bite mostly during the evening and night. Diagnosis and treatment of malaria in Cambodia in remote areas has been traditionally mostly through informal and private healthcare providers.¹¹⁸ Since 2001, the Cambodia government have rolled out Village Malaria Workers across the areas with highest malaria incidence. These are respected villagers who are given specific training and equipment for diagnosis using RDTs (**figure 1.1-12**), treatment of malaria as per national guidelines and education of villagers.¹¹⁹ This system has also allowed the collection of malaria data by passive

surveillance giving a clearer picture of the burden of disease. Malaria control in Cambodia, including the village malaria workers (VMWs), is coordinated by the Cambodia National Malaria Control Programme (CNM).



Figure 1.1-12. Boy being tested for malaria by a Village Malaria Worker, Siem Reap Province, Cambodia. An RDT is being used.

1.1.6 Malaria in Bangladesh

Bangladesh is a country of 150,500,000 people in South Asia. It shares borders with India and Myanmar. It consists of 7 Divisions, each divided into districts, and each further divided into sub districts or thana. The capital city is Dhaka. 19.1% of the population is urban.¹²⁰ The Gross National Income (GNI) per capita is \$770.¹²¹

It has an area of 147,569 square miles¹²⁰ and a tropical monsoon climate. There are six seasons, which can be divided into the wet (June to September) and the dry (October to May). Much of Bangladesh is a flood plain of the Ganges river delta. In the southeast is a forested hilly area, the Chittagong Hill Tracts (**figure 1.1-13**).



Figure 1.1-13. View of typical terrain in the north of the Chittagong Hill Tracts.

Healthcare in Bangladesh is provided through government health centres (**figure 1.1-14**) and hospitals. There is also a private sector including pharmacies, clinics and hospitals.



Figure 1.1-14. Rural Health Centre in Khagrachari District, Bangladesh.

Malaria in Bangladesh is almost exclusively in the north and east along the Indian border. Over 80 % is in the Chittagong Hill Tracts. Transmission is mostly in forests and forest fringes. The principal vectors are *Anopheles stephensi*, *dirus* and *minimus*. Much of the healthcare in remote areas of Bangladesh is provided by non-governmental organizations. Government data on malaria cases in Bangladesh are incomplete. Malaria control in Bangladesh is coordinated by the National Malaria Control Programme (NMCP).

Bangladesh shares a porous border with Myanmar and Chittagong Division is host to large numbers of refugees from that country. The high malaria prevalence and unregulated use of antimalarials in Myanmar together with population migration with Thailand and Cambodia have led to concerns of potential acquisition and spread of antimalarial drug resistance, including artemisinin resistance, through Myanmar into Bangladesh.

1.2 Introduction to the analyses of malaria epidemiology

1.2.1 Cambodia

Epidemiological data on malaria over time are essential to planning of malaria control and elimination. In Cambodia, the control and elimination of malaria has garnered much attention due the recent discovery of artemisinin resistance in Pailin Province on the Thai border.¹²² This area has historically been the source of resistance to other antimalarial drugs and if artemisinin resistance were also to spread it would be a major threat to malaria control and elimination efforts worldwide.^{123, 124} Mathematical modelling has been employed to predict the most effective interventions to try to eliminate artemisinin resistant malaria.^{23, 49} The modelling predictions are heavily dependent on accurate information on baseline malaria epidemiology.

Since 2004, CNM has used a standardized system for collecting monthly data on malaria cases across the whole country into a centralized database, the Health Information System (HIS). This includes clinical cases in each province who attend public sector health facilities. In parallel, a system of VMWs has been rolled out across the areas of the country with highest malaria prevalence. These provide diagnosis and treatment at the village level and report their activities to CNM. Between these two sources a detailed picture of malaria in Cambodia over time can be constructed.

The first epidemiological study analysed data collected through HIS and the VMW systems to produce a picture of malaria trends over time in Cambodia.

1.2.2 Bangladesh

Detailed and accurate epidemiological data on malaria in much of Bangladesh are sparse and the true burden of disease is unknown. This hampers planning of malaria control activities. Over a million suspected cases of malaria are reported annually to the WHO in Bangladesh, although the number of confirmed cases is only 60,000, and estimates of the true incidence vary widely.¹²⁵⁻¹²⁷ Cases from most medical college hospitals, specialized hospitals, NGO hospitals and private clinics and hospitals are not included and the true number is estimated to be three times higher.^{125, 128} Most malaria cases in Bangladesh did not have a confirmatory blood test until recently and clinical diagnosis of malaria is known to be unreliable.^{129, 130} Since 2000, no clear decrease in the overall annual number of cases has been documented, but this could be related to counting methods.^{126, 127, 129, 131} From 2006–2008, the number of people tested for malaria more than doubled,¹²⁹ but despite this, in 2008 only a third of 1.3 million reported suspected cases had a blood test for malaria (microscopy or rapid diagnostic test), one fifth of which (85,000) were positive.¹²⁷ The true incidence of malaria is thus probably in the range of 150,000–250,000 cases per year.^{125, 127, 128}

It is likely that an increase in the efficiency of diagnosis and reporting has masked a decline in incidence. Access to ACT has doubled since 2005¹²⁷ and annual reported numbers of confirmed deaths from malaria has decreased 10-fold since 2005 to 47 in 2009.¹²⁹ It should be noted that these figures do not include deaths in many hospitals and private facilities or those that do not reach health care and the true figure is likely to be much larger.¹²⁸

Published data on geographical distribution of malaria cases in Bangladesh are patchy and incomplete. Under-reporting is thought to be a particular problem in remote areas.¹³² The first national malaria prevalence survey in Bangladesh was undertaken in 2007. Over 85% of all cases and 95% of severe cases were reported to occur in the south of Chittagong Division, in 5 Districts: Bandarban, Chittagong, Cox's Bazar, Khagrachari and Rangamati (**figure 1.2-1**).^{132, 133} Malaria transmission in this area is highly seasonal with an estimated annual rate of infection of around 8 per 1000 people at risk.^{125, 134} The highest rates of malaria transmission are thought to be in the Chittagong Hill Tracts (CHT) i.e. Bandarban, Khagrachari and Rangamati Districts which together account for 80% of cases.¹³¹⁻¹³³ These have very low population density and are inland, mostly forested hilly areas.

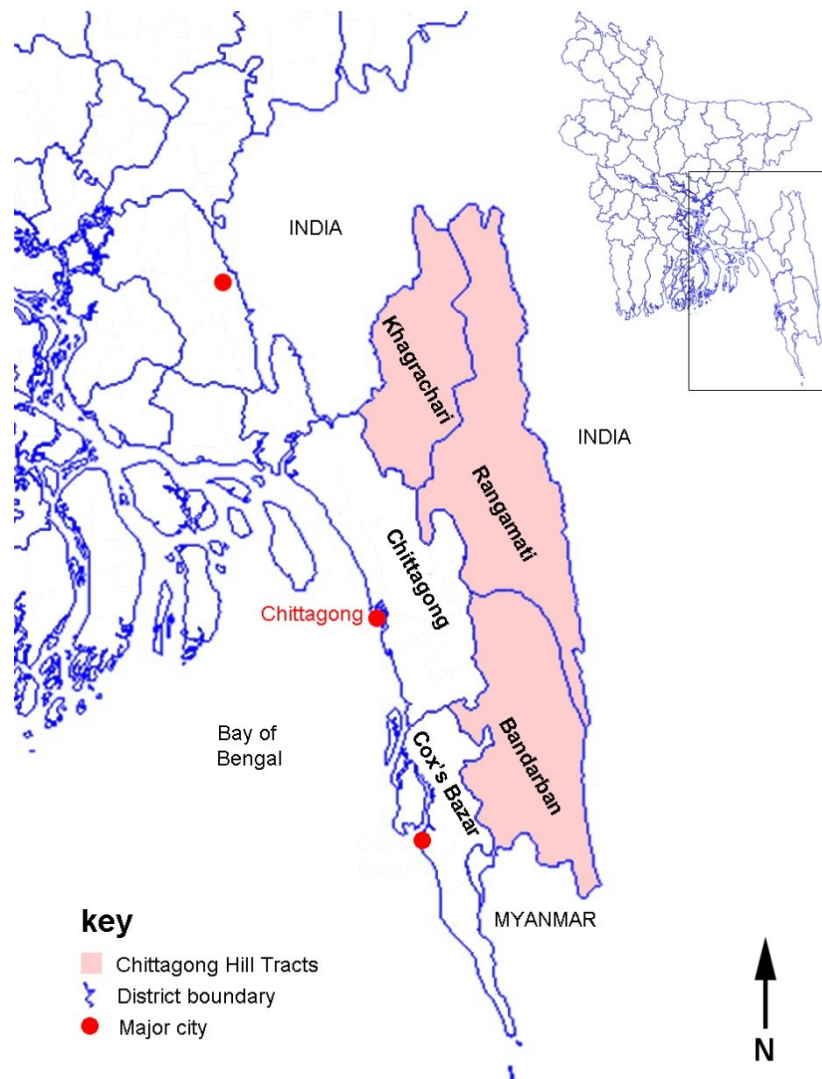


Figure 1.2-1. Map of Chittagong division.

1.2.2.1 Khagrachari

Malaria control is particularly challenging in hilly forest areas. Khagrachari District, the northern third of the Hill Tracts is thought to have the highest malaria prevalence in the country.¹³³ With a mostly rural population of 613,917,¹²⁰ much of Khagrachari District is forested with limited road access to outlying villages. Numbers of people tested and positive for malaria are collected by the Government of Bangladesh from government and non-governmental organization health facilities. These data from Khagrachari were

collected and analysed. The aims were to determine the groups most affected by malaria and to identify trends in malaria incidence over time.

1.2.2.2 Chittagong

Accurate figures for numbers of cases of severe malaria in Bangladesh are not readily available. An exception is a WHO report from 2006 reporting 513 case fatalities out of a total of 51,705 confirmed falciparum malaria cases, of which 3539 were severe.¹³² It was estimated that there were 2200-12,000 actual fatal cases in that year and 1,108,000-6,677,000 total cases of all species malaria in Bangladesh in 2006.¹²⁶ A later WHO report stated there were 1,320,581 reported cases in 2006.¹²⁷ With a case fatality rate in severe malaria of 15-20%,² the number of fatal cases suggests a total of 11,000-80,000 severe malaria cases in that year. More recently, a total number of 3591 cases of severe malaria cases were counted between July 2008 and May 2009.¹³¹ There are no published data on long-term trends and very little on the geographical distribution of severe malaria in Bangladesh.¹³¹

Mortality from severe malaria can be greatly reduced by intravenous antimalarials (particularly artesunate) and high quality supportive care.² In Bangladesh, intravenous antimalarials are widely available in local hospitals but access to advanced supportive care (e.g. blood transfusion, mechanical ventilation and renal dialysis) is much more limited. Recommended practice in Bangladesh is for all severe cases of malaria to be treated in hospital.¹³⁵ Numbers of malaria admissions may thus be a useful surrogate in the absence of reliable data on severe disease. Numbers of patients with malaria admitted

to hospital in Bangladesh have been published since 2007. There were 5678 such admissions reported in 2007, 3042 in 2008 and 3287 in 2009.¹²⁹ This is around half of the lowest estimate for the annual number of severe cases (above) and very few of these would have had access to tertiary level care. There is thus likely to be a large burden of patients who do not reach hospital, representing potentially preventable mortality. In order to improve care for people with severe malaria in Bangladesh it is important to describe this population in more detail.

The third study was undertaken using routinely collected malaria screening data from the past 13 years from Chittagong Medical College Hospital (CMCH), Bangladesh to investigate the patterns of referral of patients, temporal trends and geographical distribution of severe malaria in Chittagong Division.

1.3 Introduction to parasite clearance

1.3.1 Parasite clearance rates in Cambodia and Thailand

Drug response dynamics and recrudescence in artemisinin-resistant *Plasmodium falciparum* malaria

In Pailin, western Cambodia in 2007, the existence of artemisinin resistant *P. falciparum* malaria was confirmed for the first time.¹⁰⁴ The control group were patients with artemisinin sensitive *P. falciparum* from Wang Pha, northwest Thailand. The artemisinin resistant phenotype was prolonged parasite clearance times and a high recrudescence rate in those receiving artesunate monotherapy of 6/20 (30%). The artemisinins are recommended first line therapy for uncomplicated and adult severe malaria worldwide.¹³⁶ If artemisinin resistance increases in severity and is allowed to spread, as occurred with chloroquine, this could be disastrous for malaria control and elimination efforts worldwide. High recrudescence rates are particularly worrying as they make malaria control and elimination more difficult. Currently understanding of artemisinin resistant *P. falciparum* malaria is limited. To be best equipped to deal with it, urgent and detailed study of artemisinin resistance is a high priority on the malaria research agenda and numerous clinical and laboratory studies are underway.¹³⁷ Immediate action is needed to control and ideally eliminate artemisinin resistant malaria¹³⁷ and it is not clear what the best strategy would be to do this.

Mathematical models are currently being developed to use these data as they become available to help plan the optimal strategies to deal with artemisinin resistance.^{23, 138} For

these to give realistic and usable predictions, it is crucial to have an in-depth understanding of the behaviour of the artemisinin resistant phenotype *in vivo* and how this differs from artemisinin sensitive infections. In particular, it is not understood what happens to parasites *in vivo* below the limit of microscopic detection in peripheral blood and why this sometimes results in recrudescence. The relative fitness of artemisinin resistant versus sensitive parasites is also unknown. To help with this, the available parasite clearance data from the 2007 study in Pailin and Thailand,¹⁰⁴ together with some new data from on-going studies in Pailin from 2008 to 2010 were collated, analysed and modelled to attempt to gain some additional insights into the biology of artemisinin resistance. Aims were 1) to determine how parasite clearance behaviour of artemisinin resistant *P. falciparum* infections differs from artemisinin sensitive infections, 2) to explore the differences in parasite clearance behaviour between recrudescence and non-recrudescence infections, 3) to estimate a fitness cost for artemisinin resistant parasites, and 4) to generate and explore hypotheses for possible parasite dynamics that may result in recrudescence after treatment with artesunate.

1.3.2 Parasite clearance slope half-lives in severe malaria in Bangladesh

Evaluation of the *in vivo* responsiveness of *P. falciparum* to intravenous artesunate

Intravenous artesunate is now recommended first line treatment for severe *P. falciparum* malaria worldwide in all age groups.^{70, 73, 139} However, artemisinin resistance has been identified in western Cambodia and falling sensitivity in northwest Thailand and may already have spread elsewhere.¹⁰⁴ This could severely compromise the efficacy of

intravenous artesunate for severe malaria and pre-referral treatment with rectal artesunate, both currently being rolled out worldwide. Unfortunately, there is no reliable *in vitro* test which can identify the artemisinin resistant phenotype. Reliance is thus placed upon clinical studies and changes in parasite clearance in treated patients.^{104, 140} This is problematic as a number of factors influence the efficacy of antimalarials in an individual, of which resistance is only one. Other important factors include failure to take medication as prescribed, incorrect dosing, malabsorption and vomiting. Many of these issues apply only to oral medications and are addressed in clinical studies by concomitant measurement of artemisinin pharmacokinetics. Such studies are difficult and expensive and therefore are usually small with limited power to detect resistance. Intravenous antimalarials administered in hospital for severe malaria should produce more consistent blood levels and therefore more consistent rates of parasite clearance than oral dosing.

Artemisinin resistance in Cambodia is thought to have evolved in an environment of underdosing with oral artemisinin antimalarials for over 30 years. It is not known when or how quickly it appeared, as no historical data are available and fortunately it is the only place where resistance has thus far been identified. In Wang Pha, northwest Thailand, there have been detailed studies using artemisinin combination therapies over many years. An analysis of the malaria database there found a gradual prolongation of the clearance half-life by artemisinins since 2002,¹⁴¹ although not to the extent found in Cambodia.

If artemisinin resistance has spread it is likely to have done so via population migration to nearby countries in Southeast Asia. High on the list are Myanmar and Bangladesh. Southeast Bangladesh is a malaria endemic area with a porous border with Myanmar.

Artemisinin combination therapies were introduced there as first line treatment in 2004 and are widely available. Its' main tertiary referral hospital for malaria, Chittagong Medical College Hospital, was also one of the first places to use intravenous artesunate on a large scale.⁷⁰ More recently, rectal artesunate was trialled in large numbers of cases in Chittagong Division.⁷⁴ Pathogenesis and treatment studies on severe malaria have been carried out using intravenous artesunate in Chittagong Medical College Hospital since 2003 during which time a detailed database of enrolled patients has been maintained.

Parasite clearance and clinical data from this database were analysed in order to assess the sensitivity of *P falciparum* malaria to intravenous artesunate in Chittagong, determine how it has changed over time and compare with previously collected parasite clearance data from Wang Pha, Thailand and Pailin, Cambodia. The aims were to determine if parasite clearance half-lives due to intravenous artesunate have changed in Bangladesh over the past 9 years, if there is any evidence of artemisinin resistance in Bangladesh and whether parasite clearance half-lives affect outcome.

1.4 Introduction to the mathematical models

The Thai-Cambodian border area is historically the source of the global diaspora of anti-malarial drug resistance. Resistance to chloroquine and sulphadoxine-pyrimethamine in *P. falciparum* originated there, spread across Asia and Africa, and caused millions of deaths.¹⁰⁷ The increase in malaria mortality is now being reversed where effective vector control measures and antimalarials, principally ACT, are being deployed.¹⁴² Current initiatives to eliminate malaria are critically dependent on their continued efficacy.

In the 2006, WHO Guidelines for the Treatment of Malaria⁷¹ ACT became the recommended first-line treatment for uncomplicated *P. falciparum* malaria in all endemic areas. Intravenous artesunate became the treatment of choice for severe malaria in adults, except for children in Africa (where studies were in progress).^{70, 71} These recommendations for the large-scale use of artemisinin derivatives were based on their excellent tolerability, safety, and reliable efficacy.

In 2009, new data from western Cambodia provided the first objective evidence that efficacy of this essential drug class may be declining.^{68, 104, 143} Cure rates with ACT were worse in this area than anywhere else. This was attributed initially to resistance to the partner drugs (mefloquine¹⁰¹ and lumefantrine¹⁰²), but subsequent detailed studies show that parasite clearance times following standard doses of artesunate in uncomplicated *falciparum* malaria were significantly longer than elsewhere in the world.^{68, 104, 143} Artemisinins had been available as monotherapies in western Cambodia for over 30 years in a variety of forms and doses, whereas in most countries, other than China where they

were discovered, they have been a relatively recent introduction.⁶⁸ The extended period of often sub-optimal use and the genetic background of parasites from this region have created a dangerous milieu.¹⁴⁴ If reduced *in-vivo* parasitological efficacy was the first sign of artemisinin resistance then immediate action was required to prevent the spread of these parasites elsewhere.¹⁰⁶ Loss of these drugs would be a disaster for global malaria control and elimination prospects as there are no obvious replacements emerging from the development pipeline in the near future.³ Fortunately malaria in western Cambodia can be considered as affecting a land island with no major contiguous connections to other malaria endemic areas.¹⁴⁵ The private sector was, and still is, the main source of anti-malarial drugs in Cambodia. Self-treatment with short (inadequate) courses of oral artemisinin monotherapies was common.¹¹⁸ Many other antimalarials were available, including artesunate-mefloquine (MAS3), the nationally recommended first-line treatment for falciparum malaria since 2000. Several different interventions to contain and ideally eliminate the threat of artemisinin resistance were under consideration by the national malaria control programmes, the WHO and the malaria community.¹⁴³

In 2009, the debate centred on a number of questions including:

- Which anti-malarial should be used in different situations? Under consideration were atovaquone plus proguanil (AP) and/or dihydroartemisinin and piperaquine, both given for three days, plus one “gametocytocidal” dose of primaquine (APP).
- Who should receive these treatments? a) symptomatic patients only b) anyone with a positive blood film for malaria (‘Mass Screening and Treatment’, MSAT) c) the general population regardless of symptoms or blood film positivity (‘Mass Drug Administration’, MDA).

- How long would an intervention need to be continued? Longer interventions are more costly and difficult to sustain. The cost-effectiveness of each proposed intervention and the consequences of premature cessation had not yet been quantified.
- What is the added value of insecticide-treated bed nets (ITNs)?

These choices had to be made urgently on the basis of available evidence to reduce the risk of resistance spreading westward. Containment beyond the western border of Thailand was thought to be impossible. There were insufficient data and insufficient time to undertake adequately powered clinical studies to inform these urgently needed decisions, so the relevant agencies were relying upon expert opinion. In response, **Model 1**, a mathematical model of malaria in Cambodia focusing on the population dynamics of artemisinin resistance and its control, was rapidly developed to aid decision making.¹⁸ The intention was for this model to inform ongoing policy discussions and for it to be refined and extended as more data became available.

Elimination of malaria from much of the world became the declared aim of the WHO in 2009²¹ and is currently being attempted or planned in many countries.¹⁴⁶ As the epidemiology of malaria varies widely, malaria elimination requires a variety of approaches individually optimized for different transmission settings. It is expensive and slow, or often impossible, to develop these approaches by trial and error in the field.⁵⁰ Mathematical modelling is a rapid, low cost means of using limited available data to compare large numbers of strategies and optimize their impact. It has great potential to help guide the efforts to achieve elimination.⁵⁰ In 2009, very little mechanistic modelling

of malaria elimination had been attempted.⁵⁰ One exception was **Model 1** which had been developed for malaria elimination in the context of newly discovered artemisinin resistance in western Cambodia²³ and for which it was helping to guide planning. A major limiting factor for models of this type was that there were limited field data on which to base models of malaria elimination using modern methods.

In 2010, the results of a large field study of malaria elimination using antimalarial drugs (termed ‘FEMSE’, Fast Elimination of Malaria by Source Eradication) in southwestern Cambodia from 2004-2007, were published. Using mass drug administration and treatment with both artemisinin-piperaquine and primaquine the study was successful in reducing substantially the prevalence of malaria parasite positive individuals in most of the 26 villages studied, with elimination in 7.²⁷ As in much of the region this was an area of low, unstable, seasonal, mostly forest fringe malaria transmission. The study was in two small areas of Kampot and Kampong Speu Provinces. The overall Annual Parasite Incidence (API) in 2004 in these provinces was around 6-8 confirmed cases per 1000 population per year.^{117, 147} The results of this field study were in broad agreement with findings from **Model 1** which showed that strategies that included high coverage of treatment with ACT can achieve large reductions over a similar timescale.²³ However, the malaria elimination field trial in southwest Cambodia employed multiple strategies both simultaneously and sequentially and it was not known to what extent each strategy contributed to the successful outcomes. The execution of the trial varied geographically. This variation in the strategies employed between different sites, together with frequent monitoring of parasite rates, provided a range of data which could be used for fitting and validation of a mathematical model designed to answer specific questions about the trial.

These questions included the relative impact of mass drug administration versus augmented coverage of routine treatment (Rx) and whether adjunctive primaquine (PQ) in a single gametocytocidal dose was a worthwhile addition to either.¹⁴⁸ In the trial, primaquine MDA was given in a dose of 9mg every 10 days for 6 months, an intervention that would be very resource intensive to replicate on a large scale. In two sub studies, an additional round of MDA was tried using ACT with single dose primaquine, one study at 42 days and another at 1 year. Large studies in the Comoros (32,519 subjects)¹⁴⁹ and Cambodia (28,143 subjects)¹⁵⁰ found mass administration of repeated low dose primaquine (9mg) to be safe and well tolerated. Elsewhere, larger gametocytocidal doses of primaquine have been used^{151, 152} and currently 0.75 mg/kg base as a single dose is recommended by the WHO.² In a single dose, primaquine is currently under consideration for mass deployment in Cambodia¹¹⁵ although there remains uncertainty over the optimum dose and benefit-risk ratio for this potentially haemolytic drug. Similarly a two day regimen was used for the ACT in the field trial as opposed to the more usual three days, also recommended by WHO.² The optimal dosing for these drugs is the subject of on-going study.

There was concern, as in other areas, that reducing malaria prevalence would reduce population level immunity and a failed attempt at elimination might result in a subsequent rebound increase in malaria morbidity and mortality. The studied intervention was for three years following which malaria control measures were relaxed. Although the study population was screened for parasitaemia every 6 months, numbers of clinical cases were not recorded and active surveillance was discontinued at the end of the trial so inferences about changing population level immunity could not be made.

It was also not known if artemisinin resistance was present in the area during the study and what impact this may have had on the effectiveness of these strategies, or how these strategies may have affected the spread of drug resistance.

It was particularly urgent and important to answer these questions as ACT-based strategies were under consideration for malaria elimination throughout Cambodia¹¹⁵ and in many similar countries worldwide and there was, and is, considerable on-going debate about the possible impact of artemisinin resistance²³ and the potential role of primaquine.^{50, 148}

Detailed data from the Cambodian field study were used in combination with a range of other studies to develop and validate **Model 2**. This model was used to answer a number of specific questions for the Cambodia National Malaria Control Programme to help with their planning of malaria elimination efforts. The broad aims were: 1. separate and quantitate the effects of the various components of the strategies used in the field study and predict their long-term impact; 2. explore interaction of these strategies with artemisinin resistance; and 3. design optimal elimination strategies. The results were distilled into five key implications for malaria elimination policy.

Later in 2010, the AQUAMAT study⁷³ was published. This demonstrated the superiority of intravenous artesunate over quinine in preventing death from severe falciparum malaria in African children. Consequently, the updated WHO malaria treatment guidelines published in 2010 included the new recommendations that intravenous

artesunate should be first-line treatment for severe *P. falciparum* malaria in all age groups worldwide.² Pre-hospital administration of rectal artesunate in the community is a simple, low cost intervention that had been shown in 2009 to reduce the risk of death or permanent disability in those whose access to antimalarials would otherwise be delayed by more than 6 hours.⁷⁴ In response, the 2010 WHO guidelines for treatment of malaria also recommend rectal artesunate for all patients with severe malaria or who cannot take antimalarials orally and who do not have access to intravenous or intramuscular antimalarials within 6 hours.² The intention was for all such patients to then be referred to a health centre or clinic to complete their treatment, preferably with intravenous artesunate.^{70, 73} As a consequence of these recommendations, efforts began to roll out these two treatments worldwide.

As intravenous and rectal artesunate become more widely available, the consequent and potentially unregulated large-scale use of these artemisinin monotherapies in the community prompted concerns about their potential impact on the spread of artemisinin resistance.¹⁵³⁻¹⁵⁵ In Cambodia, the initial appearance and spread of artemisinin resistance is likely to have been due to widespread use of oral artemisinin monotherapy over many years before the introduction of ACT, the current mainstay of malaria treatment worldwide.¹⁵⁶ Artemisinin resistance has also since been found to be present and spreading in northwest Thailand¹¹² and studies are on-going to determine if it is also now present in Bangladesh (TRAC & **Sections 2.2.2, 3.2.2 and 4.2.2** of this thesis). Artemisinin resistance is a major threat to malaria elimination efforts worldwide and any treatment strategy that promotes its' spread would not be desirable.

That rectal or intravenous artesunate will significantly promote the spread of artemisinin resistance is not clear. Rectal artesunate is targeted at a minority of patients with severe malaria and those who cannot tolerate oral antimalarials. Thus when compared to the entire population receiving antimalarials this group is small. Such patients should be referred for continuation of treatment to a health centre or clinic. Most of these will be cured of malaria, many of the remaining infections being fatal. If referral were not to occur, many of those with severe disease are likely to die within a few days. Thus a large proportion of those receiving rectal artesunate will have a short duration of parasitaemia which would greatly reduce on-going transmission of any resistant parasites. The same applies to intravenous artesunate which is predominantly used in health facilities in individuals with severe malaria, almost all of whom are either cured or die from their infection and are thus not a major source of transmission.

It is not known how spreading artemisinin resistance would impact the efficacy of artemisinin antimalarials for severe malaria. No cases of severe malaria with artemisinin resistant parasites have yet been reported, although fears of possible increased mortality have led to the proposed recommendation that such patients should receive dual therapy e.g. with intravenous artesunate plus quinine. The current artemisinin resistant phenotype in Cambodia is delayed parasite clearance, the clearance half-life in resistant infections being approximately double that of more sensitive infections in Thailand.¹⁰⁴ This slower clearance would prolong the viability of highly pathogenic parasites which could theoretically delay recovery increasing severe disease and potentially increasing mortality. It is possible that parasite clearance due to artesunate is still sufficiently rapid even in infections with the current artemisinin resistance phenotype that the difference in

severe disease or mortality is minimal. Indeed, parasite clearance half-lives are not different in fatal versus nonfatal severe malaria (**Section 3.2.2**) and the incidence of severe malaria has not increased with spreading artemisinin resistance in northwest Thailand.¹⁰⁸ Until sufficient cases of severe malaria with resistant parasites are studied in detail, this will not be known.

There were several questions that urgently required answers. 1) what is the likely impact of intravenous and rectal artesunate on malaria mortality in large populations? 2) what is the potential impact of rectal and intravenous artesunate on the spread of artemisinin resistance? 3) what is the potential effect of artemisinin resistance on the efficacy of rectal and intravenous artesunate for patients with malaria? 4) How will introduction of rectal and intravenous artesunate affect the feasibility of malaria elimination? Thus **Model 3** was developed to address these questions.

1.5 Summary

This thesis presents a series of epidemiological, clinical and mathematical studies of malaria in Cambodia and Bangladesh. They are part of an on-going collaborative effort to predict the optimal strategies for tackling the newly identified problem of artemisinin resistance. First confirmed in western Cambodia in 2009, artemisinin resistance was declared by the WHO as a public health emergency and a major threat to malaria elimination efforts worldwide. The mathematical models presented here were developed in collaboration with the National Malaria Control Programme of Cambodia and WHO as a direct and timely response to this emergency to help plan the strategies to tackle artemisinin resistance. Initially very few data on malaria epidemiology and artemisinin resistance in Cambodia and Bangladesh were available. These data were collected and analysed as part of this thesis (**Sections 2.1, 2.2, 3.1 and 3.2**). The data collection and analysis and mathematical modelling were thus done in parallel from 2009 to 2012, the data analysis acting as a growing resource for these and future models. **Figure 1.5.1** illustrates how the datasets and models in this thesis are interrelated.

The first model (**Model 1**) was produced with very limited data in response to the urgent need for preliminary results to help plan malaria control activities at the time. Subsequent models (**Models 2 and 3**) were able to incorporate more detail as the data became available and have become increasingly informative and specific. This project evolved during the course of the DPhil in response to the needs of policymakers at the time. The models produced have directly informed planning of malaria control and elimination activities for Cambodia and are being further developed to examine a range of additional

strategies as well as to incorporate economic models to determine the most cost effective strategies. Data analysis and mapping of malaria as part of this thesis are also contributing to parameterization of a spatial model of malaria elimination in Cambodia.

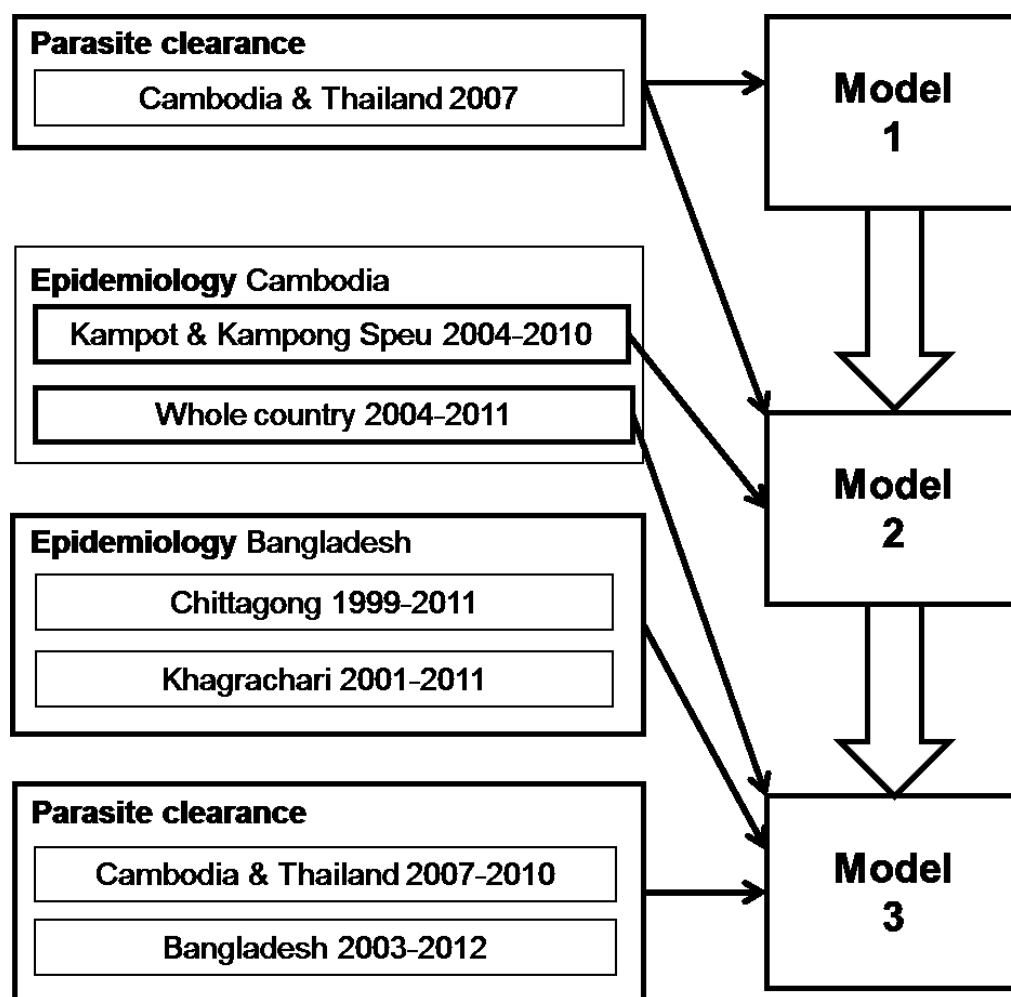


Figure 1.5-1. Summary of how the datasets and models in this thesis are interrelated.

On the left is a list of the datasets presented in **Sections 3.1 and 3.2** and on the right are the three models in order of development represented by open arrows. The thin arrows illustrate which datasets were used for which models.

1.6 Aims of this thesis

Primary aim:

To develop population level mathematical models of *P. falciparum* malaria treatment and artemisinin resistance in order to determine the optimal strategies for elimination of artemisinin resistant malaria in Cambodia and treatment of severe malaria in Cambodia and Bangladesh.

Secondary aims:

To collect and summarize available data on the epidemiology over time of malaria in Cambodia and the Chittagong Hill Tracts, Bangladesh and the epidemiology of severe malaria in Chittagong, Bangladesh.

To collect and summarise available data on the sensitivity of *P. falciparum* to artemisinin antimalarials in Cambodia and Bangladesh.

To derive parameters on malaria epidemiology and artemisinin sensitivity for population level mathematical models.

Chapter 2 : Methods

2.1 Malaria epidemiology

This section presents the methods for analysis of three data sets on the epidemiology, temporal trends and geographical distribution of malaria in Cambodia and Bangladesh to provide data with which to parameterise and validate mathematical models. The first analysis is of reported malaria cases in the whole of Cambodia broken down by OD from 2004 to 2011. The second is of reported malaria cases in Khagrachari District, Bangladesh from 2001 to 2011. The third analysis is of malaria cases admitted to hospital in Chittagong Medical College Hospital, Chittagong, Bangladesh from 1999 to 2011.

2.1.1 Cambodia

Data were extracted from the CNM HIS and VMW databases and annual reports on numbers of malaria cases from January 2004 to December 2011. Data on population and average rainfall in Cambodia were also collected.^{117, 157}

2.1.2 Bangladesh

2.1.2.1 Khagrachari

Monthly malaria screening data from all individuals tested for malaria at government and non-governmental organisation (NGO) health facilities in Khagrachari District, Bangladesh, from 2001 to 2011 were collected and analysed. Diagnosis was by smear microscopy supplemented from 2008 by HRP2 RDT. The level of detail collected increased during this period. From 2001 to 2006, only summary data on malaria positive individuals for the whole District were available. Numbers of negative individuals were

collected from 2005. From 2007 onwards, records were stratified by Thana (sub district) of residence, age group, gender and pregnancy. Rainfall data were provided by the Bangladesh Meteorological Department, Government of Bangladesh from Rangamati, as this is the nearest government weather station to Khagrachari. Data on population were taken from the 2001 and 2011 national census^{120, 158} and data on age distribution from the 2004 Sample Vital Registration Survey.¹⁵⁹

2.1.2.2 Chittagong

The study was conducted at CMCH, Chittagong, Bangladesh from January 1999 to December 2011. CMCH is a government-run 1000-bed teaching hospital and the main tertiary referral hospital for severe malaria in Chittagong Division. CMCH receives referrals from throughout southeast Bangladesh, particularly those severe cases who require more intensive management as it is the only government hospital in the south of Chittagong Division with facilities for intensive care and haemodialysis. Its patients are mostly in the lower income range. There is a high quality malaria diagnostic service on-site with a well-developed recording system for malaria screening results. As the malaria laboratory is readily accessible, being located next to the medical wards, and the test is free to the patients, most if not all patients with suspected malaria undergo testing through this facility. The vast majority of malaria patients admitted to CMCH have severe disease. The results thus provide a representative picture of severe malaria cases admitted to CMCH over time. Assuming referral patterns from outlying clinics and hospitals to CMCH have not changed significantly in this period, they may also give an indication of long-term trends of severe malaria across Chittagong Division.

Results of all screening of inpatients for malaria by the CMCH malaria diagnostic laboratory during this period were collated and analysed. Malaria diagnosis was by microscopy of thick and thin blood films. Date of testing, age, gender and smear results were collected for all malaria positive patients throughout, area of residence from 2002 (District 2002–2007, District and Thana (i.e. sub district) 2008–2011), and all of these from 2008 onwards for smear negative patients. In 2006–2011, additional data were collected from patients with malaria and their relatives to determine whether patients had travelled to another Thana in the 3 weeks before presentation. Admission GCS and outcome were also recorded during this period. Many of the malaria slide positive patients were enrolled in a series of clinical studies of severe malaria and a detailed clinical description will be presented elsewhere. Data on population density were taken from the 2001 national census¹⁵⁸ and data on age distribution from the 2004 Sample Vital Registration Survey.¹⁵⁹ Rainfall data for Chittagong were provided by the Bangladesh Meteorological Department, Government of Bangladesh.

Statistics

Statistical analyses were performed using Microsoft Excel 2010 (Redmond, WA, USA) and GraphPad Prism Version 5.04 (GraphPad Software Inc, La Jolla, CA, USA). Correlations were examined using Spearman R and significance was set at the 5% level. Maps were prepared using ARC GIS 10 (ESRI, Redlands, CA, USA) and Adobe Photoshop CS4 (Adobe Systems, San Jose, CA, USA).

2.2 Parasite clearance

This section describes the methods used for the analysis of available parasite clearance data due to artemisinins in Cambodia, Thailand and Bangladesh.

2.2.1 Parasite clearance rates in Cambodia & Thailand

Drug response dynamics and recrudescence in artemisinin-resistant *Plasmodium falciparum* malaria

Study sites and patients

The data used in this analysis were collected in 2007 to 2010 as part of clinical studies at Pailin Referral Hospital in Pailin, western Cambodia (120 patients) and in the Shoklo Malaria Research Unit (SMRU) clinic in Wang Pha, Mae Sot Tak Province in northwest Thailand (40 patients).¹⁰⁴ Malaria transmission is low and seasonal at these sites. The enrolment criteria are described in detail elsewhere.¹⁰⁴ All patients had slide-confirmed uncomplicated falciparum malaria¹³⁶ with parasitaemia above 10,000/ μ L. Patients with severe disease,¹⁶⁰ co-infection with another *Plasmodium* species on microscopy or antimalarial drug use in the 48 hours before presentation were excluded. Patients were treated with either 7 days or artesunate monotherapy (AS7) or 3 days of artesunate plus mefloquine (MAS3) on days 3 (15mg/kg) and 4 (10mg/kg). A range of doses of artesunate was used (2, 4, 6 or 8 mg/kg per day). Parasite asexual stage counts were measured on admission and 4, 8 and 12 hours thereafter and then 6 hourly until two consecutive slides were negative. Patients were followed up weekly until 63 days after enrolment and screened for possible recrudescence at each time point.

Recrudescences were confirmed by PCR comparing merozoite surface protein 1 and 2 and glutamate-rich protein genotypes in pre and post treatment samples.¹⁶¹

Pharmacokinetics

In 80 patients in the original studies, frequent samples were taken for determination of drug levels to assess the pharmacokinetics of artesunate and dihydroartemisinin up to 12 hours after the first dose. The procedure is fully described in the original paper.¹⁰⁴

Quantifying parasite clearance

Parasite count data were analysed using the World Wide Antimalarial Resistance Network (WWARN) Parasite Clearance Estimator (PCE).¹⁶² For each patient a parasite clearance rate was calculated from the linear portion of the log_e parasitaemia time profile during treatment with artesunate monotherapy. This is possible as parasite clearance in this part of the parasite clearance curve is a first order process. This method of quantifying parasite clearance has the advantage over parasite clearance times that it is independent of initial parasitaemia and is thus a better reflection of artemisinin sensitivity.

The procedure is described in detail elsewhere¹⁶³ and a summary is given below to facilitate understanding of the results. After removal of outlying data points, the WWARN PCE, the curve of log parasite count versus time was determined using a polynomial model. Tobit regression models were used to account for parasitaemias below the level of microscopic detection.¹⁶⁴ The resulting model was investigated for the presence of a lag phase (an initial flatter part of the parasite clearance curve with slower

parasite clearance) and if present, this was removed to keep only the linear portion of the parasite clearance curve. From the remaining parasite counts (minimum of 2 per patient), a parasite clearance rate constant (K) was calculated for each patient. This was defined as the fraction by which $\log_e$ parasite count (P) fell per unit time (t) such that $P_t = P_0 \exp(-Kt)$. Taking log of both sides, $\log_e(P_t) = \log_e(P_0) - K \cdot t$ and therefore $K = (\log_e(P_0) - \log_e(P_t)) / t$. This value was derived by estimating the absolute value of the slope of a linear regression model of log parasite count against time in hours. K is directly and inversely related to the clearance slope half-life ($T_{1/2}$), the time needed for parasitaemia to be reduced by half such that $T_{1/2} = \log_e(2) / K$. Patients with an initial parasite count <1000 per microlitre or final parasite count >1000 per microlitre after accounting for points below the limit of detection were excluded from the analysis.

Statistical analysis

Statistical analyses were performed using Excel 2007 (Microsoft, USA) and GraphPad Prism version 5.04 (GraphPad Software Inc., San Diego, USA). Clearance rates were compared between cleared and recrudescence infections, between different dose regimens and between Pailin and Wang Pha. A significance level of 0.05 was used throughout. For normally distributed data means were compared using the t test and for non-normally distributed data medians were compared using the Mann-Whitney U test. Proportions were compared using Chi Square or Fisher's exact tests as appropriate.

Modelling

Simple mathematical modelling was used to explore possible parasite dynamics that may result in recrudescence after treatment with artesunate. This was done by extrapolation

from the available parasite clearance and recrudescence data combined with logical deduction and evidence where available. Of particular interest were the dynamics below the limit of microscopic detection in the peripheral blood and any fitness cost of artemisinin resistance. The methods used and the rationale behind them are outlined in detail below.

Parasite numbers below the limit of detection

In vivo investigations of parasite clearance have an important limitation. The sensitivity of the diagnostic test used for detecting parasites means there is a minimum parasite count below which an infection will no longer be detected. Currently, microscopy of peripheral blood remains the most reliable quantitative measure of parasite burden. Using standard techniques, the lowest parasite counts detectable are 1 parasite per examination. For a highly competent microscopist examining a thick film for 1 parasite per 500 white blood cells, with a white cell count of 80,00 per μl , this would give $1 \times 8000 / 500 = 16$ parasites per μl . This equates to a total parasite biomass in a 60 kg person of $16 \times 60 \times 80,000 = 7.7 \times 10^8$ per μl . Below this level, parasite counts will usually be reported as negative (**figure 2.2-1**). The time at which the first of two consecutive negative slides is collected is the parasite clearance time and this is typically less than three days after starting treatment with artemisinins. This is important when considering parasite clearance as an individual's parasites have not actually cleared until their parasite *biomass* is zero and this may be several days later.

In the absence of adequate quantitative methods, it is not certain what happens to parasite numbers below the limit of detection. For artesunate, several assumptions can be made

which help to explore this by extrapolation from available data. As artesunate has a short half-life of much less than 24 hours, if the same dose of artesunate monotherapy is given each day, it can be assumed that the log linear parasite clearance that occurs above the detection limit continues below it until the drug ceases to act. This is not likely to apply to artesunate-mefloquine due to the residual effect of the mefloquine which has a half-life of 2-3 weeks. Thus in the example in **figure 2.2-1**, imaginary patient 1 had artemisinin sensitive *P. falciparum* infection. By extrapolating the parasite clearance slope, true parasite clearance was estimated to occur in around 5.5 days for this patient. Here, 7 days of treatment would be sufficient to achieve clearance, as in 90% of individuals with artemisinin sensitive infections who receive oral artesunate monotherapy.⁶⁸ In individuals with artemisinin resistant infection, parasite clearance is slower and thus treatment should be less likely to fully clear infection in 7 days. It can therefore be hypothesized that such patients would be more likely to have a recrudescence infection and this may be the explanation for the high recrudescence rate found in the study in Pailin.¹⁰⁴ This is illustrated in **figure 2.2-1** by hypothetical patient 2 with artemisinin resistant *P. falciparum* malaria. Due to slower clearance, elimination of parasites was not achieved and they went on to recrudescence.

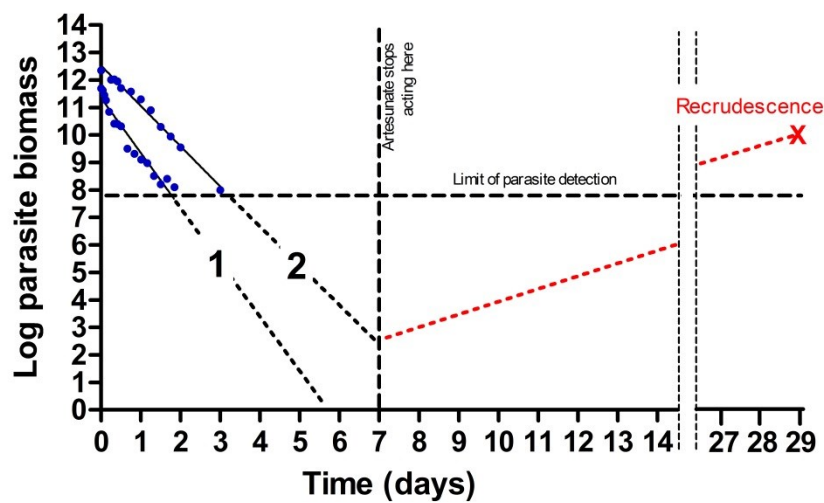


Figure 2.2-1. Parasite clearance slope for two hypothetical individuals receiving 7 days of artesunate monotherapy.

1 is a non-recrudescent infection and 2 a recrudescent infection. The blue dots represent actual parasite biomass over time calculated from peripheral blood microscopy parasite counts. The black line through the dots is the clearance slope estimated by the WWARN PCE. The dotted portion is the same slope extrapolated below the limit of microscopic parasite detection. The red 'X' represents a recrudescence on day 29 with parasite multiplication shown by the red dotted line.

Fitness cost

Rough estimates for a possible fitness cost of artemisinin resistance were derived as follows. As parasites multiply in a log linear fashion, a line was drawn from the point of recrudescence (the 'X' in **figure 2.2-1**) with a gradient determined by the parasite multiplication rate back to the point at which the recrudescence began. In **figure 2.2-1**, this point is at the end of a failed treatment (day 7) due to a slow parasite clearance rate because of artemisinin resistance. By comparing this multiplication rate in recrudescent,

presumed artemisinin resistant, infections (m_R) with the normal multiplication rate for *P. falciparum* (m_f , roughly a 6 to 10 fold increase per 48 hours¹⁶⁵), an estimate for fitness cost of artemisinin resistance *in vivo* was derived. The fitness cost was equal to $1 - (m_R / (\log(m_f)/2))$ (Method 1). For patients whose projected parasite counts reach zero before 7 days, this calculation was done using the parasite count of zero at the end of treatment as the origin of the recrudescence slope, as parasite counts can't be negative (Method 2). This would give a conservatively low estimate of fitness cost as the parasite count at the end of treatment was likely to be significantly greater than zero. As additional estimate was derived by using the multiplication rates of recrudescence infections in Wang Pha that received the same treatment as the comparator.

Using these methods of extrapolation from the parasite clearance slope and back extrapolation from the recrudescences, the parasite clearance behaviour below the limit of parasite detection was modelled (using Microsoft Excel 2007 (Microsoft, USA)) for all recrudescence infections who received artesunate monotherapy. In this way, several hypotheses were generated for the different drug response dynamics of recrudescence and non-recrudescence artemisinin resistant infections. These hypothesis and the associated analyses are presented in **Appendix 6.1**.

2.2.2 Parasite clearance slope half-lives in severe malaria in Bangladesh

Evaluation of the *in vivo* responsiveness of *P. falciparum* to intravenous artesunate

Ethics

Ethical approval for the studies in which these patients were enrolled was obtained from OXTREC, the University of Oxford Tropical Research Ethics Committee and either the Bangladesh Medical Research Council ethics committee or the Chittagong Medical College ethics committee.

Study sites and enrolment criteria

The data were collected from inpatients at Chittagong Medical College Hospital (CMCH), Chittagong, Bangladesh from 2003 to 2012. These patients had been enrolled in multiple previous studies for which these data were originally collected. All included patients had received intravenous artesunate 2.4mg/kg body weight as per WHO guidelines,¹³⁹ and administered according to a strict protocol by specifically trained study doctors. The data were analysed retrospectively. Inclusion criteria were: 1) asexual forms of *P. falciparum* malaria in peripheral blood film, 2) patient received ≥ 2 doses of intravenous artesunate. Patients with an initial parasite count < 1000 per microlitre or final parasite count > 1000 per microlitre after accounting for points below the limit of detection (see below) were excluded from the analysis.

Where available, indicators of severity (blood haematological and biochemical indices, parasite count and staging and Glasgow Coma Scale (GCS)), outcome measures

(mortality and coma recovery time), and artemisinin pharmacokinetics from these patients were compared to derived parasite clearance slope half-lives.

Other data for comparison were 120 patients from Pailin, Cambodia and 40 from Wang Pha, Thailand, as described in **Section 2.2.1** Of these, one patient from Pailin was excluded as their initial parasite count was $<1000/\mu\text{l}$. Additional data used for comparison were historical clearance slope half-lives from a published study in 3202 hyperparasitaemic ($>4\%$ infected erythrocytes) patients receiving a variety of oral artemisinin-based regimens in Mae Sot, northwest Thailand from 2001 to 2010.¹⁰⁸

Procedures

Parasite count and relevant clinical data for patients in Bangladesh were extracted from the anonymised database for all patients who met the above criteria.

Parasite count data were analysed using the World Wide Antimalarial Resistance Network (WWARN) Parasite Clearance Estimator (PCE)¹⁶² as described in **Section 2.2.1**. For each patient a parasite clearance slope half-life was calculated.

Statistics

A significance level of 0.05 was used throughout. Median half-lives were compared using the Mann-Whitney U test rather than geometric means as log transformation of the parasite clearance half-lives did not give a normal distribution for Pailin. Correlations of clearance slope half-life with time were sought by Spearman r , univariate and multivariate analyses using Stata Version 11 (Statacorp LP, Texas, USA).

2.3 Mathematical models

This section describes the process of development and final structure of three mathematical models. Each is a population dynamic model comprising ordinary differential equations. They all share the same basic structure of the malaria parasite life-cycle in humans and the responses of each life cycle stage to antimalarial drug treatment. With each successive model, there was an increase in model complexity. This allowed incorporation of additional aspects of infection in the human host, host behaviours and details of the various malaria control strategies being considered at the time. These were added in an iterative process response of ongoing discussion with policymakers and model refinement so the models could best answer the scientific questions being posed. This additional complexity included the addition of immunity in **Models 2** and **3** and severe and fatal malaria in **Model 3**. The models were run in the Berkeley Madonna™ software package (California, USA). Equations, assumptions and parameter tables for each model and the Berkeley Madonna code for **Model 1** are given in the appendix.

2.3.1 Model 1

The structure of the basic population dynamic model is shown in **figure 2.3-1**. For **Model 1**, a deterministic structure was used to conduct sensitivity analyses and to make an initial assessment of the relative effectiveness of the various containment interventions proposed. Results of interest were then confirmed in a stochastic framework with the same structure and parameters. Seasonality was incorporated to reflect the highly seasonal transmission intensity in this region. In order to maintain simplicity and therefore flexibility and interpretability of the model, a number of assumptions were made

(**Appendix 6.3, tables 6.3-1 and 6.3-2**). The model was run from 1960-1975 to achieve a treatment free equilibrium for a population prevalence of malaria parasitaemia (of any density) of 16%. This equilibrium was calculated by the numerical solution of the treatment-free sub-model equilibrium equations. Artesunate monotherapy was then introduced as the only treatment in 1975 (when artesunate was first used in Cambodia). A single patient with artemisinin-resistant infection was introduced in 1980. By 2008, the mean population prevalence of malaria in the high transmission season was 7.5% (compared to estimate from field data of 7.4%¹⁴⁵ and the prevalence of artemisinin resistance in the model was 10.6% (expert opinion estimates this to be around 10%) (this whole process is shown in **figure 3.3-1**). The interventions were then introduced in 2009, to reflect the current plans for containment.

The full list of parameters is shown in **Appendix 6.4 tables 6.4-1 and 6.4-2**. The interventions that were introduced into the model, alone and in combination, were:

- a) Eliminating the use of artemisinin monotherapies (artesunate in the model) and replacing them with ACT in the private sector (i.e. for symptomatic patients only).
- b) One or more annual three-month pulses of MSAT with ACT or AP during the low transmission season.
- c) One or more annual three-month pulses of MDA with ACT or AP during the low transmission season.
- d) As for b) and c) plus primaquine.
- e) Distribution of LLITN (or comparable treated materials).

For each pulse of MDA and MSAT, each patient received a three-day course of treatment (ACT or AP) on one occasion only.

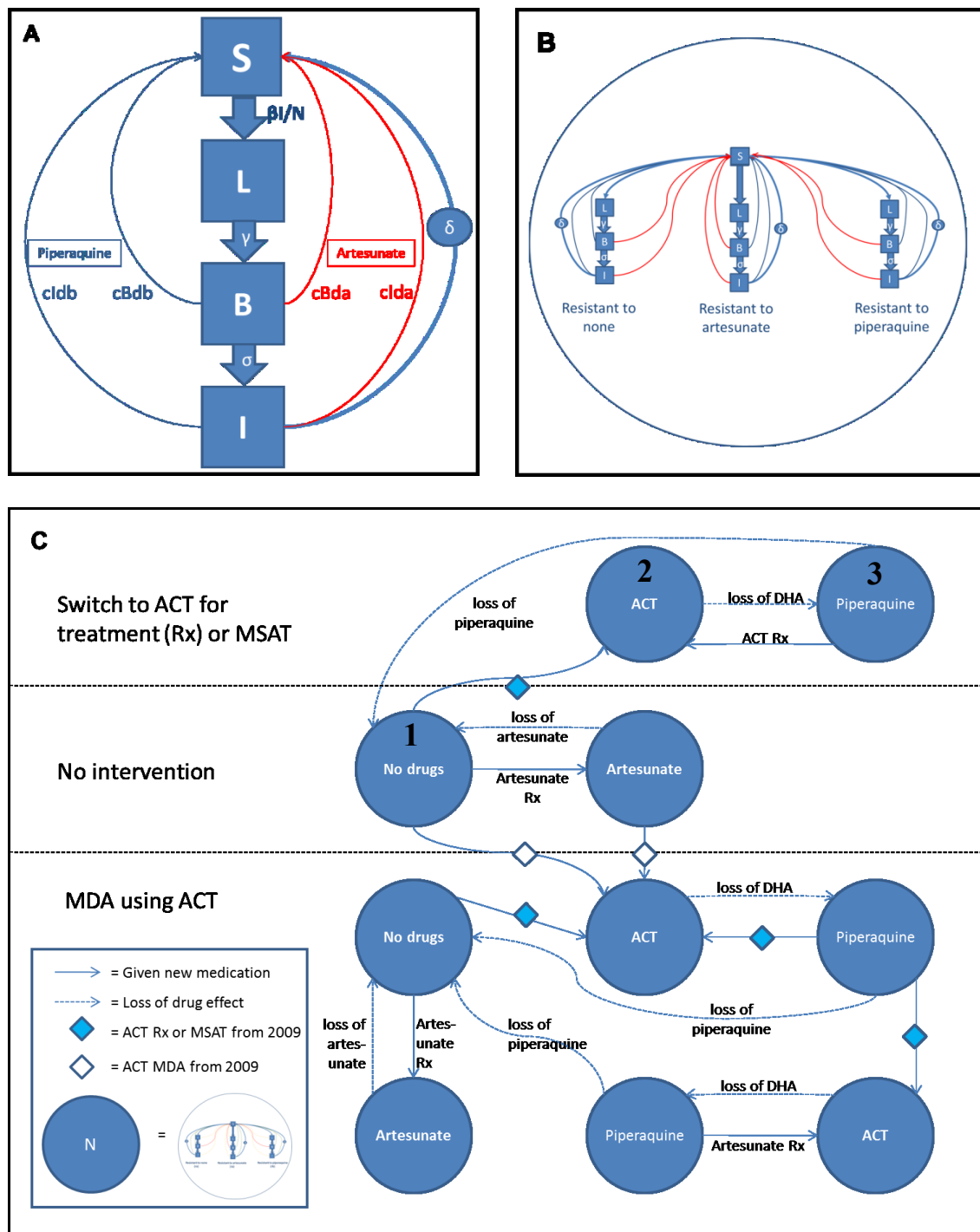


Figure 2.3-1. Schematic diagram of the structure of the mathematical modelling framework for Model 1.

The structure of the model was built up as follows: natural history and pharmacodynamics incorporated as a repeating unit **A** with four compartments – **susceptible** people, people with **liver stage** infection, people with non-infectious **blood**

stage infection (asexual stage parasites in the peripheral blood but no gametocytes) and people with **infectious** (blood) **stage** infection (gametocytes), rates of flow between these compartments ($\beta I/N$, δ , γ and σ) and rates of recovery due to each of artesunate and piperazine treatment (c_{Bda} , c_{Ida} , c_{Bdb} and c_{Idb}) (**Appendix 6.4 tables 6.4-1 and 6.4-2**) are shown. The times to recovery ($1/\text{rate}$) following treatment were then adjusted by a multiplying factor (e_{rada} or e_{rddb}) ($0 \leq e \leq 1$) depending on the degree of resistance to each drug, giving three possible linked variants of unit **A** (resistant to no drug, artesunate only and piperazine only) making up a repeating pattern **B**. Finally the population dynamics of transmission are shown in **C**. This consisted of multiple repetitions of **B** with different rates of flow between them at different time points depending on which treatments and interventions were used. For example, for individuals with blood stage infections to begin treatment with ACT, they moved from the ‘No drugs’ box (**1**) to the equivalent parts of an ‘ACT’ box (**2**) at a rate determined by the time to begin treatment. The dynamics in the ‘ACT’ box were different from the ‘No drugs’ box as these individuals were subjected to faster rates of recovery due to the ACT. Each box was also subject to pharmacokinetic dynamics independent of infection dynamics. This was in the form of waning pharmacodynamic drug effect over time (‘loss of...’) with sequential loss of DHA and then piperazine. This resulted in a percentage of the entire unit moving to a new box ‘Piperazine’ (**3**) which again had different dynamics representing the effect of piperazine on recovery rates. Interventions shown here are elimination of artemisinin monotherapy and replacement with ACT (‘Switch to ACT’) and MSAT and MDA with ACT. Each circle represents a population exposed to a particular drug or combination. Key: ACT = dihydroartemisinin/piperazine combination therapy, Rx = treatment, DHA = dihydroartemisinin. (For more details, please see the full model code in **Appendix 6.6**)

2.3.2 Model 2

The structure of **Model 2** is shown diagrammatically in **figure 2.3-2** and as equations in **Appendix 6.2.2**. It differs from that for **Model 1** in that antidisease immunity and asymptomatic infection have been added,¹⁶⁶ and the strategies used have been changed to match those used in the Cambodia field trial which it was designed to reproduce.²⁷

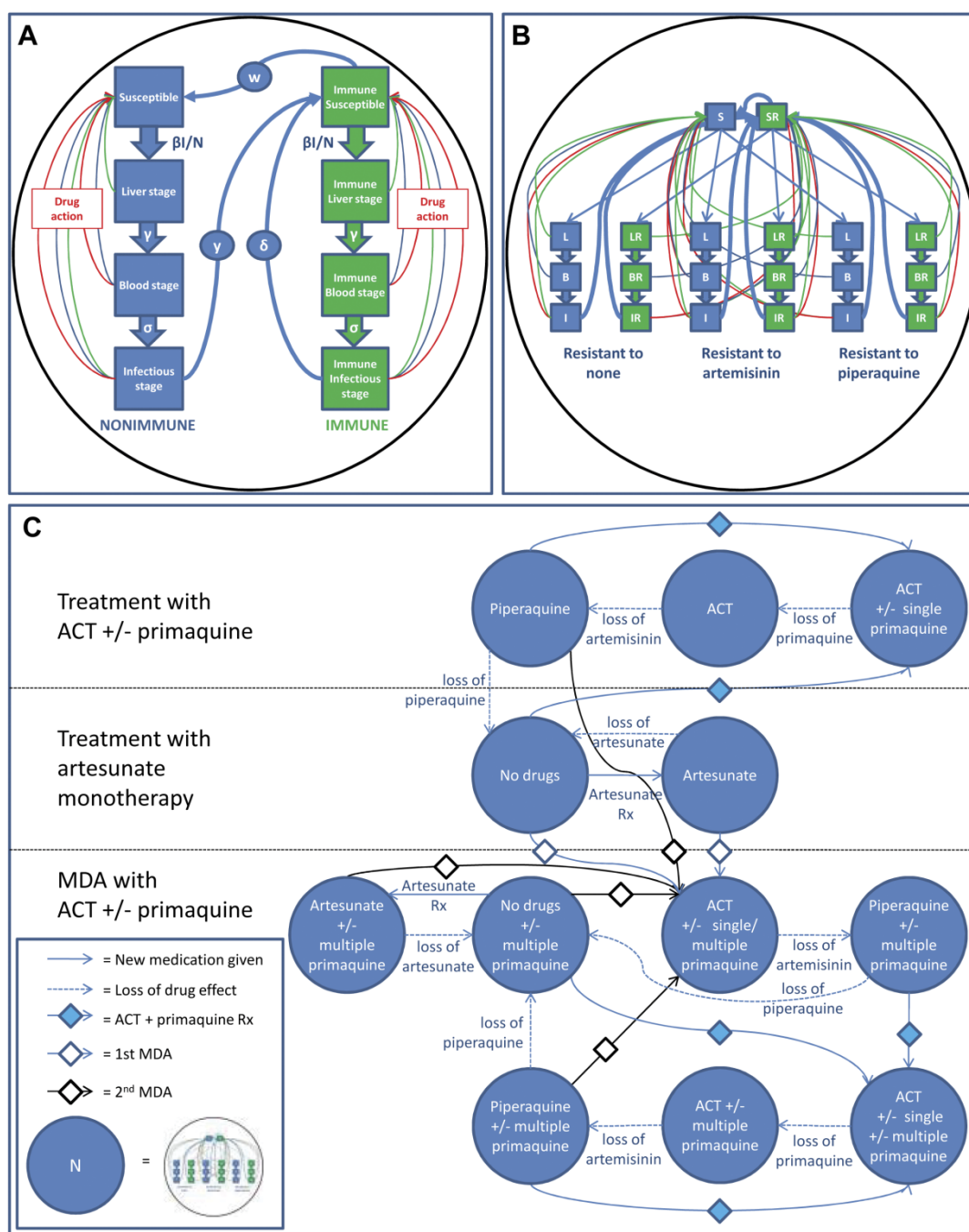


Figure 2.3-2. Summary of structure of Model 2.

A shows the basic model unit with parasite life cycle stages in the human host, antimalarial drug action and immunity. **B** shows the unit in **A** repeated three times to track parasites resistant to artemisinin and ACT partner drug. **C** shows multiple repetitions of **B** to reproduce the various strategies used in the trial.

The basic framework was developed from that of **Model 1**²³ with major additions and modifications including the addition of host immunity and asymptomatic infections¹⁶⁶ using a method based on that of Aguas et al⁴⁰ and formal and extensive model fitting to, and validation with, malaria surveillance data (**figure 3.3-4**) and results from the field study (for details see **Appendix 6.5.1**). Symptomatic infection was assumed to occur only in those with asexual parasites in the peripheral blood. Artemisinin resistance was modelled as previously using increased parasite clearance rates derived from field studies in Pailin, Cambodia,²³ which first identified prolonged parasite clearance rates¹⁰⁴ although the prevalence was varied to explore its effect on the impact of the strategies under consideration. Parameters for malaria epidemiology were matched to those for the field study area and the strategies used in the trial were replicated in detail. Although similar reductions in parasite prevalence were found for *P. vivax* and *P. malariae* in the trial, only *P. falciparum* was modelled.

The modelled strategies were various combinations of:

- Treatment of symptomatic cases:
 - artemisinin-piperaquine ACT
 - adjunctive single dose of primaquine

- Mass drug administration :
 - artemisinin-piperaquine ACT
 - adjunctive single dose of primaquine
 - multiple doses of primaquine: one dose given every 10 days for 6 months
- LLITN

ACT was given as a 2 day course of artemisinin-piperaquine (Artequick®, Guangzhou, People's Republic of China; 125 mg and 750 mg once a day respectively for adults) and each dose of primaquine was 9 mg. In the figures, for brevity, an adjunctive single dose of primaquine is referred to as 'single primaquine' and multiple doses of primaquine MDA as 'multiple primaquine'.

Model Assumptions are listed in **Appendix 6.3 tables 6.3-1 and 6.3-3**. Model parameters and their sources are listed in **Appendix 6.4 tables 6.4-1 and 6.4-3**.

The fitted model was used to answer the following questions:

- What was the contribution of each component of the trialled strategies to the reductions in *P. falciparum* malaria burden?
- What will happen to the *P. falciparum* malaria burden (clinical cases and asymptomatic parasitaemic individuals) once these interventions are stopped at the end of the trial?
- What was the relative effect of the different strategies on population level immunity i.e. proportions of symptomatic versus asymptomatic cases?

- What would be the effect of artemisinin resistance on the effectiveness of these strategies and how do they affect its spread?
- What is the optimal design for an elimination strategy using these methods to achieve maximum long-term impact on *P. falciparum* malaria parasite prevalence?

2.3.3 Model 3

To produce **Model 3**, **Model 2** was extensively revised and updated with the addition of explicit modelling of the spectrum of disease (uncomplicated, severe and fatal malaria) and patient referral pathways within the community and from community to hospital. Immune individuals had a lower probability of developing both symptomatic infection and severe disease. New parameter values were taken from a wide range of clinical and epidemiological studies and the model was fitted to and validated with multiple independent data sets. The novel aspects of the updated model structure are shown diagrammatically in **figure 2.3-3**. The model is also defined as a set of differential equations in **Appendix 6.2.3**. Model Assumptions are listed in **Appendix 6.3 tables 6.3-1 and 6.3-4**, the parameter values and their sources in **Appendix 6.4 tables 6.4-1 and 6.4-4** and details of the fitting and validation in **Appendix 6.5.2**.

The model was developed to be generally applicable to any transmission setting. Although it can easily be adapted to a range of settings, this model was applied in detail to Khagrachari District, Bangladesh as a case study. The model was then used to explore the same scenarios in the whole malaria endemic southeast of Bangladesh and all of Cambodia, for which less detailed data were available. There were several reasons for this. The epidemiology of malaria in this area is similar to that in other nearby countries in South and Southeast Asia. Bangladesh was a major field site in the two major studies of prereferral rectal artesunate and a large trial of intravenous artesunate versus quinine for severe malaria thus detailed data are available from these. There were also detailed epidemiological data available on malaria from multiple independent sources with which to parameterise the model. These data were used to build an accurate model with which a

broad range of scenarios was explored to generate results that are broadly applicable to a wide range of countries.

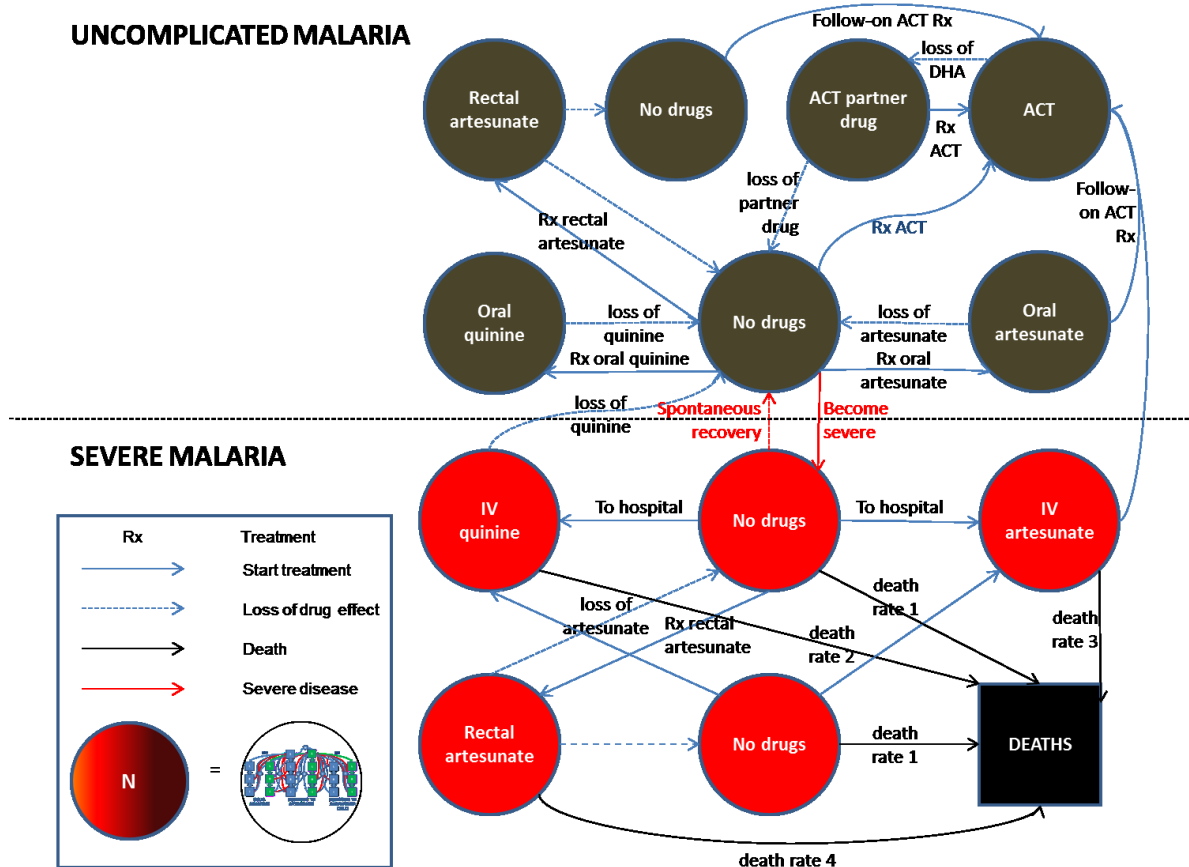


Figure 2.3-3. Summary of structure of Model 3.

Each circle represents a cycle of malaria transmission within a population being exposed to a particular antimalarial drug, as for **Model 2**. As for **Models 1** and **2**, people move between these units as the drugs to which they are exposed change, either by them starting a treatment or by drug metabolism resulting in loss of antimalarial effect. Oral, intravenous and rectal treatments for uncomplicated and severe malaria and the different possible referral pathways from community to hospital are included. Black arrow = death, blue arrow = treatment, blue dotted arrow = loss of drug effect.

Detailed data on malaria epidemiology were obtained from Bangladesh and Cambodia for fitting of the model to determine parameters for baseline transmission dynamics and the burdens of non-severe and severe disease. This included increasing coverage with ACTs replacing oral quinine from 2007. Detailed analyses of these data are presented elsewhere (**Sections 3.1.1 and 3.1.2**).¹⁶⁷ To determine parameters for the efficacy of the different combinations of intravenous artesunate, intravenous quinine and rectal artesunate, the model was used to reproduce a large hospital-based trial of intravenous artesunate versus quinine (SEAQUAMAT study)⁷⁰ and a large trial of pre-referral rectal artesunate versus placebo (Study 13)⁷⁴ and fitted to the results. To reproduce these clinical trials, adherence to antimalarial drugs was assumed to be perfect. Other parameter values were taken from the published literature, as listed in **Appendix 6.4 tables 6.4-1 and 6.4-4**. The model was then validated against two cross sectional surveys using RDTs in Khagrachari in 2007,^{133, 168} one in southeast Bangladesh,¹⁶⁹ one using microscopy in Cambodia¹⁴⁵ and separate longitudinal data on cases of severe malaria admitted to Chittagong Medical College Hospital, Chittagong, Bangladesh.¹⁶⁷ Other model parameters for access to healthcare, antimalarial drug effects and artemisinin resistance were derived from largely unpublished data from two large community-based trials of single dose rectal artesunate (Study 13⁷⁴ and Study 18 (TDR, on-going)), the SEAQUAMAT study⁷⁰) and recent data on artemisinin sensitivity from Cambodia, Thailand and Bangladesh (**Sections 3.2.1 and 3.2.2**). The current artemisinin sensitive and resistant phenotypes were modelled using parasite clearance slope half-lives derived from updated analyses of field studies of oral artesunate for uncomplicated malaria in Pailin, Cambodia (resistant parasites) and Wang Pha in Thailand (sensitive parasites) (**Section 3.2.1**) and intravenous artesunate for severe malaria in Chittagong, Bangladesh (**Section 3.2.2**).

Treatments included in the model were ACT, oral quinine and oral artesunate (uncomplicated malaria) and rectal artesunate, intravenous artesunate and intravenous quinine (severe malaria). The antimalarial treatments chosen to be included in the model were to reflect changing policy over time. This included the switch from oral artesunate and other non-artemisinin drugs to ACT in Cambodia in 2000 and large scale use of ACTs in Bangladesh from 2007-2008 following their introduction in 2004. Treatment for severe malaria in the model was with intravenous quinine until its' replacement by intravenous artesunate in 2013. Rectal artesunate was introduced in 2015 in the model.

The model was used to answer the following questions: 1) what is the likely impact of intravenous and rectal artesunate on malaria mortality in large populations? 2) what is the potential impact of rectal and intravenous artesunate on the spread of artemisinin resistance? This was assessed relative to ACT for a variety of possible baseline scenarios of resistance prevalence. 3) what is the potential effect of artemisinin resistance on the efficacy of rectal and intravenous artesunate for patients with malaria? This was assessed for the current known phenotype of artemisinin resistance and for increasingly resistant possible future phenotypes. 4) How will introduction of rectal and intravenous artesunate affect the feasibility of malaria elimination? If they promote the spread of artemisinin resistance, to what degree might this compromise the efficacy of ACT, the cornerstone of current strategies?

Various scenarios were considered including the introduction of these therapies alone and in combination, at different levels of coverage, in a variety of transmission settings and with varying efficiency of the referral system.

Chapter 3 : Results

3.1 Malaria epidemiology

3.1.1 Cambodia

HIS

Total cases

From 2004-2011, 1,328,686 people screened for malaria in Cambodia were recorded by the government HIS system.

Of these, 434,285 (32.7%) were positive, 334,828 (77.1%) with *P. falciparum* and 80,183 (18.5%) with *P. vivax*. 19,274 (4.4%) had mixed infection. 596,324 people were recorded as treated for malaria. Thus at least 162,039/596,324 (27%) of those treated did not have confirmed malaria. During this period, there were 2027 (0.5% of confirmed cases) recorded deaths from malaria. The yearly average number of confirmed malaria cases in the Cambodia HIS was 54,286. Diagnosis was by RDT or microscopy. The proportion of HIS patients diagnosed by microscopy fell from 52% in 2007 to 29% in 2010.

Seasonality

The number of cases of both *falciparum* and *vivax* was highly seasonal with high numbers in July to December with a peak in September (**figure 3.1-1**). This corresponded with peak rainfall.

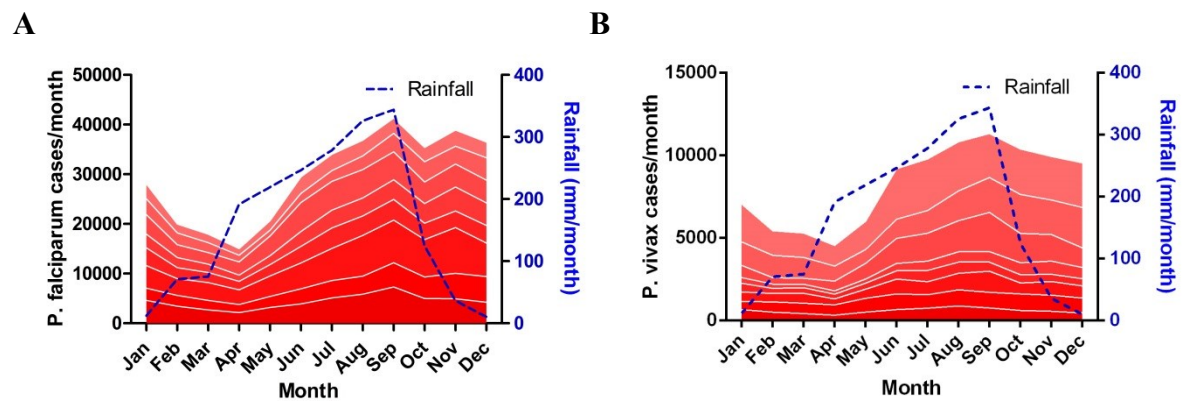


Figure 3.1-1. Cumulative seasonal pattern of A *P. falciparum* and B *P. vivax* malaria cases from HIS and average monthly rainfall in Cambodia from 2004 to 2011.

Each band represents a single year from 2004 (bottom) to 2011 (top).

Temporal trends

From 2004 to 2011 there was no overall change in the number of cases of *P. falciparum* ($p=0.18$) (**figure 3.1-2A**). Since 2008 there has been a marked increase in the number of *P. vivax* (**figure 3.1-2A**, Pearson $R=0.9896$, $p=0.01$), the total number being 4.9 times greater in 2011 than in 2008 and 47% of the total in 2011. From 2004 to 2008, the number of people screened for malaria and percent of screens positive for malaria did not change (**figure 3.1-2B**, $p=0.11$), indicating that the overall trends in cases detected were not an artefact due to changing surveillance efforts. In 2010 and 2011, the percent of screens positive in the low season was lower than in previous years, suggesting a relative intensification of malaria screening in the low season in those years.

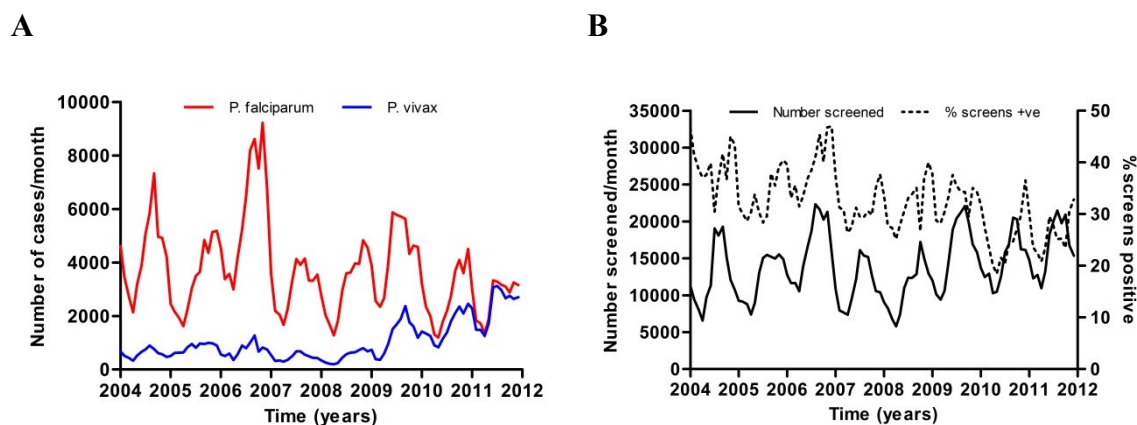


Figure 3.1-2. A Number of reported malaria cases, and B people screened for malaria in Cambodia HIS from 2004-2011.

Numbers for each species include those with mixed infection.

From 2004 to 2011, the number of deaths from malaria and % mortality decreased (**figure 3.1-3**, Pearson $R=-0.8654$, $p=0.006$ and $R=-0.9252$ and $p=0.0001$ respectively). As it was not recorded, it was assumed all deaths were due to *P. falciparum*.

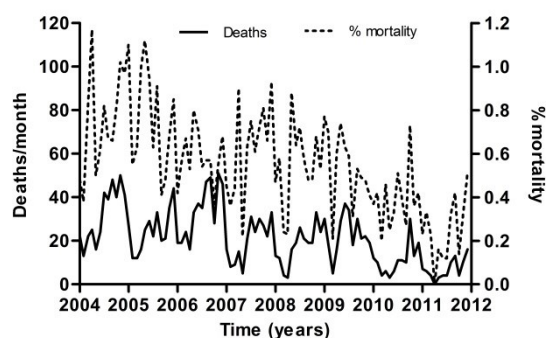


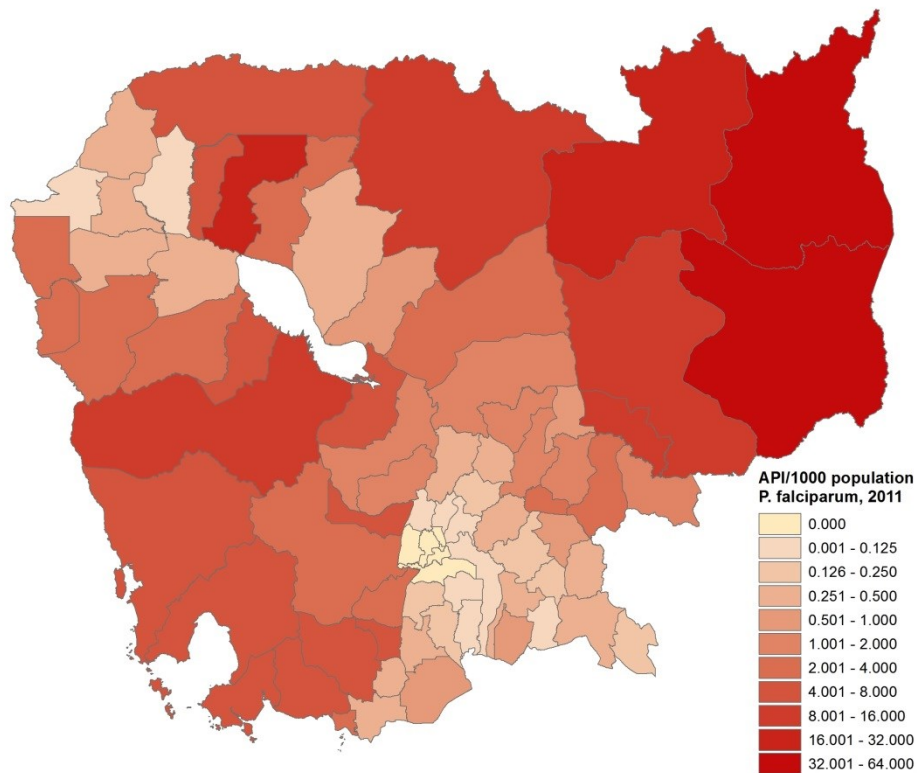
Figure 3.1-3. Numbers of deaths and percent mortality from *P. falciparum* malaria in Cambodia from 2004 to 2011.

Spatial distribution

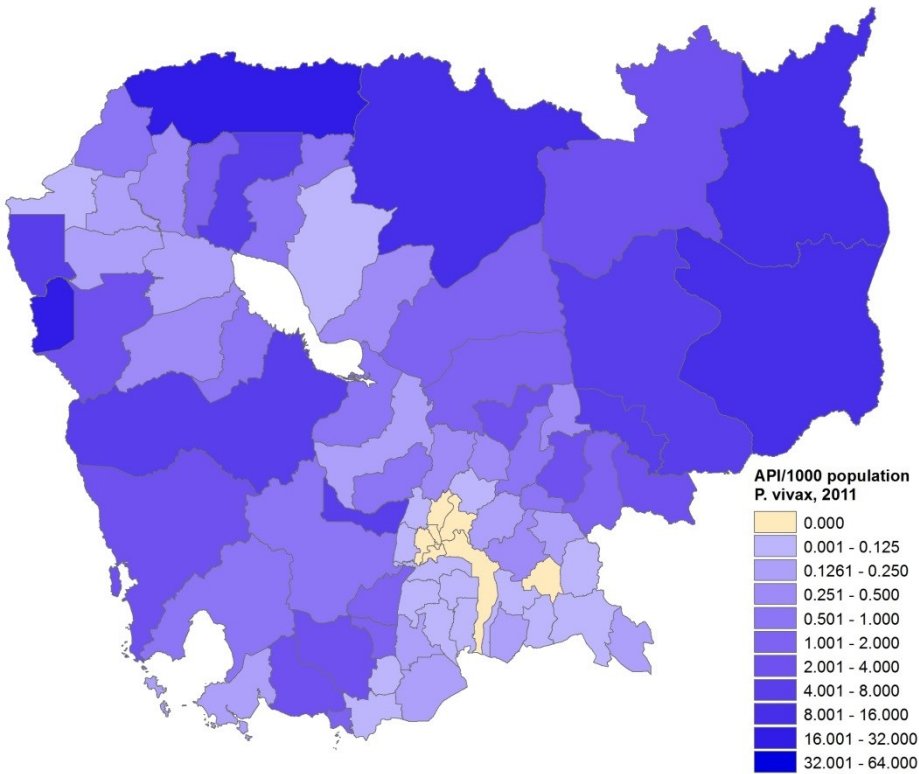
The spatial distribution of malaria cases was similar for *P. falciparum* and *P. vivax*. In 2011 (**figure 3.1-4**), the highest transmission intensity for both species was in the north

and northeast of the country, areas which are heavily forested. The ODs with the highest transmission for *P. falciparum* (API/1000 >10) in descending order were Sen Monorom (45.6, northeast), Rattanakiri (44.8, northeast), Ankor Chum (21.6, north), Steung Treng (20.3, northeast), Kratie (15.4, northeast) and Preah Vihear (15.3, north). Although, the four provinces in the northeast of Cambodia (Sen Monorom, Rattanakiri, Steung Treng and Kratie) had only 3% of the population, they had 31% of the falciparum malaria and 21% of the vivax malaria cases in 2011.

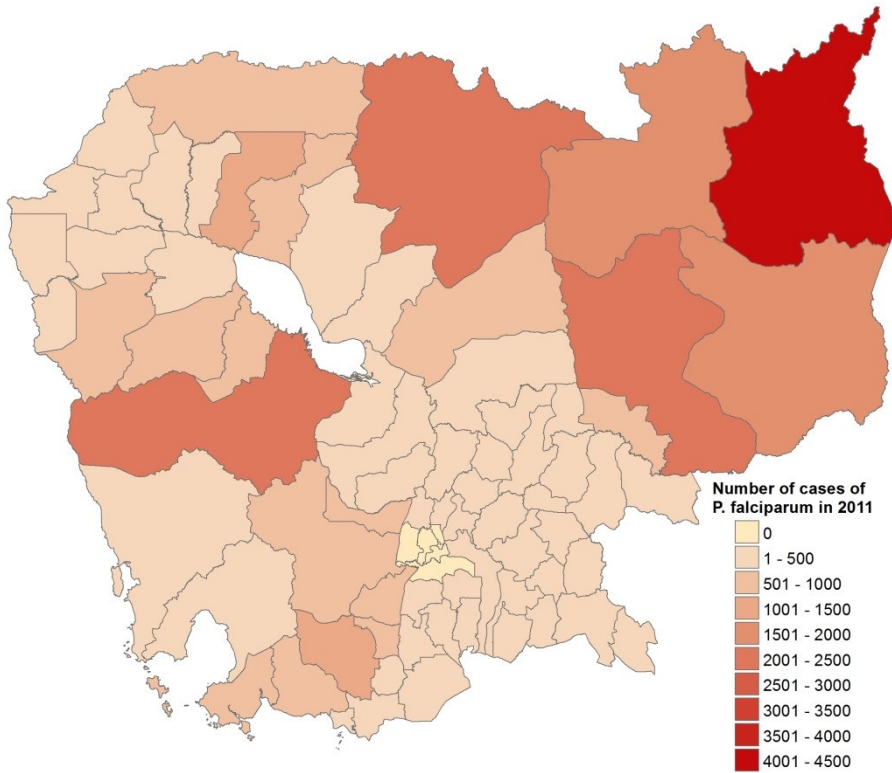
A



B



C



D

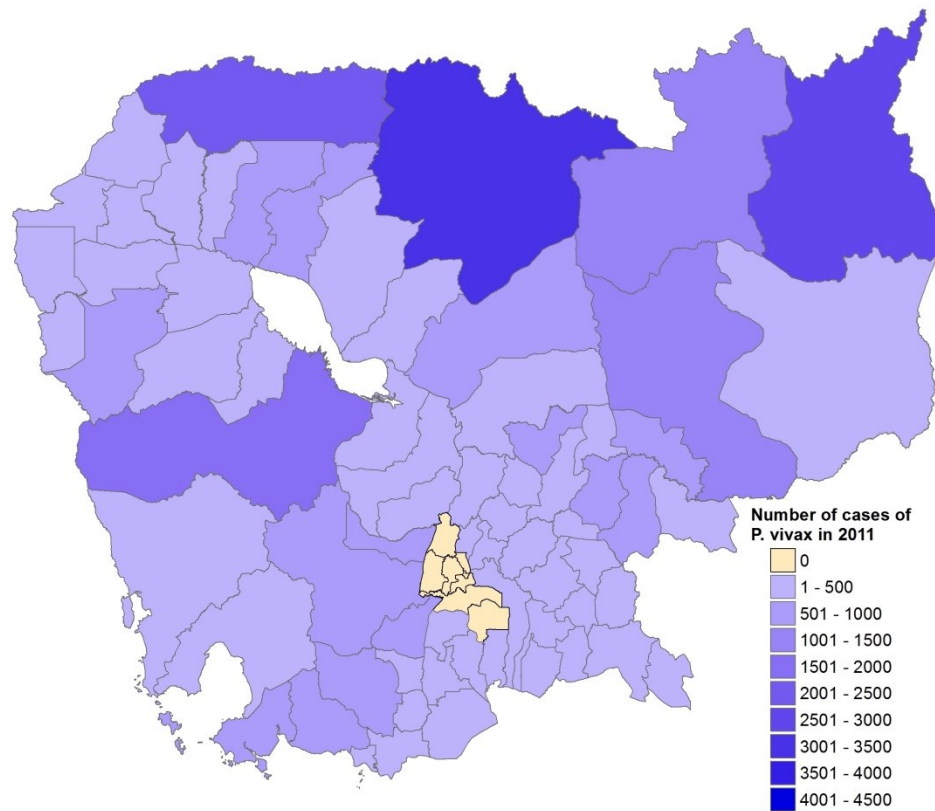


Figure 3.1-4. Geographical distribution of *P. falciparum* and *P. vivax* malaria in Cambodia in 2011.

A and **B** Annual Parasite Index (API, the annual rate of malaria positive individuals per 1000 population) in 2011, **C** and **D** number of cases.

The geographical distribution of malaria in Cambodia has changed from 2004 to 2011. There were several major trends. These included a marked decrease in *P. falciparum* in the west (Pailin, Battambang, Banteay Meachey, Pursat, Siem Reap and Otdar Meanchay provinces) from 2007 onwards (**figure 3.1-5A**), particularly in Pailin (**figure 3.1-5B**, the OD with the highest transmission intensity from 2004-2006) and the surrounding Province of Battambang (**figure 3.1-5C**, with historically much lower

transmission than Pailin,). The ODs along the border with Thailand (in the north and west), with the exception of Battambang and Pailin, did not experience a sustained decrease in transmission (**figure 3.1-5D**). This was also the case in the Provinces of Kampot and Kampong Speu in the south where the modelled trial presented in **Sections 2.3.2 and 3.3.2 (Model 2)** was performed (**figure 3.1-5E**). In these areas there was a short-term decrease in *P. falciparum* early during the trial but this was not sustained, probably because malaria control efforts in the area were not sustained. Details of numbers of *P. falciparum* malaria cases over time in Kampot are presented in **Section 3.3.2**. There was an increase in malaria in the northeast of Cambodia (Sen Monorom, Rattanakiri, Steung Treng and Kratie provinces) (**figure 3.1-5F**) where numbers of *P. falciparum* cases increased from 2007 to 2010 and *P. vivax* from 2008 to 2011.

From 2004 to 2011 *P. falciparum* remained widespread across Cambodia with low and unstable transmission in the southeast. *P. vivax* transmission was much less stable, with low and unstable transmission in many areas, particularly in a band from the southeast to the northwest of the country. *P. vivax* transmission was noticeably most stable in the ODs along the borders with Thailand and Laos in the west and north.

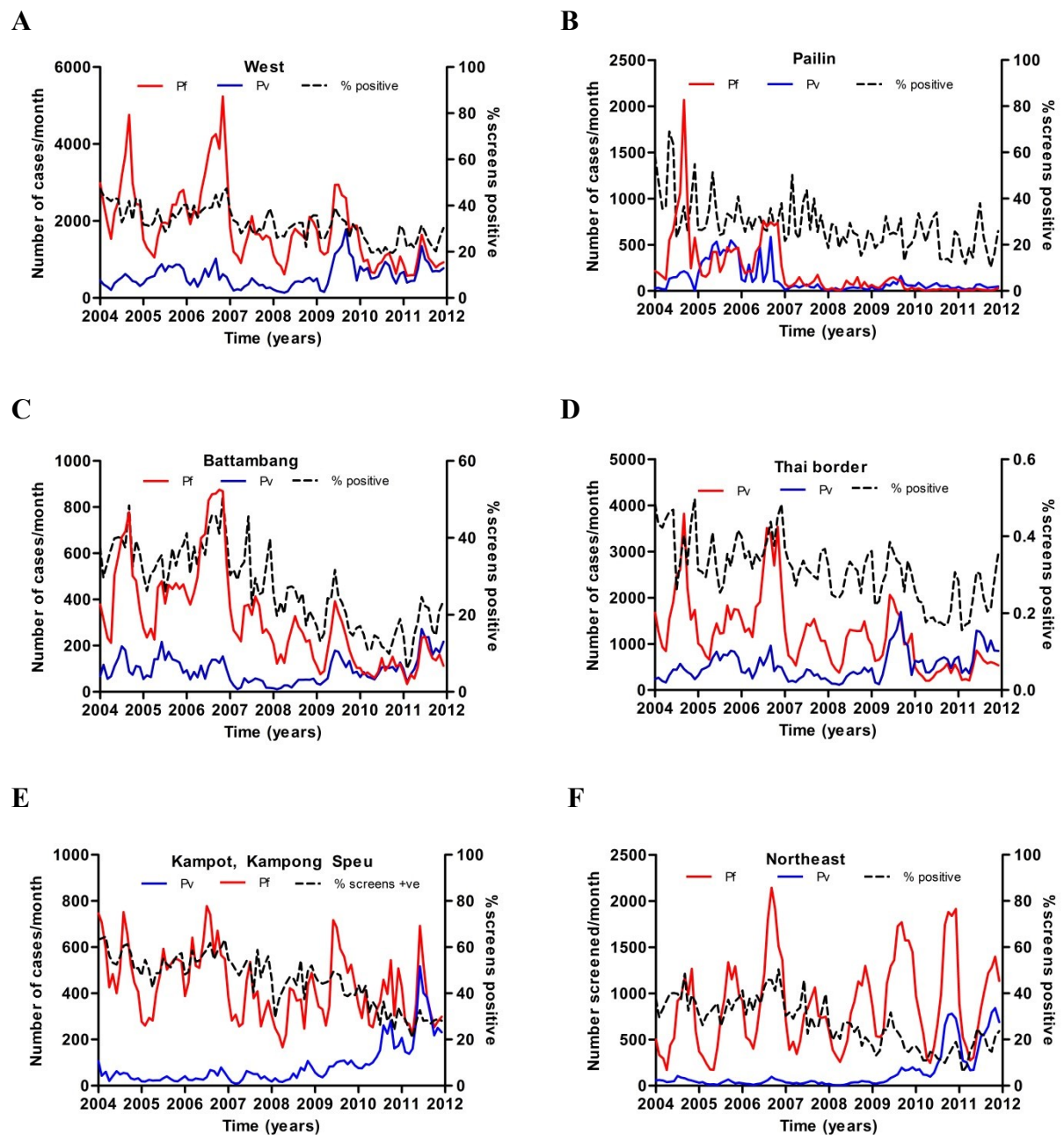


Figure 3.1-5. Number of cases of *P. falciparum* and *P. vivax* malaria in selected regions of Cambodia.

A west, **B** Pailin, **C** Battambang Province, **D** the Thai-Cambodian border (excluding Pailin), **E** Kampot & Kampong Speu Provinces and **F** northeast Cambodia.

Malaria Control Activities

During the period of the data collection, the sale of ACTs for treatment of malaria was increased, insecticide treated bed net distribution was scaled up and long-lasting insecticide treated bed nets were introduced in 2007. Detailed data on actual numbers of ACT treatments taken and bed net usage are not included in this analysis, thus the figures for sales and distribution were used as an approximate surrogate. There were no clear relationships between these measures of malaria control activities and the number of malaria cases reported through HIS (**figure 3.1-6**).

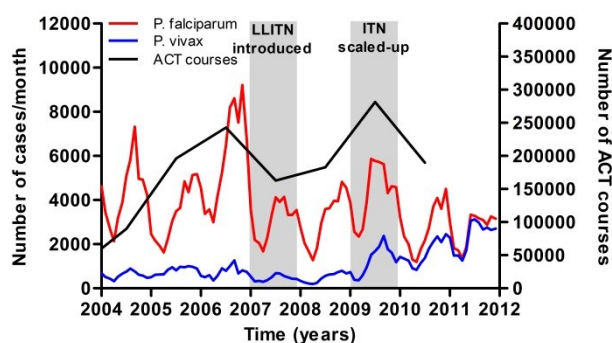


Figure 3.1-6. Malaria control activities and monthly numbers of cases in Cambodia.

VMW

The number of VMWs has gradually increased in Cambodia since their introduction in 2003 (**figure 3.1-7**). Being predominantly in more remote areas with higher malaria prevalence, they covered 1618 villages by the end of 2011 (11% of the national total and 25% of the villages in the 34 ODs currently included in the VMW programme, **figure 3.1-8**). Since their introduction, VMWs have detected 300,051 RDT positive cases of malaria. 22,614 (7.5%) of these were referred to government facilities due to being severely unwell, elderly or pregnant. Most of those referred would thus also have been

captured in the HIS data. Thus at least 277,437 additional cases of malaria not included in the HIS data were detected by VMWs. In 2009, 2010 and 2011, 42%, 48% and 47% of total cases were detected by VMWs.

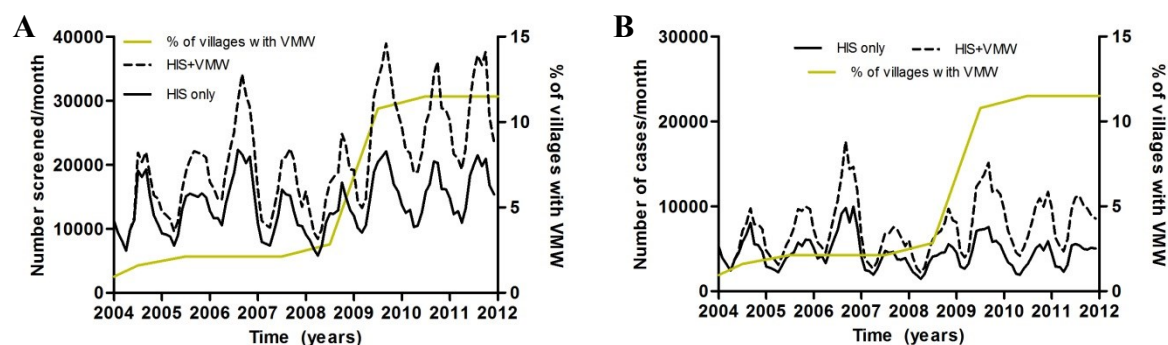


Figure 3.1-7. Number screened for malaria over time in HIS and VMW data.

Individuals screened for malaria (A) and positive cases (B) over time in HIS (solid lines) and HIS plus VMW data (dotted line) with % of villages in Cambodia with a VMW (green line).

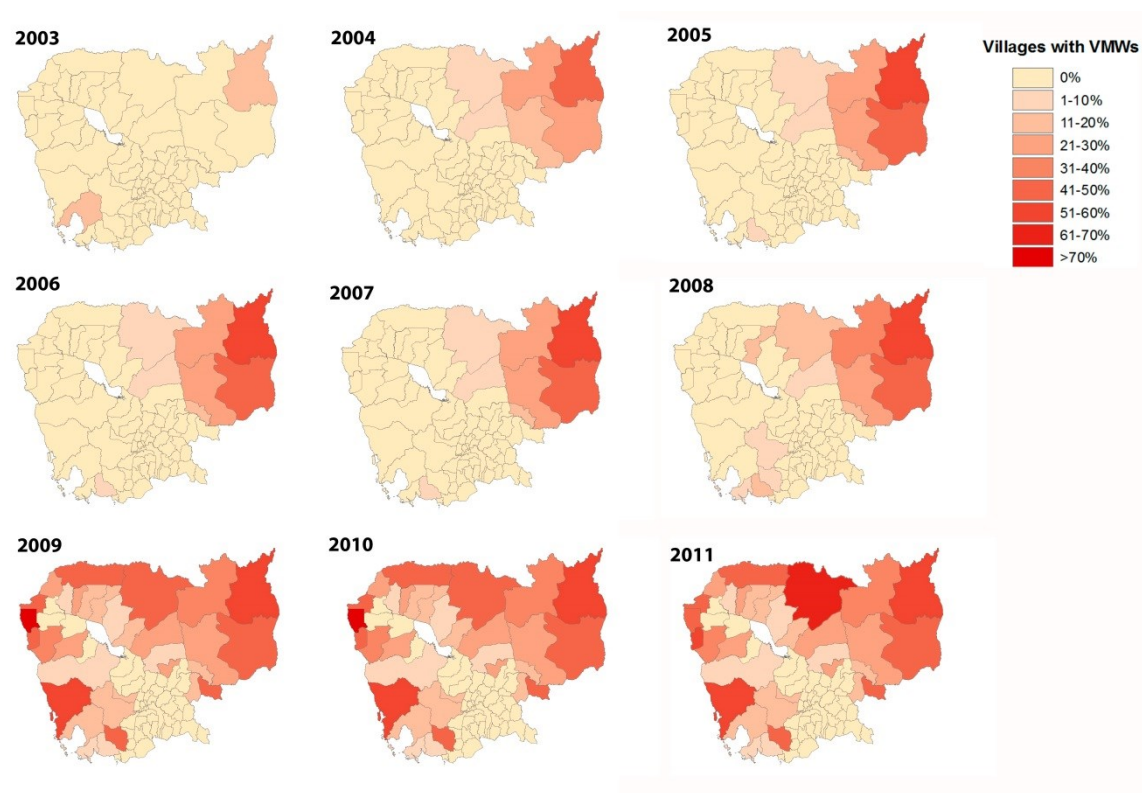


Figure 3.1-8. Coverage of VMWs by OD in Cambodia from 2003-2011.

Until 2009, VMWs used only p-HRP2 based *P. falciparum* RDTs to screen people for malaria thus only *P. falciparum* cases were detected (**figure 3.1-8**). Since 2009, these have gradually been replaced by LDH plus HRP2-based RDTs which can detect both *P. falciparum* and *P. vivax*.

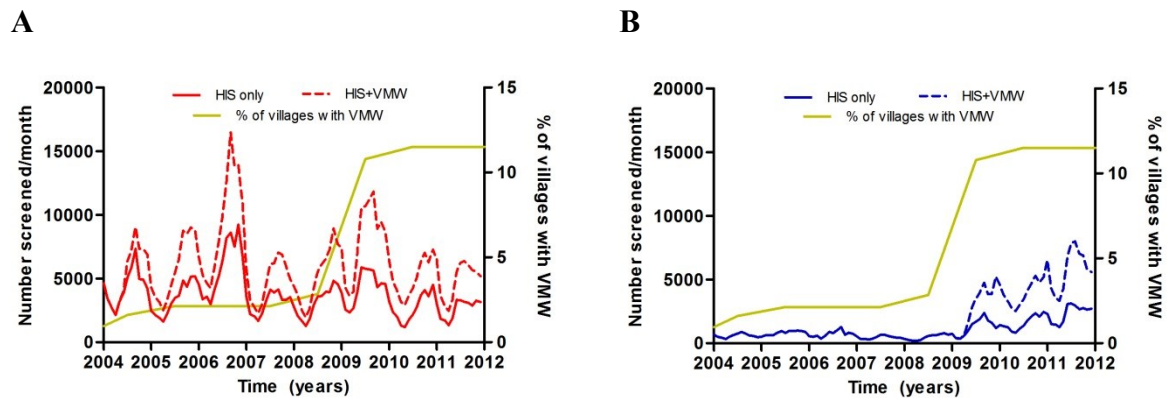


Figure 3.1-9. Additional monthly malaria cases detected by VMWs in Cambodia by species. A *P. falciparum* and B *P. vivax*.

With the scale-up of VMWs, the number of *P. falciparum* malaria cases detected by HIS in ODs with VMWs has decreased in 2010 and 2011 (**figure 3.1-9A**) compared to those ODs without VMWs (**figure 3.1-9B**). The decrease in malaria mortality was also more marked in ODs with VMWs (**figure 3.1-9C**). Numbers of cases of *P. vivax* malaria have increased in both.

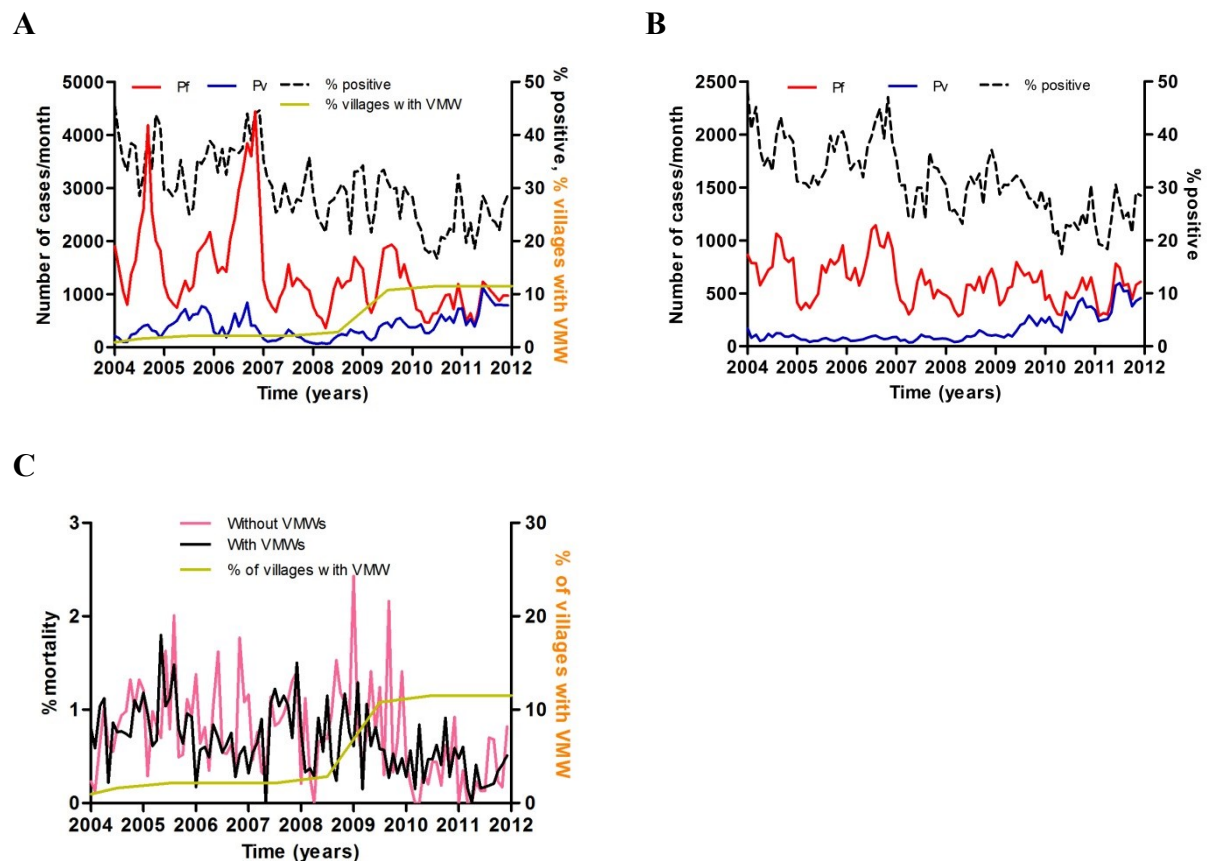


Figure 3.1-10. Malaria cases and deaths over time in villages with and without VMWs.

Cases reported through HIS in ODs with (A) and without (B) Village Malaria Workers. C % mortality in ODs with and without village malaria workers.

Since 2009, there have been VMWs in 41/85 (48%) villages in Pailin OD. The number of *P. falciparum* cases detected by VMWs in Pailin greatly decreased after 2009, similar to the decrease seen in the HIS data (figure 3.1-10A). The number of *P. vivax* and percent of tests which were positive did not change from 2009 to 2011 (figure 3.1-10B and C).

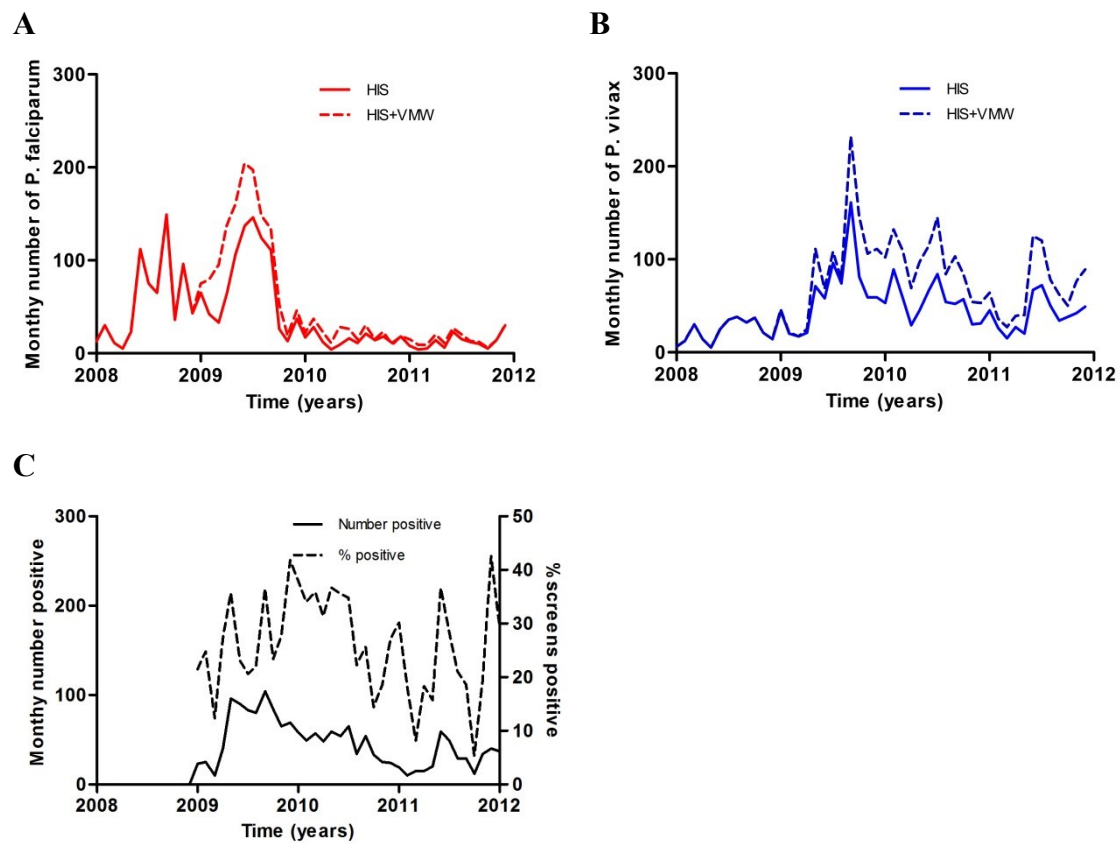


Figure 3.1-11. Additional monthly malaria cases detected by VMWs in Pailin by species.

A *P. falciparum* and **B** *P. vivax* and **C** numbers of positive tests and % of tests positive.

In the northeast, VMWs started work in 2004 and the number of cases of *P. falciparum* detected by them has remained steady since (**figure 3.1-11A**). Numbers of *P. vivax* detected by VMWs in the northeast increased from mid-2009, similar to the number reported through HIS (**figure 3.1-11B**).

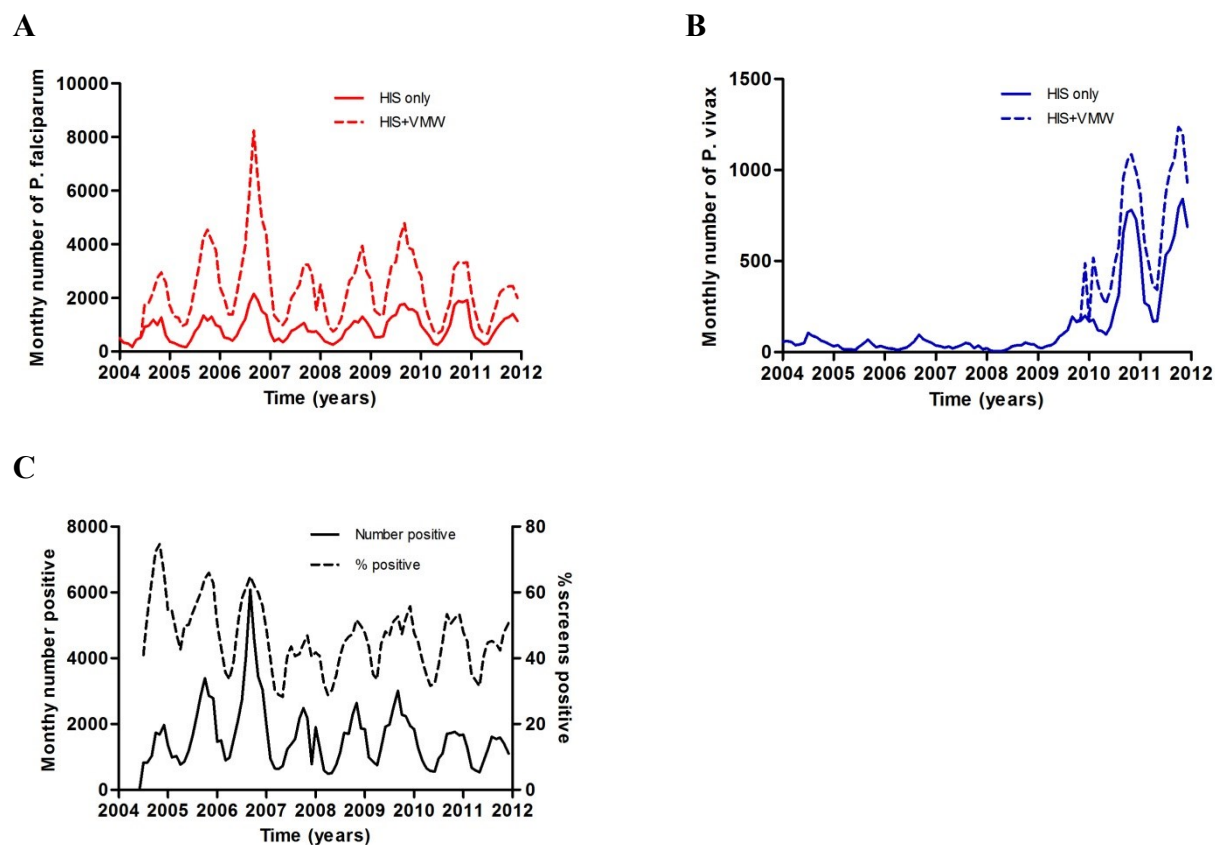


Figure . Additional monthly malaria cases detected by VMWs in the northeast of Cambodia by species.

A *P. falciparum* and **B** *P. vivax*.

3.1.2 Bangladesh

3.1.2.1 Khagrachari

From 2001 to 2011, 692,931 tests were done for malaria in Khagrachari District, Bangladesh, of which 153,321 (22%) were positive. There were on average 13,939 positive cases per year, an API of 25 per 1000 population. 78/71,636 (0.1%) positive cases since 2007 were pregnant. *P. falciparum* alone was found in 92% of malaria cases, 8% *P. vivax* and 0.4% mixed infection. Transmission was markedly seasonal (**figures 3.1-12**) with a peak in *P. falciparum* positive cases in July coinciding with peak rainfall (**figure 3.1-13A**). *P. vivax* cases peaked in June with an additional smaller peak in November (**figure 3.1-13B**). Despite on-going malaria control activities in the area there was no decrease in numbers of *P. falciparum* cases during this period. From 2007, the numbers were higher than in preceding years. *P. vivax* cases decreased after 2009. This is despite an increase in the population of Khagrachari District from 2001 to 2011 of 17%.^{120, 158}

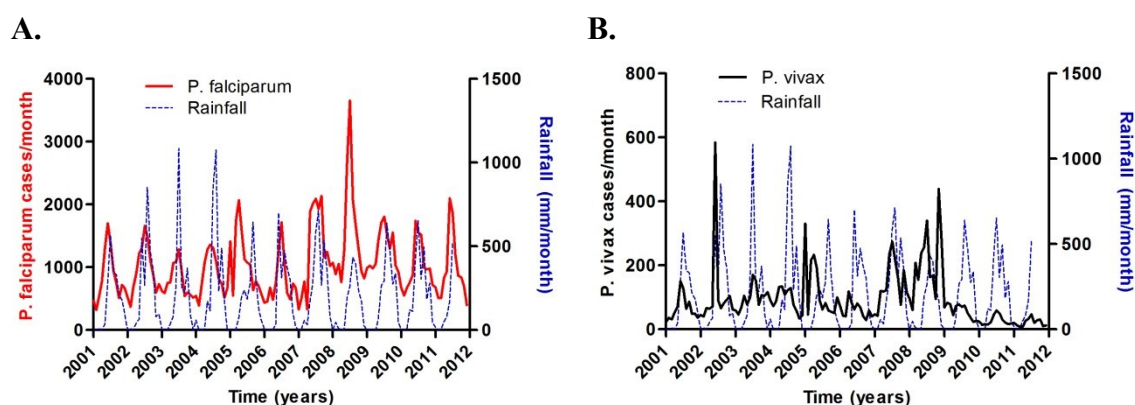


Figure 3.1-12. Number of cases of malaria and rainfall in Khagrachari District and rainfall from 2001 to 2011.

A *P. falciparum* and **B** *P. vivax*.

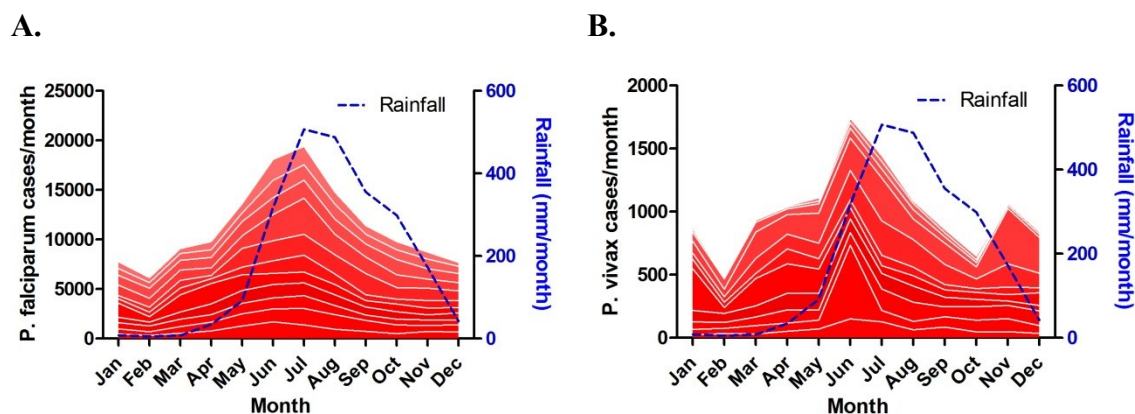


Figure 3.1-13. Cumulative monthly malaria cases and mean monthly rainfall in Khagrachari District from 2001 to 2011.

A *P. falciparum* and **B** *P. vivax*.

The number of people tested for malaria increased from 2006 to 2009 then levelled off (**figure 3.1-14A**). There was a marked increase in testing for malaria during the malaria season. The proportion of tests which were positive remained stable year on year with little seasonal variation. Rapid Diagnostic tests were introduced in 2008, following which fewer tests were done by microscopy (**figure 3.1-14B**).

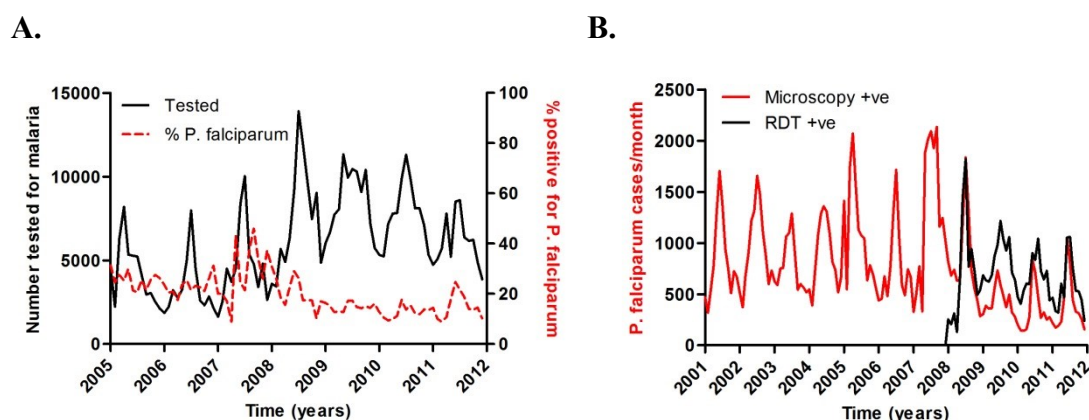


Figure 3.1-14. Monthly malaria tests performed from 2005 to 2011.

A number of people tested and proportion positive for *P. falciparum*. **B** number of people tested by microscopy and RDT.

From 2007 to 2011, 53% of those with *P. falciparum* and 52% of those with *P. vivax* were male. 50% of those with *P. falciparum* and 43% of those with *P. vivax* were aged 15 or over and 18% and 26% under 5 years. In the general population in Bangladesh, it was estimated 2004 51% were male, 62 % age 15 and over and 38% under 5 years.¹⁵⁹ The proportion of *P. falciparum* positive individuals under 1 years of age decreased markedly during this period. That for *P. vivax* did not change. The proportions over 15 years and in males both increased for both species (**figure 3.1-15**).

There was geographic heterogeneity in the distribution of cases (**figure 3.1-16**). Numbers of *P. falciparum* decreased in several sub districts but increased in Dighinala. The distribution of *P. falciparum* cases in 2011 is shown in **figure 3.1-17**. The thana with the highest number of cases in 2011 was Dighinala (32% of the total) with an API of 35/1000 population. Laxmichari thana had the highest API of 62/1000 population.

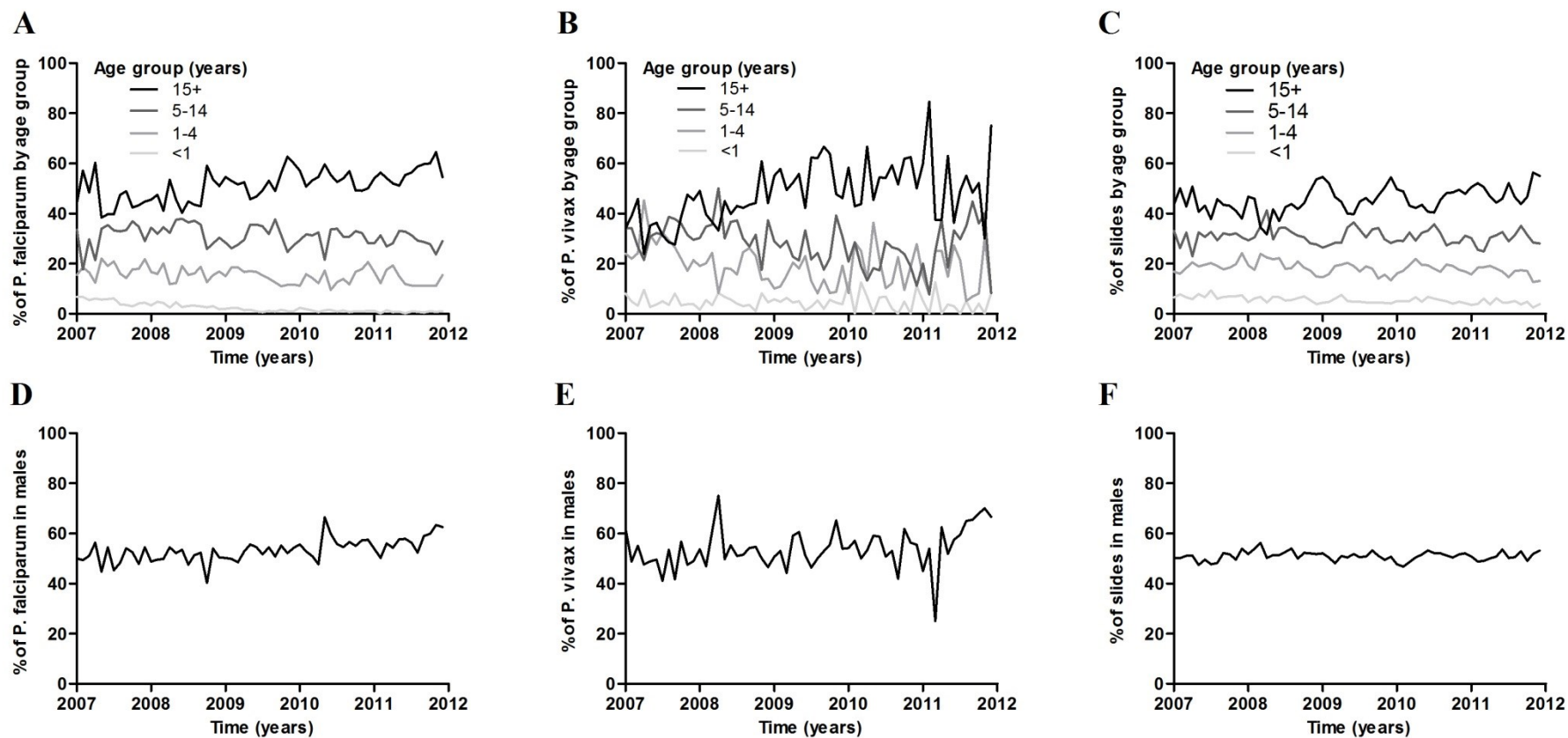


Figure 3.1-15. Proportion of malaria positive individuals by age group and gender in Khagrachari District from 2007-2011.

A age for *P. falciparum*, **B** age for *P. vivax*, **C** age for all tested for malaria with slide, **D** % male for *P. falciparum*, **E** % male for *P. vivax* and **F** % male for those tested by slide.

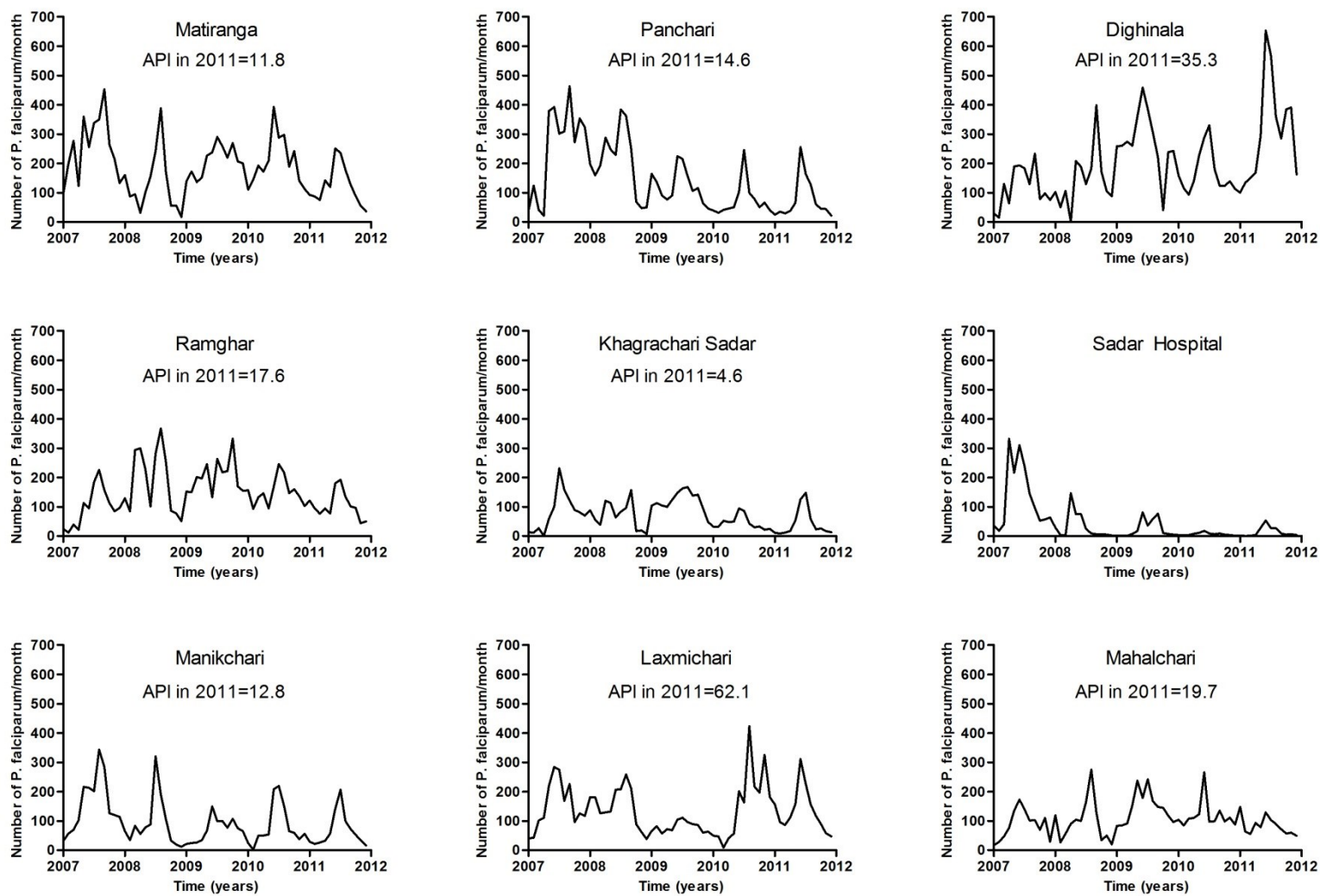


Figure 3.1-16. Number of *P. falciparum* malaria cases in each sub district in Khagrachari District from 2007 to 2011.

API per 1000 population is shown for each sub district.

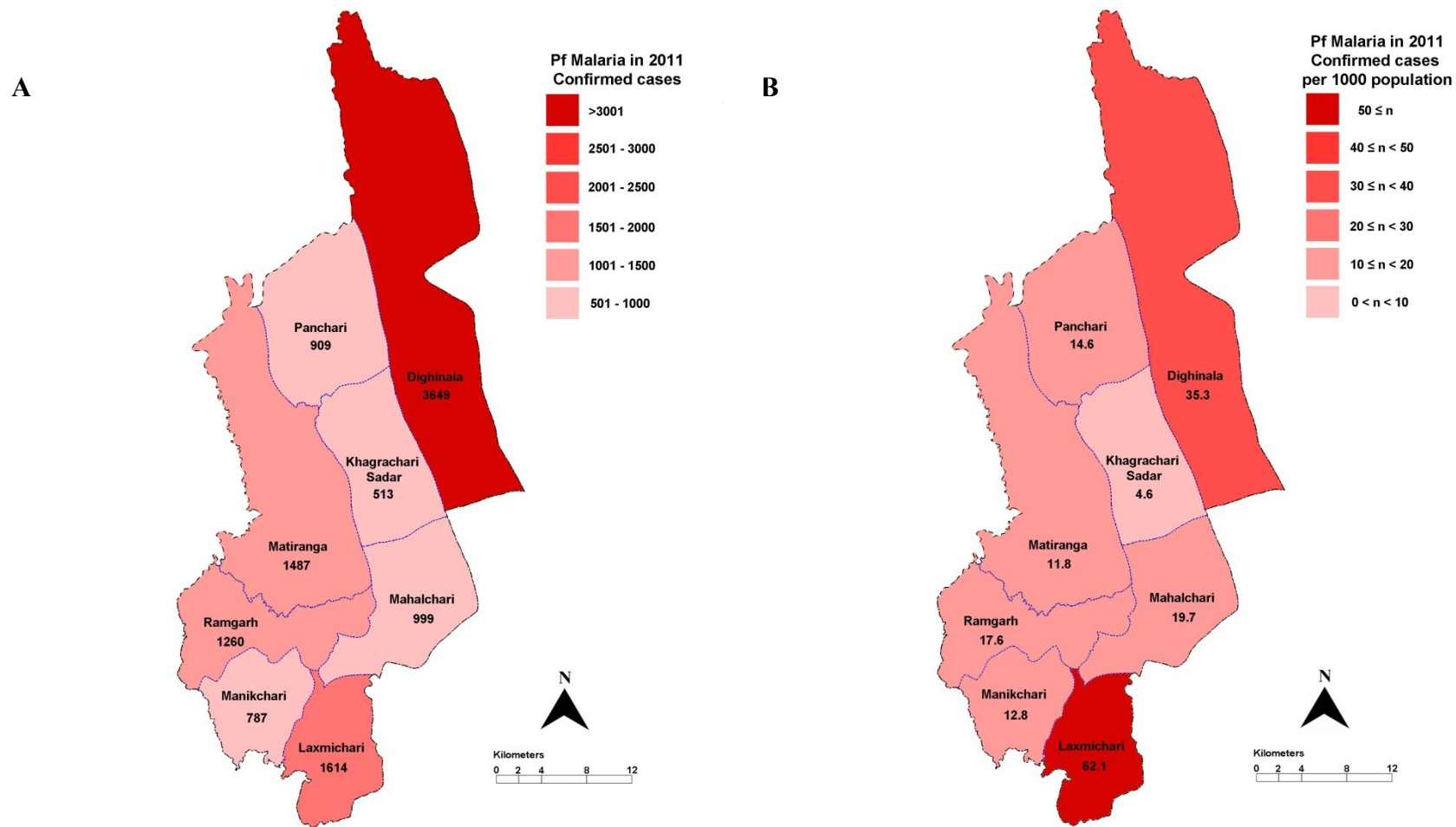


Figure 3.1-17. Geographical distribution of *P. falciparum* malaria cases in Khagrachari District in 2011.

A Absolute numbers of cases, **B** cases per 1000 population.

3.1.2.2 Chittagong

In total, there were 22,785 inpatients screened for malaria at CMCH between January 1999 and December 2011. Of these, 2,394 (11%) were positive, 2295/2,394 (96%) with *P. falciparum* (mean of 177 cases per year), and 93/2,394 (4%) *P. vivax*. One patient (1/2,384 (0.04%)) had *P. malariae*. The median (IQR) age of those who were parasite negative was 25 (15–45) years and those with *P. falciparum* 26 (18–40) years, $p = 0.51$. Of those with *P. falciparum*, 67% were male whereas 59% of parasite negative patients were male ($p = 0.002$). For 251 unselected patients with *P. falciparum* between 2008 and 2011, admission Glasgow Coma Scale and outcome were recorded. GCS was <11 in 103/251 (41%) and 44/251 (18%) died.

The proportions of patients screened for malaria broken down by age group roughly mirrored the structure of the general population (**figure 3.1-18A**). There were two exceptions to this. Those age 5–19 years were under-represented and those age 20–29 years were over-represented. *P. falciparum* was commonest in those aged 15–34 years and the highest proportion positive was in those age 35–39 years (**figure 3.1-18B**). Of those with *P. falciparum*, 24.0% were age 18 years or less. Few *P. falciparum* positive patients were ≤ 10 years (11.6% of positives versus 19.5% of those screened) or over 50 years old (10.1% of positives versus 16.3% of those screened). The age profile of those who died was no different to that of those who survived (**figure 3.1-18C**).

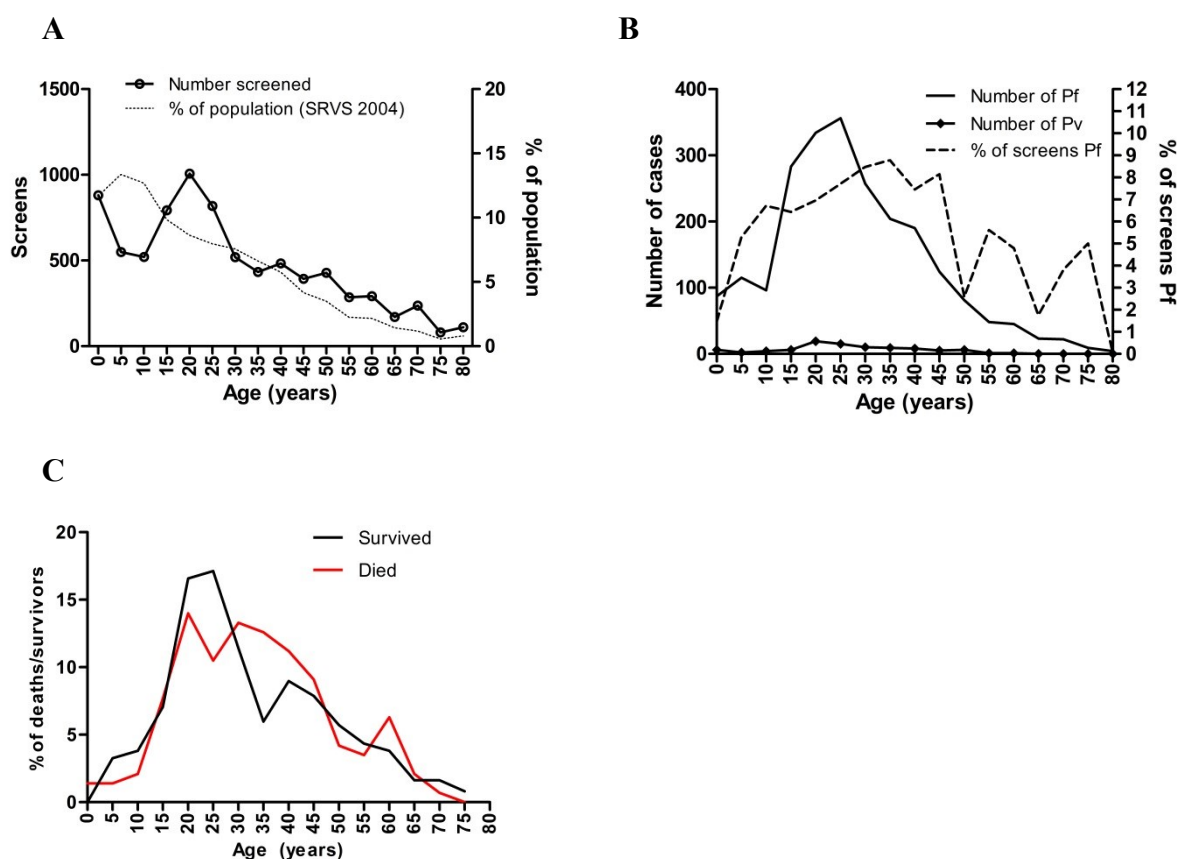


Figure 3.1-18. Age profiles in 5 year age groups.

A screened patients, **B** *P. falciparum* (Pf) and *P. vivax* (Pv) positive patients and **C** percent of total deaths and percent of total survivors. The solid lines are numbers of individuals and the broken lines are percentages. % of population is the percentage of the population in that age group in the 2004 Sample Vital Registration Survey (SRVS).¹⁵⁹

Temporal trends

The annual number of patients screened for malaria at CMCH was highest in 2008–2009 (**figure 3.1-19A**). Both the number and proportion that were positive for *P. falciparum* decreased dramatically from 2007 onwards (**figures 3.1-19A and 3.1-19B**). The number with *P. vivax* decreased from 1999 (**figure 3.1-19B**). The median age of people with *P. falciparum* infection did not change from 1999–2011 (**figure 3.1-19C**). The proportion of

patients with *P. falciparum* from the CHT increased from 2008, although the absolute number did not change (**figure 3.1-19D**).

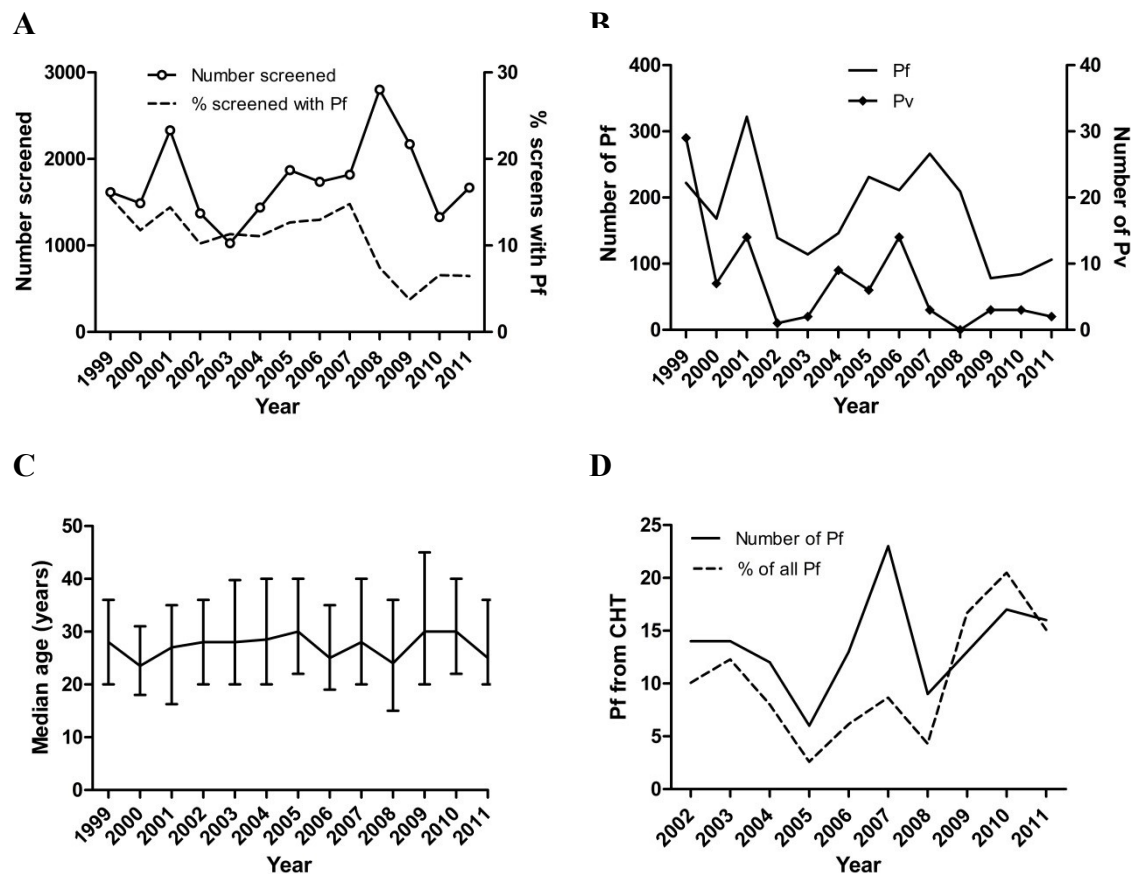


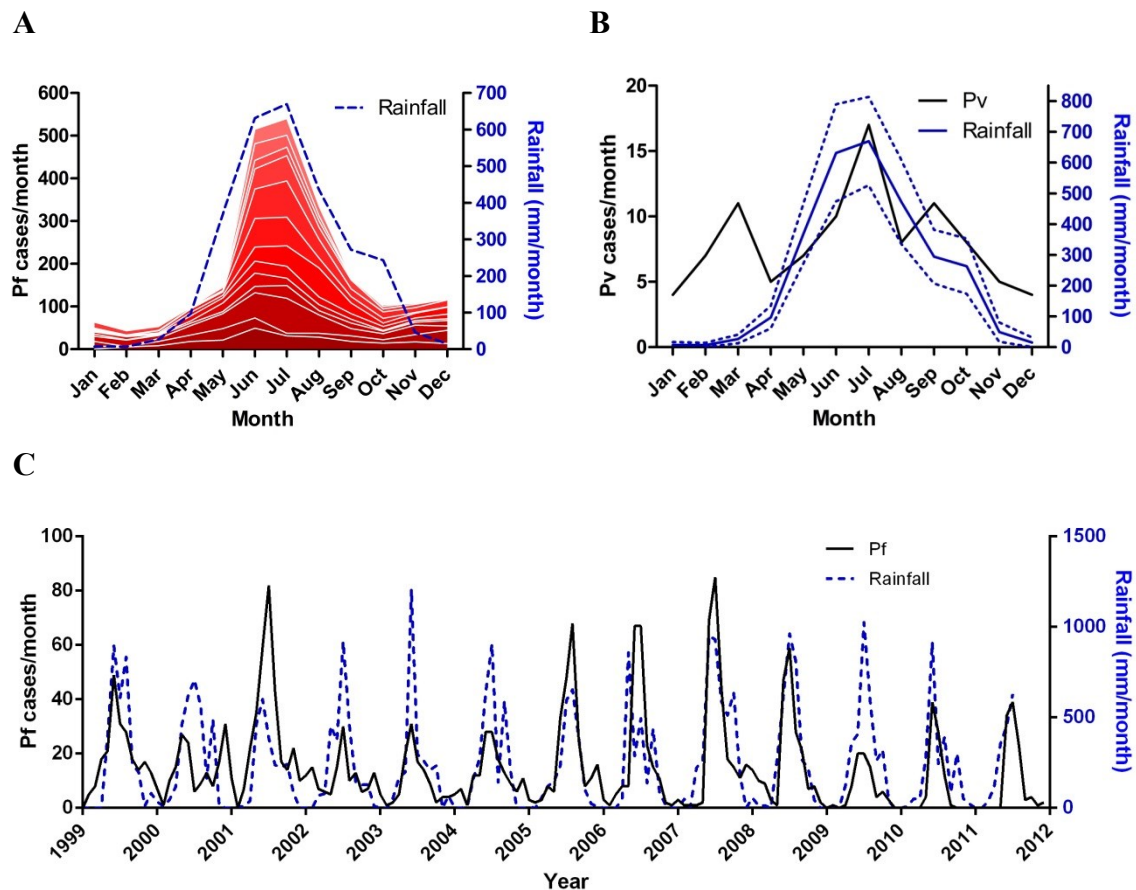
Figure 3.1-19. Long term trends.

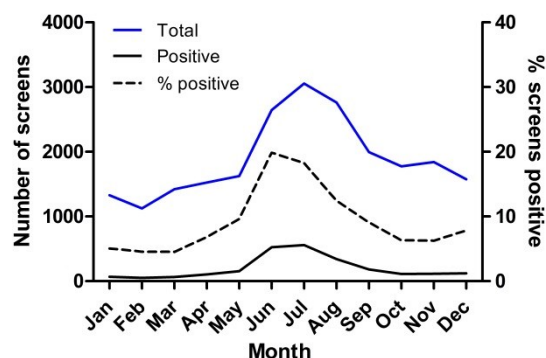
A annual number of individuals screened for malaria and % positive for *P. falciparum*. **B** annual numbers of individuals positive for *P. falciparum* (Pf) and *P. vivax* (Pv) from 1999–2011. **C** Median (IQR) age for those with *P. falciparum* from 1999–2011. **D** Annual number and % of individuals with *P. falciparum* from CHT 2002–2011 (data on area of residence were not collected before 2002).

Seasonality

The number of *P. falciparum* positive cases was highest from June to August and this was highly consistent from 1999 to 2011 (**figure 3.1-20A**). There was a clear association with

the timing of peak rainfall (**figure 3.1-20A**). The amount of rainfall per month correlated with the number of malaria cases in the same month ($p < 0.0001$, $R^2 = 0.40$), consistent with a peak of malaria cases in the wet season (**figure 3.1-20C**). However, the annual amount of rainfall and the total in the wettest three months (June-August) were unrelated to the number of cases of malaria in the same or the following month. There was a different seasonal pattern in *P. vivax* caseload, with a broader peak and an additional peak in February to March 1999 (**figure 3.1-20B**). During the peak transmission season, both the total number of patients screened and the proportion of those that were positive for *P. falciparum* increased (**figure 3.1-20D**). The amplitude of seasonal variation of *P. falciparum* was around 80-90%.



D**Figure 3.1-20. Seasonality.**

Cumulative numbers of monthly cases of *P. falciparum* (Pf) (**A**) and *P. vivax* (Pv) (**B**) at CMCH and rainfall from 1999 to 2011 (shown as average with 95% confidence interval).

In **A**, the bottom band is 1999 and the top band 2011. **C** Cases of *P. falciparum* each month with monthly rainfall. **D** Monthly cumulative numbers screened for malaria with proportion and number positive for *P. falciparum*.

Geographical distribution

Maps of the annual numbers of *P. falciparum* malaria cases by area of residence from 2002 to 2011 are shown in **figure 3.1-21**.

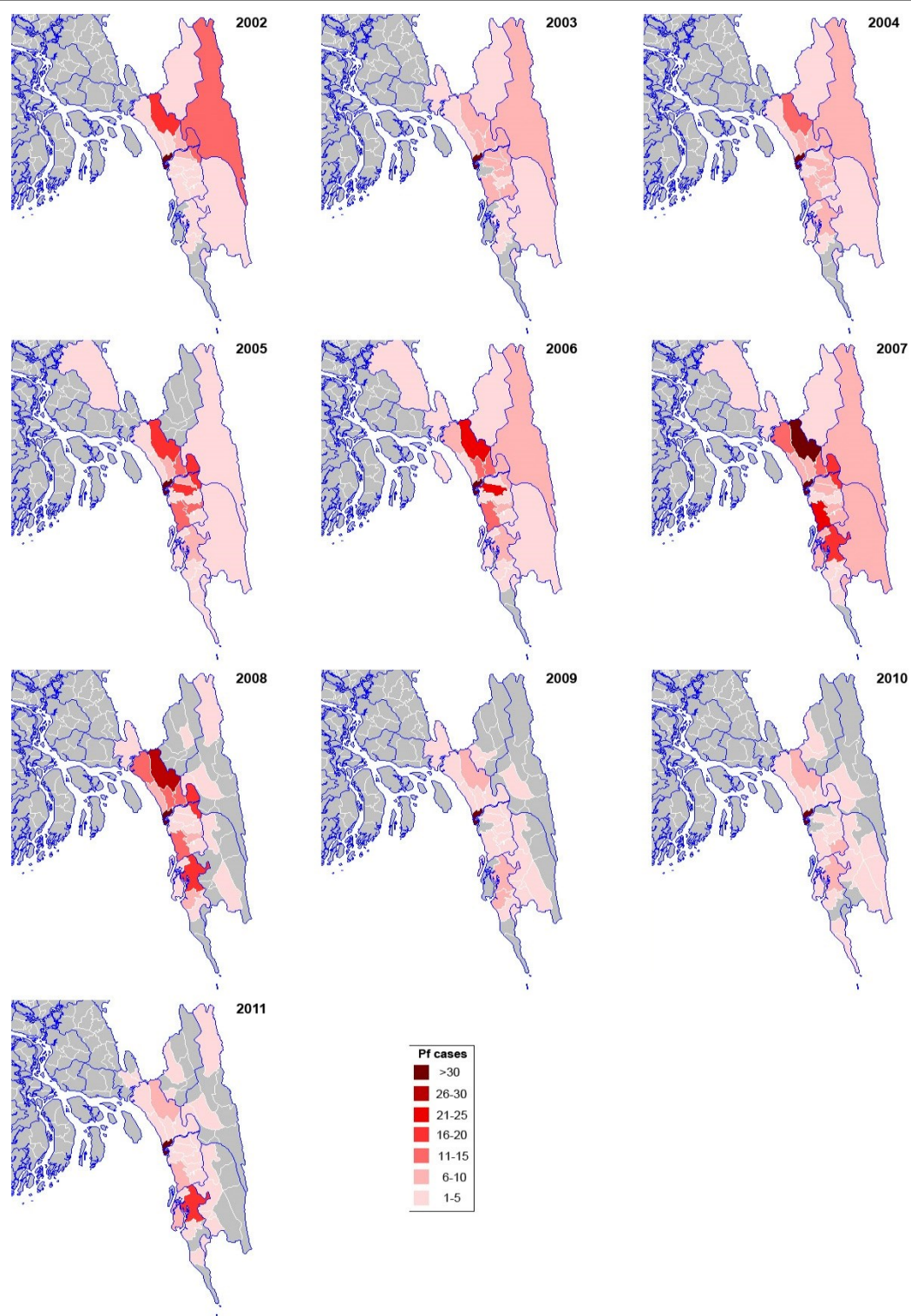


Figure 3.1-21. Maps of annual number of cases of *P. falciparum* in CMCH by area of residence from 2002-2011.

Before 2008, data on Thana of residence were not available for those from the CHT.

The numbers of malaria cases from each District are summarized in **table 3.1-1**. The geographical distributions of patients screened and positive for malaria are shown in **figures 3.1-21 and 3.1-22**. In **figure 3.1-22A** the three Districts in pale yellow on the right are the CHT. Overall, 400/7950 (5%) screened patients and 57/484 (12%) malaria positive patients (96% *P. falciparum*) were resident in the CHT in 2008–2011. 86/1147 (7.5%) malaria positive patients from 2002–2007 were resident in the CHT. The annual number of malaria cases that lived in the CHT varied from 2002–2011 although numbers were small and there was no clear overall trend (**figure 3.1-19D**).

Table 3.1-1. Number screened, *P. falciparum* (Pf) and *P. vivax* (Pv) positive cases by District of residence from 2008-2011.

District	Screened (%)		Pf (%)		Pv (%)		% of screens positive
Chittagong	6576	(82.7%)	310	(65.0%)	4	(50.0%)	5%
Cox's Bazar	696	(8.8%)	91	(19.1%)	1	(12.5%)	13%
Bandarban	153	(2.0%)	28	(5.9%)	1	(12.5%)	19%
Khagrachari	100	(1.3%)	8	(1.7%)	1	(12.5%)	9%
Rangamati	147	(1.8%)	19	(4.0%)	0	(0.0%)	13%
Feni	156	(1.9%)	13	(2.7%)	1	(12.5%)	9%
Other (Chittagong Division)	61	(0.8%)	0	(0.0%)	0	(0.0%)	0%
Other (Other Divisions)	11	(0.1%)	3	(0.6%)	0	(0.0%)	27%
Unknown	50	(0.6%)	5	(1.0%)	0	(0.0%)	10%
Total	7950		477		8		6%

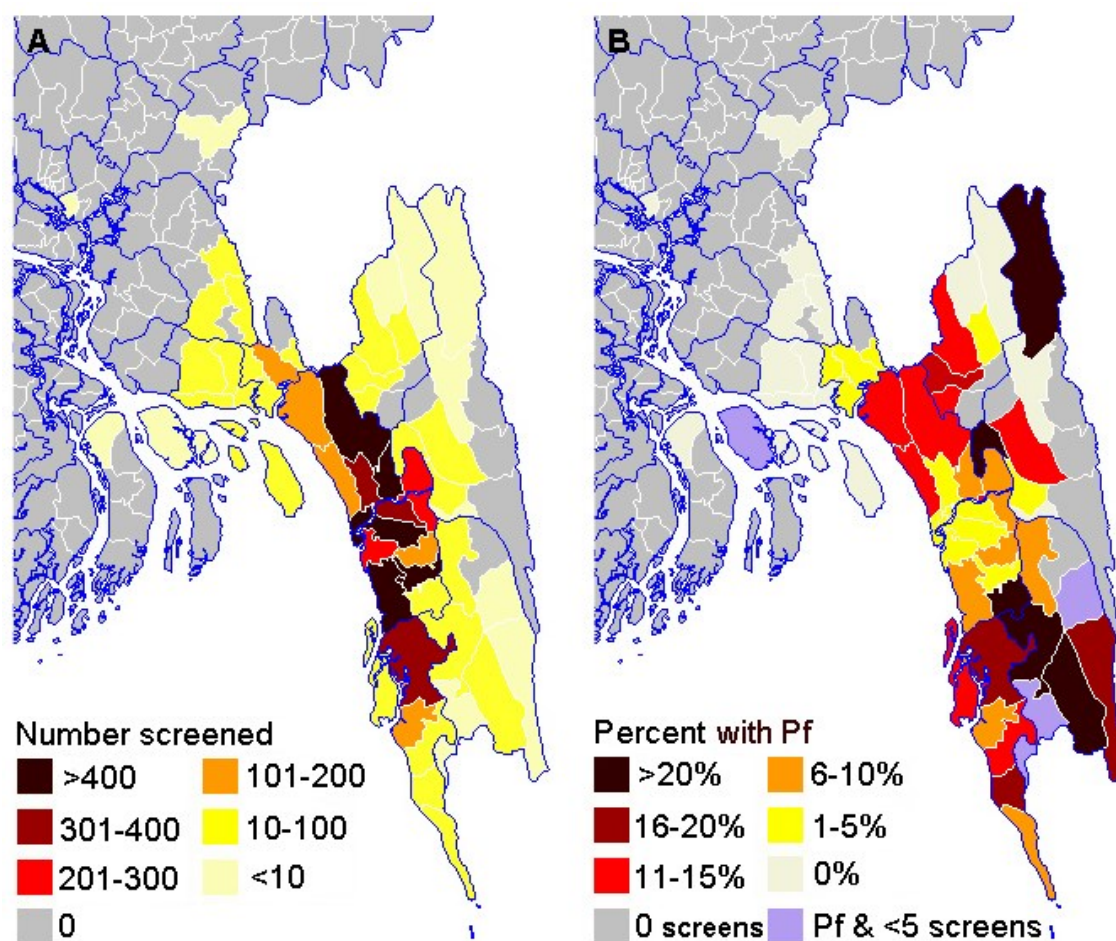


Figure 3.1-22. Geographical distribution of malaria.

A patients screened for malaria at CMCH 2008–2011 shown as number per Thana. **B** Percent of those screened in each Thana who were positive for *P. falciparum*. Where a Thana had case(s) of *P. falciparum* but less than 5 individuals were screened, percentages are not shown. Thana boundaries are white and District boundaries blue.

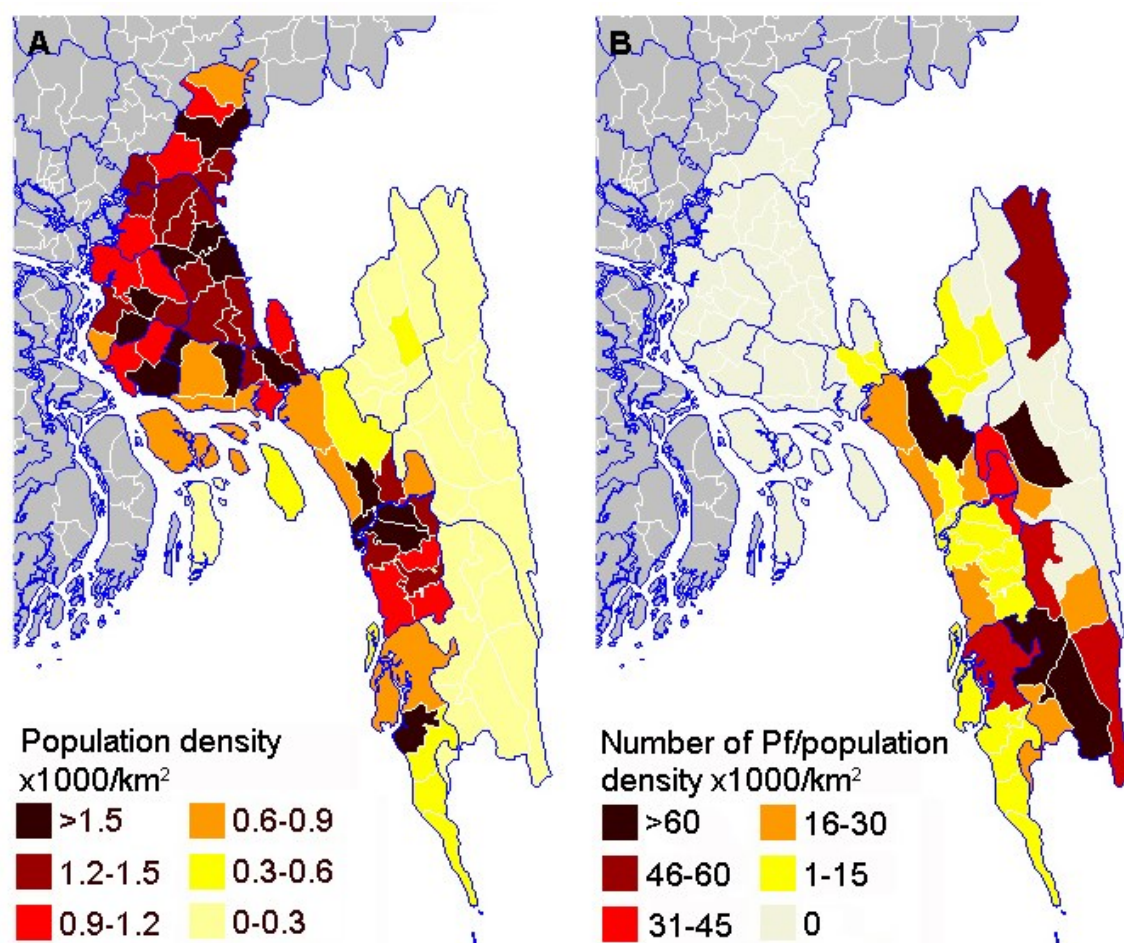


Figure 3.1-23. Population density and malaria.

A Population density in Chittagong Division from the 2001 census.¹⁵⁸ The CHT are the three Districts in pale yellow on the right. **B** Number of *P. falciparum* malaria cases seen at CMCH 2008–2011 per 1000 population km⁻². Thana boundaries are white and District boundaries blue.

Travel

In 2006 to 2011 travel in the 3 weeks before admission was recorded for 266 unselected patients with severe malaria (**table 3.1-2**). All patients surveyed were resident in Chittagong Division, 41/266 (15%) of whom lived in the CHT. A total of 74/266 (28%)

had visited another Thana in the preceding 3 weeks of whom 47/74 (64%) had visited the CHT but did not live there. Thus, in total, 88/266 (33%) had been in the CHT in the preceding 3 weeks. All patients who travelled to Thana in the CHT were resident outside of the CHT. 10/266 (3.8%) of the patients lived in Chittagong City and had not travelled, thus suggesting there is malaria transmission in the city itself.

Table 3.1-2. Travel to another Thana within the 3 weeks preceding admission.

Place	Travelled				Did not travel	
	Destination		Residence		Residence	
Chittagong District	20	(27.0%)	45	(60.8%)	104	(54.2%)
Chittagong city	5	(6.8%)	36	(48.6%)	10	(5.2%)
Other	15	(20.3%)	9	(12.2%)	94	(49.0%)
Cox's Bazar District	2	(2.7%)	13	(17.6%)	36	(18.8%)
Chittagong Hill Tracts	47	(63.5%)	0	(0.0%)	41	(21.4%)
Bandarban District	19	(25.7%)	0	(0.0%)	19	(9.9%)
Khagrachari District	8	(10.8%)	0	(0.0%)	8	(4.2%)
Rangamati District	10	(13.5%)	0	(0.0%)	14	(7.3%)
Thana not specified	10	(13.5%)	0	(0.0%)		(0.0%)
Unknown	5	(6.8%)	16	(21.6%)	11	(5.7%)
Total	74		74		192	

Responses to the question “did you travel anywhere within the previous 3 weeks?” for 266 patients with *P. falciparum* malaria from 2006–2011 and Thana of residence for those who did and did not travel.

3.2 Parasite clearance

3.2.1 Parasite clearance rates in Cambodia and Thailand

Drug response dynamics and recrudescence in artemisinin-resistant *Plasmodium falciparum* malaria

From Pailin, data from 120 patients were included and from Wang Pha, 40 patients. One patient in Pailin was excluded as their parasite count was too low to calculate a clearance rate constant (<1000 parasites/ μ l). Because of the different trials they were enrolled in, patients received a variety of oral treatment regimens, as shown in **table 3.2-1**.

Parasite clearance

Parasite clearance rate constants were lower in Pailin (median (interquartile range (IQR)) 0.12 (0.10-0.15)) than in Wang Pha (0.22 (0.18-0.32), $p<0.0001$, **figures 3.2-1A and 3.2-1B**). Although there was overlap between the groups, the majority of patients in Pailin (105/120 (88%)) had clearance rates below the 25th percentile for Mae Sot. Parasite clearance rates were more broadly distributed in Wang Pha. There was a 6.8-fold difference between the highest and the lowest clearance rates in Wang Pha and 4.7-fold in Pailin. In Pailin, 32/120 (27%) patients had a lag phase before parasite clearance of median (IQR) 8 (6-12) hours. In Wang Pha, 10/40 (25%) had a lag of 6 (4-8) hours.

Table 3.2-1. Numbers of patients, treatment regimens and numbers of recrudescent infections in patients with *P. falciparum* malaria in Pailin and Wang Pha.

The percentages are the proportion in each group which recrudesced. AS7=artesunate for 7 days, MAS3=artesunate mefloquine i.e. artesunate for 3 days with mefloquine 15mg/kg on the third day and 10mg/kg on the fourth day, mg=mg/kg of artesunate, od=once a day, bd=twice a day.

Treatment	Pailin				Wang Pha			
Code	Cleared	Recrudesced	Total		Cleared	Recrudesced	Total	
AS7,2mg od	14	6 (30%)	20		18	2 (10%)	20	
AS7,6mg od	10	0 (0%)	10		0	0	0	
AS7,3mg bd	10	0 (0%)	10		0	0	0	
MAS3,4mg od	20	1 (5%)	21		19	1 (5%)	20	
MAS3,6mg od	11	4 (27%)	15		0	0	0	
MAS3,3mg bd	15	0 (0%)	14		0	0	0	
MAS3,8mg od	14	0 (0%)	15		0	0	0	
MAS3,4mg bd	15	0 (0%)	15		0	0	0	
Total	109	11 (9%)	120		37	3 (8%)	40	

This difference in clearance rates between the two sites was true for both artesunate monotherapy (0.12 (0.10-0.17) versus 0.20 (0.16-0.26), $p=0.0002$) and artesunate-mefloquine (0.12 (0.10-0.15) versus 0.26 (0.18-0.34), $p<0.0001$), despite higher doses being used in many patients in Pailin, and regardless of the dose of artesunate received (**figure 3.2-1C**). Parasite clearance rates did not change significantly with increasing doses of artesunate (**figure 3.2-1D**) and did not correlate with log peak plasma levels of

either artesunate or dihydroartemisinin at either site or for either treatment. Patients in the two sites had similar peak artesunate and DHA levels in their blood (median (IQR) [artesunate] 279 (126-429) ng/mL in Pailin versus 201 (138-336) ng/mL in Wang Pha, $P=0.44$ and [DHA] 1030 (731-1530) ng/mL versus 1077 (704-1564) ng/mL, $P=0.93$). 7/40 (18%) of the patients in Wang Pha had clearance rates similar to the majority in Pailin. These 7 patients had similar peak artesunate and DHA levels to the other 33 from Wang Pha with higher clearance rates (median (IQR) [artesunate] 145 (131-231) ng/mL versus 206 (143-343) ng/mL, $P=0.23$ and [DHA] 1076 (426-1134) ng/mL versus 1101 (723-1668) ng/mL, $P=0.23$).

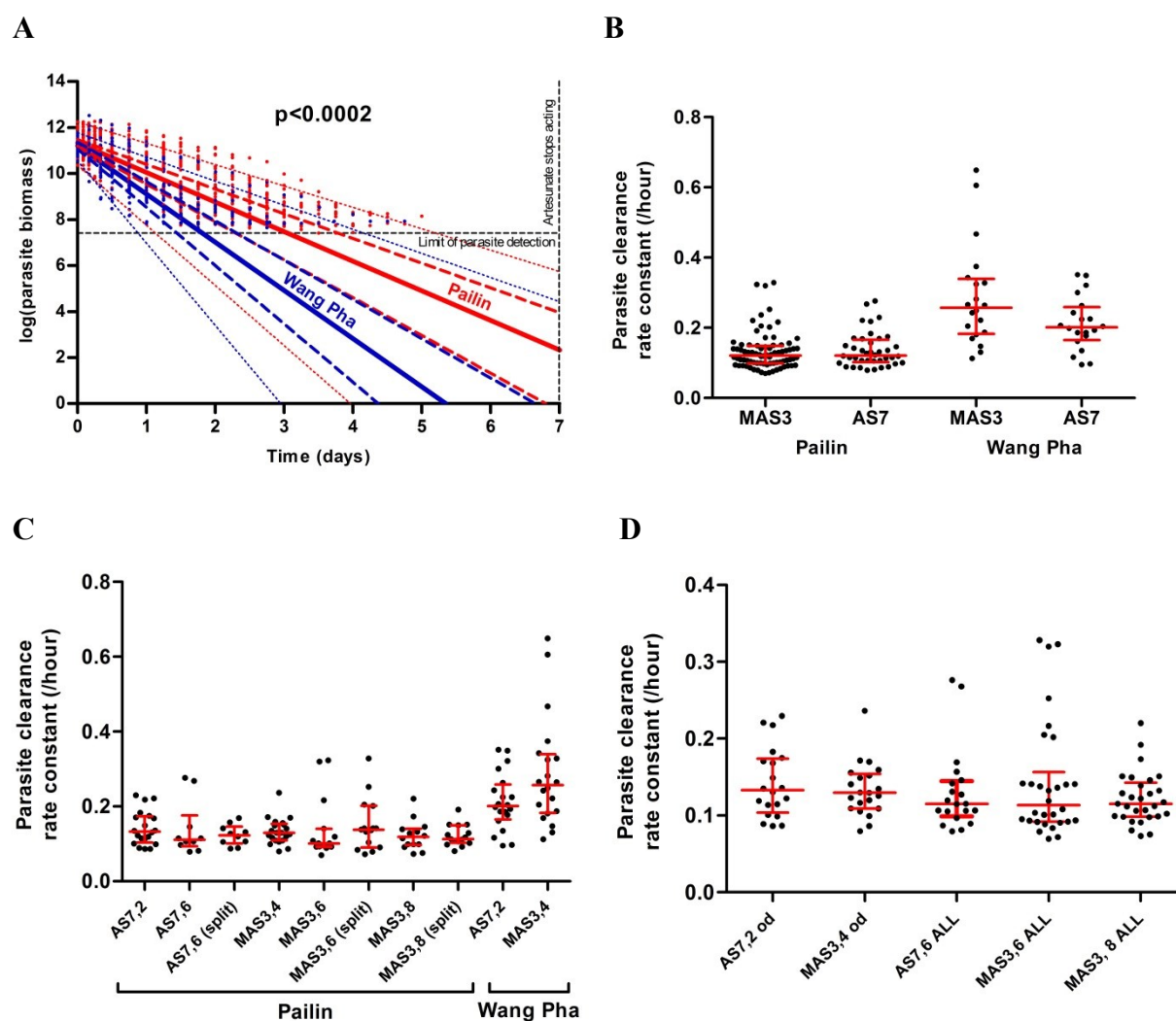


Figure 3.2-1. Parasite clearance in Pailin compared to Wang Pha.

A log parasite biomass against time showing raw data for all patients receiving 7 days of artesunate monotherapy with median (solid lines), quartile (coarse dotted lines) and range (fine dotted lines) parasite clearance lines shown for Wang Pha (blue) and Pailin (red). **B** Parasite clearance rate constants for all those who received artesunate monotherapy (AS7) compared to artesunate-mefloquine (MAS3). **C** all dosing regimens given in the trial are shown separately. For each, the number shown after the comma is the dose of artesunate/kg given, od=once a day, bd=twice a day. **D** clearance rates due to different

doses/kg/24 hours artesunate in Pailin. ALL=od plus bd. In **B**, **C** and **D** the lines indicate medians and IQRs.

Recrudescence

The numbers of recrudescences in each group are shown in **table 3.2-1**. In Pailin, 109/120 (91%) patients were successfully treated and 11 (9%) had recrudescence infections. In Wang Pha, 3/40 (7.5%) were recrudescence infections ($p=1.0$). There was no difference in the median (IQR) parasite clearance rate constants in recrudescence versus non-recrudescence (cured) infections at either site or with any dose regimen. In Pailin, the median (IQR) parasite clearance rate in recrudescence infections was 0.13 (0.09-0.22)/hour and non-recrudescence infections 0.12 (0.10-0.15)/hour, $p=0.60$, **figure 3.2-2A**). There were no differences in the proportion with, or length of, lag phase before parasite clearance between recrudescence and non-recrudescence infections in Pailin (3/11 (27%) versus 29/109 (27%), $P=1.0$). In non-recrudescence infections that were treated with 7 days of artesunate, in Wang Pha, 5/18 (27.7%) and in Pailin 27/34 (79%) did not reach parasite biomass of zero before 7 days and thus did not clear their infection.

In Pailin, 6/40 (15%) who received 7 days of artesunate monotherapy had a recrudescence and 5/80 (6%) of those who received artesunate-mefloquine ($p=0.18$). In those who received monotherapy, the median (IQR) clearance rate for recrudescence infections ($n=6$) was 0.19 (0.12-0.22)/hour and for cleared infections ($n=34$) 0.12 (0.10-0.15)/hour ($p=0.085$, **figure 3.2-2B**). All 6 of these recrudescences were in the 20 patients that received 2mg/kg once a day (6/20 (30%)) with none in those that received 6mg/kg/day (0/20 (0%), $P=0.02$). Of the 5 recrudescence infections in Pailin that received MAS3 one

received 4mg/kg/day (1/21 (5%)) and 4 received 6 mg/kg/day (4/30 (13%)). In Wang Pha, 2/3 recrudescences were in patients who received 2mg/kg/day artesunate monotherapy for 7 days (2/20 (10%) vs. 6/20 (30%) in Pailin: $P=0.24$) and the other patient with a recrudescence received MAS3 with 4mg/kg/day artesunate (1/20 (5%)). The peak concentrations of artesunate and dihydroartemisinin were the same in cured and recrudescence infections at both sites (in Pailin median (IQR) [artesunate] 264 (52-644) ng/mL in recrudescence infections versus 282 (142-397) ng/mL in cleared infections, $P=0.93$, and [DHA] 1080 (614-1480) ng/mL versus 1030 (741-1830) ng/mL, $P=0.81$).

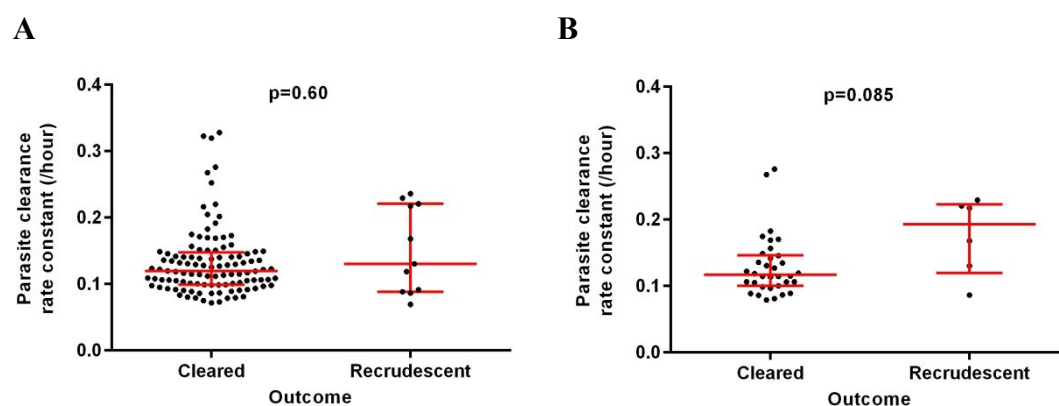


Figure 3.2-2. Parasite clearance rate constants in cleared and recrudescence infections in Pailin.

A 120 patients in Pailin comparing non-recrudescence ('cleared', $n=109$) with recrudescence ($n=11$) infections and **B** 40 patients in Pailin treated with AS7 only; cleared ($n=34$) versus recrudescence ($n=6$). The red lines are medians and IQRs.

The median clearance slopes for recrudescence and non-recrudescence infections who received artesunate monotherapy in Pailin are shown in **figure 3.2-3A** and the individual clearance slopes for the 6 who had a recrudescence in **figure 3.2-3B**.

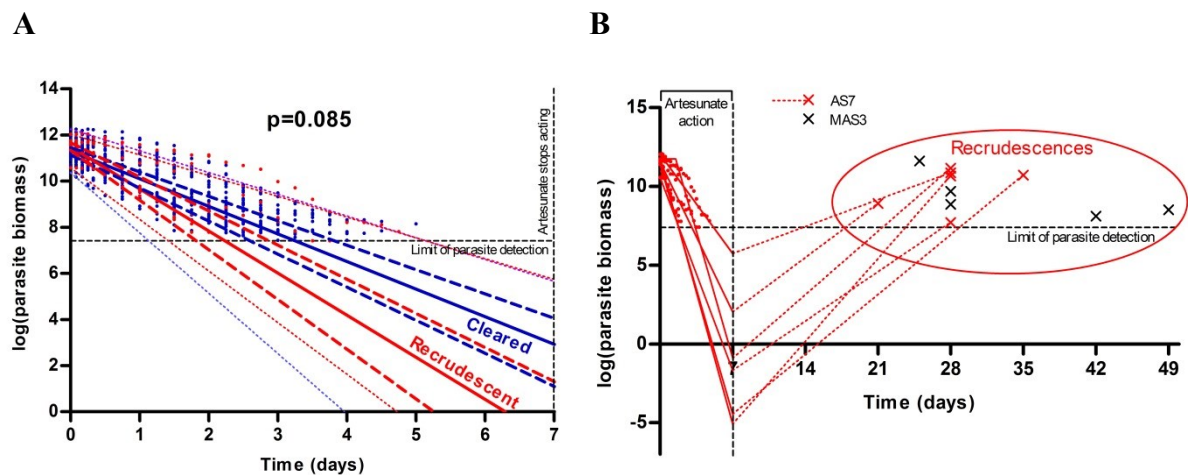


Figure 3.2-3. Parasite clearance slopes and recrudescence in Pailin.

A median parasite clearance slopes and individual parasite biomasses over time for 6 recrudescence and 34 non-recrudescence infections who received 7 days of artesunate 2mg/kg once a day in Pailin. **B** individual parasite clearance slopes for the 6 recrudescence infections. Recrudescences are shown as red crosses with dotted lines extrapolated back to meet the clearance lines at 7 days. The 5 recrudescences in patients who received MAS3 are shown in black.

Fitness cost

The estimated fitness costs for patients receiving artesunate monotherapy are given in **table 3.2-2**. Of these infections, 4/6 (67%) reached a projected biomass of zero before 7 days, thus requiring method 2, and 2/6 (33%) did not, requiring method 1. In Wang Pha, two patients who received artesunate monotherapy recrudescence and their multiplication rates were 0.78/hour and 0.39/hour (mean 0.59/hour i.e. an 11.2-fold increase per 48 hours).

Table 3.2-2. Estimated fitness cost of presumed artemisinin resistant recrudescent infections in Pailin following 7 days of treatment with artesunate monotherapy.

‘6-fold’, ‘8-fold’ and ‘10-fold’ refer to the fitness cost calculated using the assumed multiplication rates for fit parasites (0.39/hour, 0.45/hour and 0.50/hour respectively).

‘Wang Pha’ is the fitness cost calculated using the mean multiplication rate of recrudescent infections in Wang Pha (0.59/hour) as the comparator value. Method 1 and Method 2 are the methods used to calculate the fitness cost and are explained in the Methods section.

Fitness cost in Pailin vs.:						
Patient	Method	Multiplication				
		rate (log/24h)	6-fold	8-fold	10-fold	Wang Pha
1	2	0.46	-18%	-2%	8%	22%
2	1	0.49	-27%	-9%	1%	16%
3	2	0.30	23%	34%	40%	49%
4	2	0.41	-6%	9%	18%	30%
5	1	0.25	37%	46%	51%	58%
6	2	0.33	16%	28%	35%	44%
Mean		0.37	4%	17%	25%	37%

3.2.2 Parasite clearance slope half-lives in severe malaria in Bangladesh

Evaluation of the *in vivo* responsiveness of *P. falciparum* to intravenous artesunate

Three hundred and forty four patients from Chittagong with severe malaria were screened for eligibility. Of these, 137 (40%) were excluded from the analysis; 16/137 (12%) because they had received intravenous quinine. Of the other 121 excluded patients, 64/121 (53%) had too few parasite counts to calculate a clearance half-life, 24/121 (20%) had an initial parasite count of $<1000/\mu\text{l}$ and 33/121 (27%) a final parasite count greater than $1000/\mu\text{l}$. 64/121 (53%) of these patients died, 38/121 (31%) within 12 hours of the first parasite count being taken.

The remaining 207 patients were included in the analysis. All had severe *P. falciparum* malaria treated with intravenous artesunate. Although not part of the treatment protocol, all patients had cleared parasites from the peripheral blood before switching to oral treatment thus all clearance half-lives were during treatment with intravenous artesunate only. An initial lag phase of median (interquartile range (IQR)) duration of 7.4 (6.0-12.9) hours was present in 57/207 (28%) patients. A terminal tail was present in 32/207 (15%) patients and outliers in 2/207 (2%) patients. The median (IQR) number of remaining parasite counts during artesunate monotherapy treatment in these patients was 9 (5-13). The parasite clearance half-lives over the period of the study are shown in **figure 3.2-4**. The median (IQR) parasite clearance half-life was 2.8 hours (2.1-3.7 hours) and median parasite clearance rate constant 0.25/hour (0.19-0.34/hour). There was a wide (9-fold) variation of parasite clearance half-lives between individuals in all patient groups.

In Chittagong, there was an increase in parasite clearance half-life over time from 2004-2012 (Spearman $r=0.172$ (95% CI=0.033-0.305), $P=0.013$) (**figure 3.2-4**). After accounting for age, gender, whether or not the patient lives in CHT and or Cox's Bazar (high malaria prevalence areas), duration of fever prior to admission, haematocrit, initial parasitaemia, initial parasite biomass and whether or not the infection was fatal the increase in parasite clearance half-lives over the period of the study remained significant ($p=0.001$) with a total magnitude of 5% (95% CI =2-8%). This is similar to the magnitude of increase seen in Mae Sot over the period 2003-2010 (Spearman $r=0.094$ (95% CI=0.055-0.132), $P<0.0001$), although the numbers in the Mae Sot study were much larger. Parasite clearance half-lives increased from a median of 2.3 (1.8-3.1) hours in 2004 to 3.1 (2.6-4.5) ($p=0.02$) in 2012 compared to Wang Pha with 2.6 h (IQR 2.5-2.7) in 2001 and 3.7 h (3.6-3.8) in 2010 ($p<0.0001$). According to the definition used previously¹⁰⁸ 9/207 (4.3%) of infections in Bangladesh were slow clearers (half-life ≥ 6.2 hours). There were 0-2 slow clearing infections each year and the proportion of slow clearers per year did not change over time.

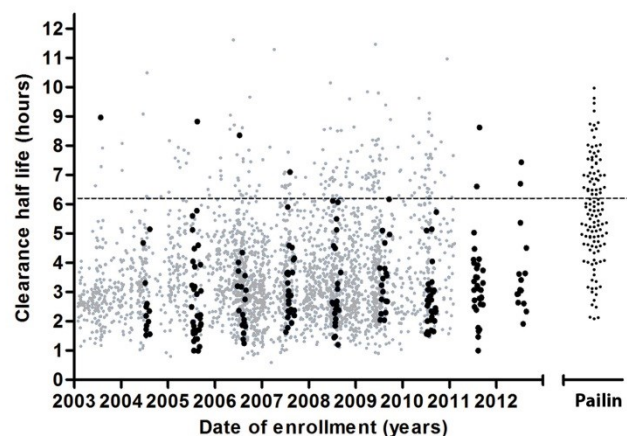


Figure 3.2-4. Parasite clearance half-lives from 2003-2012 in Thailand (blue) and Bangladesh (black).

For comparison, parasite clearance half-lives from 119 patients in Pailin Cambodia are shown on the right.

Parasite clearance half-lives in fatal cases ($n=45/207$ (21.7%), median (IQR) 2.7 hours (2.0-3.5)) were no different to those in cases who survived (2.9 hours (2.1-3.8), $p=0.37$) (**figure 3.2-5**). There was no association between parasite clearance half-lives and coma recovery times to GCS >10 ($p=0.55$) or GCS 15 ($p=0.31$).

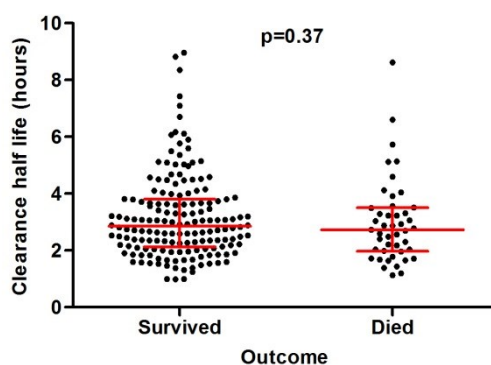


Figure 3.2-5. Distribution of parasite clearance half-lives in patients with severe malaria from Chittagong who survived ($n=162$) and died ($n=45$).

Median parasite clearance half-lives in Chittagong in patients treated with 2.4mg/kg intravenous artesunate were no different from those with oral artesunate in 40 patients in Wang Pha, northwest Thailand (median (IQR) 3.1 hours (2.2-3.9), $p=0.37$), but significantly faster than in 120 patients in Pailin, western Cambodia (median (IQR) 5.7 hours (4.6-7.0), $p<0.0001$), where artemisinin resistance is known to be present (**figure 3.2-6A**).

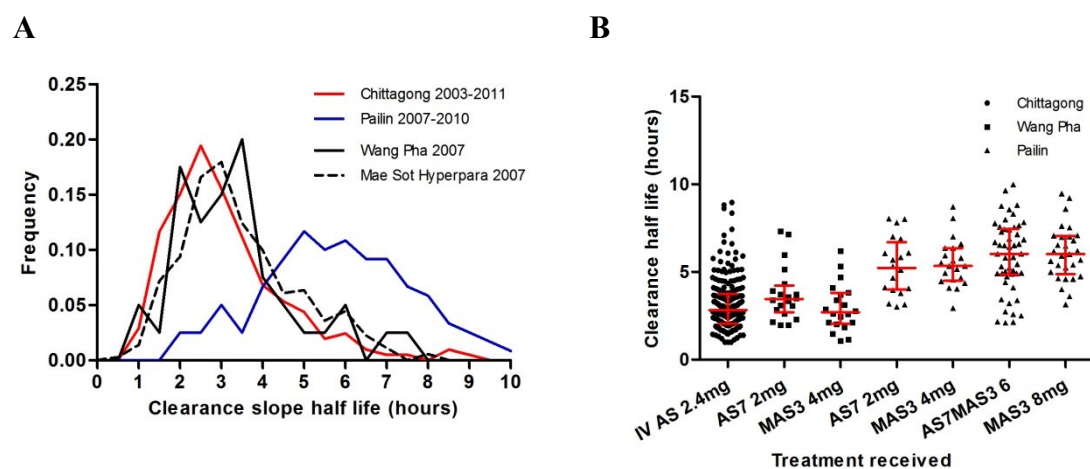


Figure 3.2-6. Parasite clearance half-lives in Chittagong, Wang Pha and Pailin.

A all regimens combined. **B** different dosing shown separately. Chittagong = red/circles, Wang Pha = black/squares and Pailin = blue/triangles. IV AS = intravenous artesunate 2.4 mg/kg/dose, AS7 = oral artesunate for 7 days, MAS3 = oral artesunate for 3 days plus mefloquine on days 3 and 4. 2mg=2mg/kg/day, 4mg=4mg/kg/day, etc.

A variety of doses and dosing schedules were used for oral artesunate in Pailin and Wang Pha (**figure 3.2-6B**). Median parasite clearance half-lives were shorter with intravenous artesunate in Chittagong than with oral artesunate in Pailin for all dose schedules: 2mg/kg/24 hours (20 patients, median (IQR) 5.2 hours (4.0-6.7), $p<0.0001$), 4mg/kg/24 hours (21 patients, median (IQR) 5.3 hours (4.5-6.4), $p<0.0001$), 6mg/kg/24 hours (50

patients, median (IQR) 6.0 hours (4.9-7.5), $p<0.0001$) and 8mg/kg/24 hours (29 patients, median (IQR) 6.0 hours (4.9-7.0), $p<0.0001$). In Wang Pha, median parasite clearance half-lives with 4mg/kg (20 patients (2.7 hours (2.0-3.8), $p=0.78$) were no different from those in Chittagong but they were longer in those who received 2mg/kg (20 patients, median (IQR) 3.5 hours (2.7-4.2), $p=0.039$). There were no differences in clearance half-lives between doses of artesunate at either site.

In Pailin 32/120 (27%) had a lag phase of median (IQR) duration 6.3 (4.4-8.5) hours and 10/40 (25%) in Wang Pha with duration 8.0 (6.0-12.0) hours (**figure 3.2-7**). These were no different to the proportion with, or duration of ($p=0.53$), lag phase for those receiving IV artesunate in Chittagong.

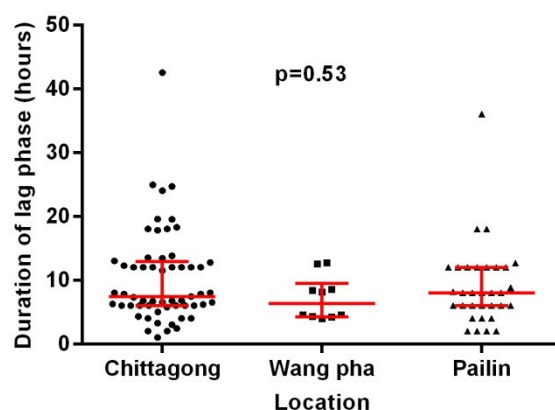


Figure 3.2-7. Duration of lag phase in patients from Chittagong, Pailin and Wang Pha.

The parasite clearance half-lives in individuals from higher transmission areas (Chittagong Hill Tracts) were no different from those from lower transmission areas (outside Chittagong Hill Tracts) ($p=0.32$). There was also no correlation between age of

the infected individual and clearance half-life ($p=0.54$). Clearance half-lives were not different between male and female patients ($p=0.26$).

Parasite clearance half-lives were found to be independent of the proportion of late parasite stages in the peripheral blood on enrolment, initial log parasite biomass, Glasgow Coma Score, haemoglobin, haematocrit, white blood cell count, lactate and plasma bicarbonate.

12/207 (6%) patients had pharmacokinetic sampling done for another study in which they were enrolled. There were no correlations between parasite clearance half-lives and either area under the curve or peak concentration of either artesunate or dihydroartemisinin.

There was a negative correlation of clearance half-life constants with platelet counts ($p=0.006$) (**figure 3.2-8**).

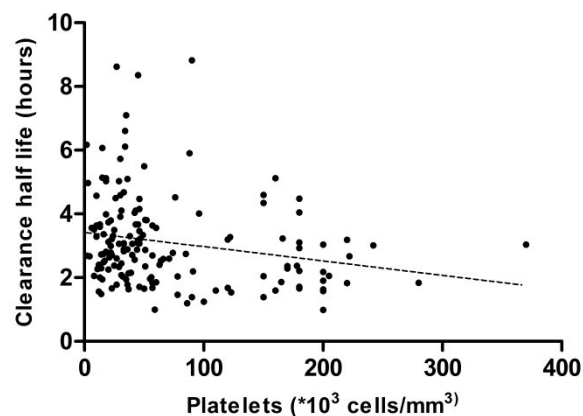


Figure 3.2-8. Parasite clearance half-lives and platelet counts.

The linear regression line is shown, $R^2=0.046$, $P=0.006$.

3.3 Mathematical models

3.3.1 Model 1

Continuing availability of artesunate monotherapy

The model predicted that if there was no intervention, and use of artemisinin monotherapies continued, there would be an exponential rise in the proportion of resistant infections and a slowly increasing prevalence of infection. By 2030, the model predicted that the prevalence of malaria would have doubled compared to 2008 and resistance to the artemisinins would be approaching 100% (**figure 3.3-1**).

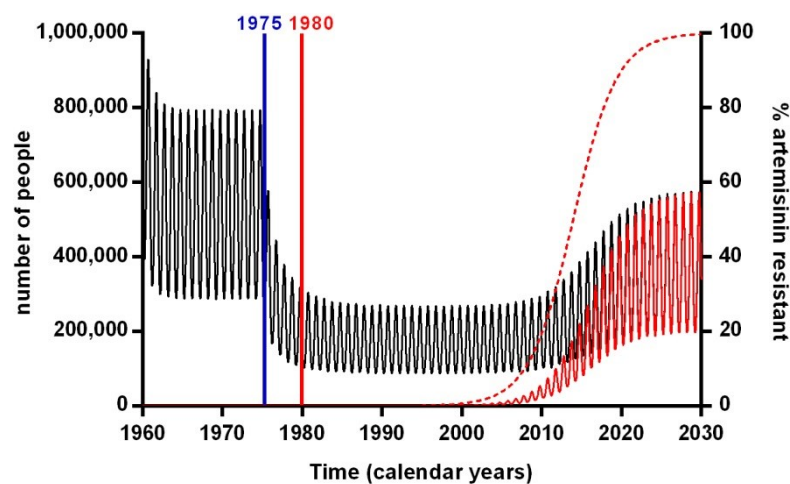


Figure 3.3-1. Effect of continuing availability and use of artemisinin monotherapy on the total number of malaria infections (black line), the number of artemisinin-resistant infections (red line) and percentage of infections resistant to artemisinin (red dotted line) over time. Artesunate monotherapy was introduced as treatment in 1975 and a single artemisinin-resistant infection in 1980.

Eliminating artemisinin resistance

The model predicted that it is possible to achieve elimination of artemisinin resistance using medications alone, but in all scenarios elimination of malaria was required in order to eradicate artemisinin resistance.

The most effective single intervention to achieve elimination of artesunate resistance was ceasing the use of inadequate courses of artesunate monotherapy and replacement with ACT with high coverage (a). This would be achieved if all monotherapies were actively replaced by ACT in the private sector and there was adequate continued supply of good quality, affordable ACT. Using this strategy and with an introduction of the ACT over three months it was possible in the model to achieve elimination of artemisinin-resistant malaria within four years in 70% of cases (mean time to elimination 3.42 years (95% CI 3.32-3.60 years), (**figure 3.3-2**). The stochastic model was run 200 times and elimination was achieved with this strategy in 100% of cases, suggesting this strategy was highly likely to be successful. The downside was that, because a lot of artemisinin was being used, the prevalence of artemisinin resistance just before elimination drastically increased. It reached 82% in the deterministic model and 100% in 58% of cases in the stochastic model (**figure 3.3-3** illustrates one of these cases).

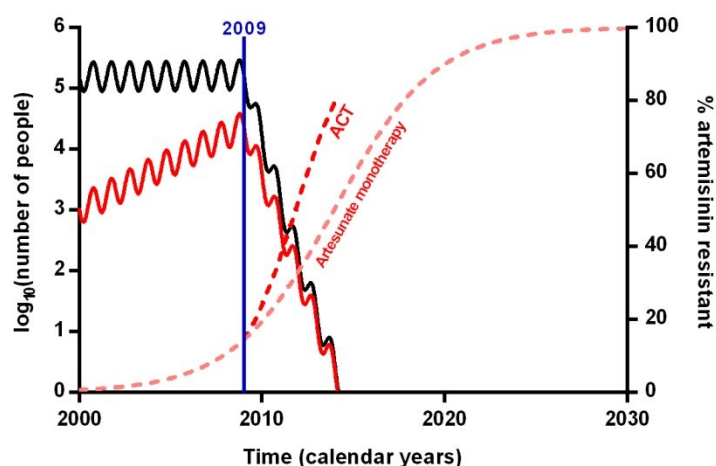


Figure 3.3-2. Effect of eliminating artesunate monotherapy and replacement with ACT in 2009 for treatment of symptomatic cases

on the total number of malaria infections (black line), the number of artemisinin-resistant infections (red line) and the percentage of infections resistant to artesunate (dotted lines, pink=artesunate red=ACT) (mean-field approximation).

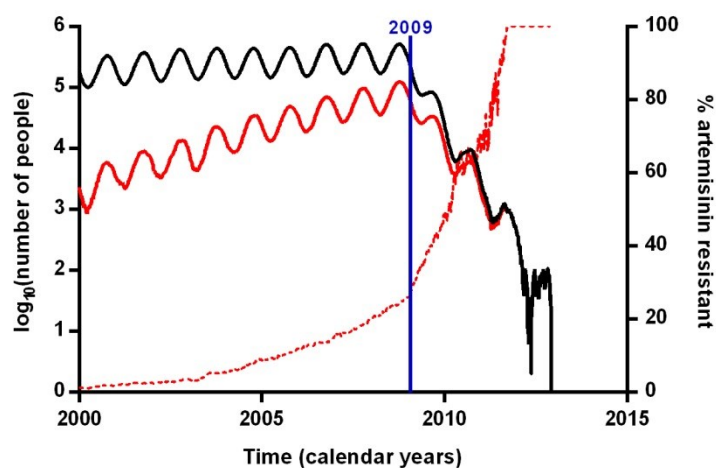


Figure 3.3-3. Example of a single stochastic output illustrating the effect of a switch of treatment to ACT in 2009.

The proportion of infections resistant to artemisinins then remained at this high level provided that there was no importation of new infections from elsewhere. This suggests

that if an intervention were to fail, or was discontinued prematurely, the prevalence of resistance would be much higher subsequently than if no intervention had taken place.

If artemisinin monotherapies remain available, one three-month pulse of MSAT or MDA with either ACT or AP (b or c) reaching 80% of the infected population would have a significant short-term impact, but a negligible long-term impact on the numbers of resistant infections. This was because although such interventions reduced infections they were not sufficient to completely interrupt transmission at the population level. For example, AP MSAT was the most effective of these strategies but one pulse of this only produced a 35% reduction in total malaria infections and 31% reduction in the number of artemisinin-resistant infections. If a three-month pulse of MSAT or MDA was used in addition to a switch to ACT for treatment then there was negligible additional effect. Repeated annual three-month pulses of MSAT or MDA were also insufficient to achieve elimination when used alone, regardless of the drugs used. This was because the number of infections increased again in the other nine months of the year with an increasing proportion of artemisinin-resistant infections each time due to on-going artemisinin use. When used in this way, AP had greater impact than ACT. For example, the maximum effect of long-term annual MSAT with AP on the numbers of artesunate resistant infections was at two years. At that time, the decrease in malaria infections was 55% and artemisinin-resistant infections had fallen to 39% compared to 2009. Following this trough, the number of artemisinin-resistant infections rose again although the total number of malaria infections did not reach a minimum until after 7 years (80% below that for 2009). For comparison, if ACT MSAT was used, the lowest number of artesunate resistant infections was in the first year with a 32% decrease in malaria prevalence and

31% fall in artesunate resistant infections compared to 2009. The number of artesunate resistant infections then increased again but the total number of malaria infections did not reach a minimum until after five years (61% lower than in 2009).

If the MDA or MSAT was carried out in the peak transmission season for malaria, the maximum decrease in the number of artemisinin-resistant infections was half that which could be achieved in the low transmission season. The frequency of MSAT and MDA was varied and it was found that if carried out twice a year then elimination became possible. If MSAT with AP was undertaken at a maximum of four times a year (it took three months to complete one round) then elimination could be achieved in eight years. All this was predicated upon resistance not emerging to AP.

The addition of primaquine to annual MDA or MSAT (d) reduced the trough in the number of artemisinin-resistant infections by 20% and the total number of malaria infections by 40%. This extra effect was still insufficient to achieve elimination.

Assuming that malaria vectors bit after people were in or near their beds, then insecticide treated bed nets (ITN) (e) had a relatively large effect and accelerate the eradication of resistance. The longer that nets were used for, the larger the effect; for example, if the effect of bed nets lasts around four years (the equivalent of optimal LLITNs) and they reduced transmission by 30%, then time to eradication was reduced by about 50%. Thus a modest, but sustained, protective effect from bed nets or other transmission blocking methods could have a significant impact on the time to elimination.

When a fitness cost of artemisinin resistance was included in the model, the rate of increase of resistant infections was slower (around 33% slower for fitness cost of 5%) and the rate of elimination by an intervention was marginally faster (19% for a fitness cost of 5%). The fitness cost must be less than 7.5% for the number of artemisinin-resistant infections to increase over time. There was no information on fitness cost of artemisinin resistance or the form it might take when this model was developed. In **Model 1**, fitness cost manifested as a reduction in transmissibility. Data on transmissibility of artemisinin resistant infections were not available at the time of this thesis. A later attempt was made to estimate another possible manifestation of fitness cost in **Section 3.2.1**, but the results were insufficiently conclusive to include in these models.

Sensitivity analysis

For all interventions, the following were varied in turn: the coverage with ACT from 0 to 100%, the effectiveness of dihydroartemisinin and piperaquine against resistant infections and the cost of piperaquine resistance between 0 and 100%, the cost of artemisinin resistance in terms of fitness between 0 and 5%, the average time to receive antimalarials during an intervention from 7 to 90 days, and time to natural recovery from infection (i.e. without treatment) from 60 to 200 days. Duration of drug effect was also varied for atovaquone from 10-15 days and for piperaquine from 20-30days.

The model was most sensitive to ACT coverage, effectiveness of artemisinins against resistant infections, fitness cost of ACT resistance, and time to receive antimalarials.

There was a threshold coverage with ACT of 47% below which the time to eradication was more than a decade. With ACT coverage of <28%, elimination was impossible. The

effectiveness of artemisinins against resistant infections was determined from field data to reflect current phenotypes¹⁰⁴ but it is likely that this will decrease over time with continued use of artemisinins. The model predicted that if this effectiveness halved compared to 2009, time to eradication would take 50% longer. If time to receive antimalarials (ACT or AP) doubled (from 16 to 32 days after developing blood stage infection) time to elimination increased more than threefold, whereas if people received antimalarials in half the time (eight days after blood stage infection), time to elimination was three times less. The time to eradication was unaffected by natural recovery rate from infection, and the effectiveness of, and fitness cost of, resistance to piperaquine. Changing the duration of atovaquone and piperaquine effect had minimal impact on the results. To explore the effect of synergy between the components of ACT, the rate of clearance of infection was varied from $1/3 \text{ days}^{-1}$ to $1/7 \text{ days}^{-1}$. This had negligible effect on the time to elimination but decreasing the clearance rate of gametocytes by ACT to $1/7 \text{ days}^{-1}$ decreased the percentage of artemisinin-resistant infections at the time of elimination from 82% to 54%.

3.3.2 Model 2

Fitting and validation of model with field data

The model was able to reproduce closely malaria surveillance data from the national malaria control programme (**figure 3.3-4A**) and the results of the study (**figure 3.3-4B-E**) with realistic values for coverage with the various components of the strategies employed. Further details of the fitting and validation are given in the **Appendix 6.5.1** with derived coverages for the various components of the interventions shown in **table 6.5-1**.

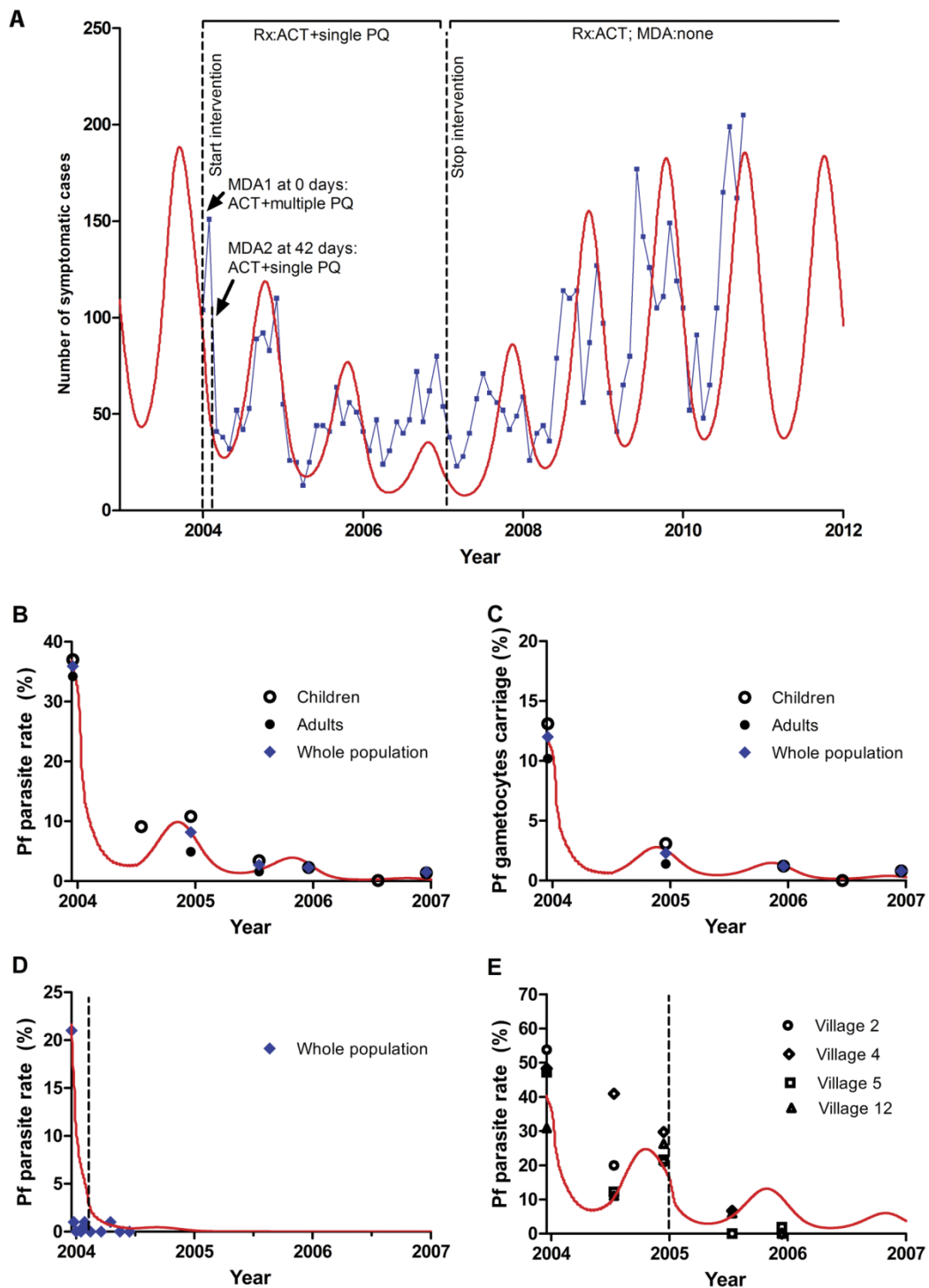


Figure 3.3-4. Model fits to data and validation.

Model predictions are shown as red lines and surveillance data in black (subgroups) or blue (summary). A Model validated with surveillance data for Kampot OD (2004-2010).

The malaria control strategies used are shown. **B-E** Model fitted to data from the field study (2004-2007). **B-C** Reductions in *P. falciparum* asexual stage parasite (**B**) and gametocyte (**C**) prevalences in 17 villages in Kampong Speu OD. The strategy used was treatment with ACT plus single dose adjunctive primaquine for three years and a single MDA with ACT and multiple rounds of primaquine MDA. **D** Reduction in *P. falciparum* asexual parasite prevalence in 3 villages in Kampot OD with MDA and treatment as above, although with lower coverage, followed by a second MDA of ACT plus single dose adjunctive primaquine with higher coverage (dotted line) at 42 days. **E** Reduction in *P. falciparum* asexual parasite prevalence in 4 villages in Kampong Speu OD with the same strategy as **D** but with the second MDA (dotted line) at 1 year.

Analysis

Contribution of each component. Figure 3.3-5 shows the modelled effect of each component of the elimination strategies employed in the study using the coverages of 78% for treatment and 95% for MDA derived from fitting the model to data (**Appendix 6.5.1, table 6.5-1**). Results for gametocyte carriage are shown in **figure 3.3-6**.

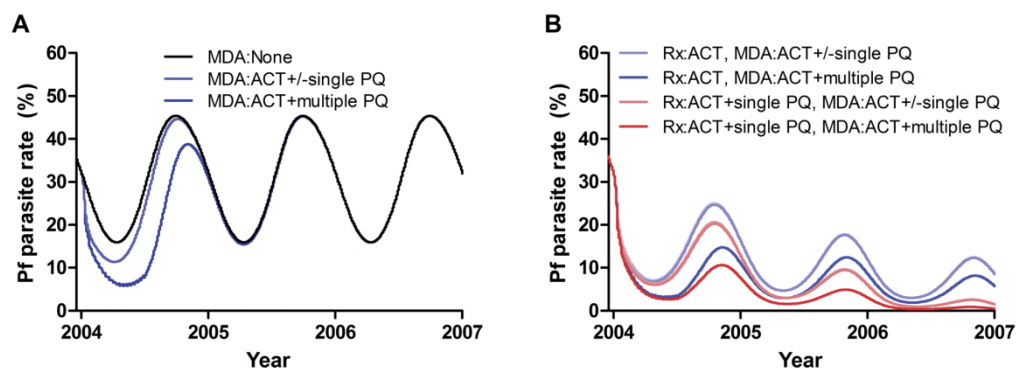


Figure 3.3-5. Contribution of each component of the strategies employed on *P. falciparum* overall parasite prevalence.

Each panel shows the additional effects of adding primaquine to MDA with ACT. **A** MDA alone and **B** MDA combined with simultaneous introduction of ACT plus or minus single dose adjunctive primaquine for treatment. Black line is no treatment, blue lines are no treatment (A) or treatment with ACT (B), red/pink lines are treatment with ACT plus primaquine.

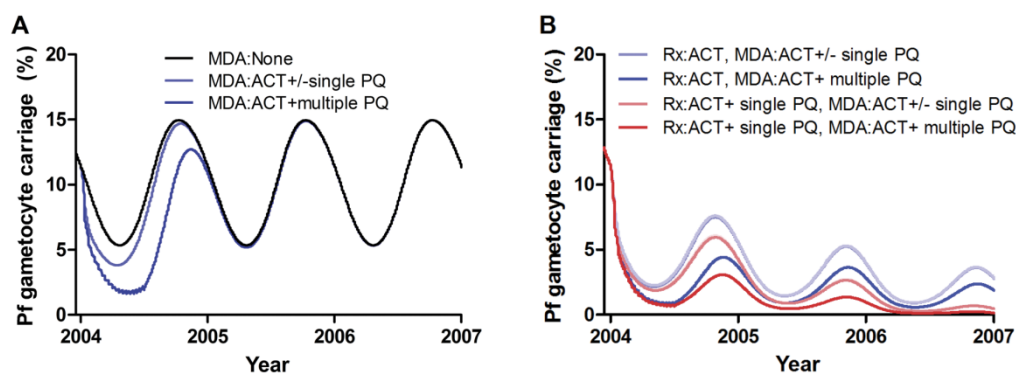


Figure 3.3-6. Contribution of each component of the strategies employed on *P. falciparum* gametocyte carriage.

Each panel shows the additional effect of adding primaquine to MDA with ACT. **A** MDA alone and **B** MDA combined with simultaneous introduction of ACT plus or minus single dose adjunctive primaquine for treatment. Black line is no treatment, blue lines are no

treatment (A) or treatment with ACT (B), red/pink lines are treatment with ACT plus primaquine.

The model showed that MDA produced most of the initial reduction in parasite prevalence. ACT MDA reduced parasite prevalence by 26% and the addition of multiple rounds of primaquine MDA increased this to 65% (**figure 3.3-5A**), whereas adding a single dose of primaquine to ACT MDA had <1% additional effect. Timing of the primaquine dose simultaneously with the ACT was partly responsible for this limited effect observed in the model, and later doses had more effect. Introducing ACT treatment without MDA produced an initial drop in parasite prevalence of 23%, less than that due to MDA alone at the same coverage. Changing treatment to ACT plus single dose primaquine added a further 20% initial drop in prevalence to that due to MDA. MDA alone at an estimated coverage of 95% was insufficient to achieve elimination (defined as <1 malaria parasitaemic individual), producing only a temporary reduction in the number of cases lasting under 1 year following which the parasite prevalence returned to the pre-intervention equilibrium. This was because the timing of the MDA was not optimal (see below), coverage and adherence were not 100% and it was not logistically feasible to provide MDA to the whole population simultaneously. Transmission occurred from infected people who had not yet received MDA and even with repeated rounds of MDA, there was on-going transmission in the time between rounds.

The long-term decrease in prevalence was mostly due to the introduction and continuation for 3 years of high coverage ACT for treatment of people with fever (**figure 3.3-5B**). This effect was significantly enhanced (10-13% additional decrease in parasite prevalence) by

the addition of a single dose of primaquine treatment. In the field study the high coverages were achieved by use of trained village malaria workers and a high profile advertising strategy.

Sensitivity analyses found the relative effects of different strategies to be robust to changes in coverage for the interventions, duration of immunity and the proportion of immune patients who became symptomatic. Changes in immunity did not alter the effect of MDA but did change the impact of treatment. Varying the duration of immunity from 1 day to 5 years produced a diminishing decrease in the size of the initial drop in parasite prevalence due to introducing high coverage with ACT treatment from 34% to 13%. Varying the proportion of immune patients who became symptomatic from 0-100% changed the initial drop in prevalence resulting from this ACT treatment from 13-34%.

The predicted time to elimination (<1 parasitaemic individual) for the main study intervention of a combination of simultaneous introduction of high coverage with ACT plus single dose primaquine for treatment and MDA with ACT plus multiple doses of primaquine every 10 days for 6 months was 4.2 years. A second round of single dose MDA made little difference to this result. Without primaquine treatment, continued use of high coverage ACT treatment after MDA with multiple doses of primaquine eliminated malaria more slowly over 7.3 years. These times are relatively long because of the high baseline parasite prevalence in the study population and incomplete adherence to the medication (assumed for this study to be 77%).

The predicted times to elimination were affected greatly by parameter values used for immunity despite immunity having no direct effect on transmissibility in the model. This was because immunity affected the proportion of cases that became symptomatic and were treated. Assuming 10% of immune patients were symptomatic, changing the duration of immunity between e.g. 1 day, 1, 2 and 3 years increased the time to elimination exponentially from 1.2, 4.1, 9.2 and 23.2 years and longer durations of immunity precluded elimination. If 20% of immune patients were symptomatic, these times were reduced to 1.1, 3.3, 6.1 and 9.2 years with elimination in 21.3 years with immunity lasting 5 years. Greater percentages of immune patients being symptomatic further reduced the times to elimination as more of them received treatment.

The results were robust to altering the parameters for primaquine efficacy. The effect of low dose primaquine (9 mg) on liver stage parasites is unknown and probably very small. Varying the rates of clearance of liver stage parasites due to primaquine in the model made no noticeable difference to the relative effect of strategies including primaquine or the times to elimination. The efficacy of this low dose of primaquine on gametocytes is also uncertain, although known to be significant at larger doses. A recent review of the evidence suggests 0.75mg/kg and 0.25 mg/kg may be equally effective.¹⁷⁰ Its effectiveness was thus varied from that estimated for 0.75mg/kg (see **Appendix 6.4.2, table 6.4-2**) to an effect 4 times smaller. Large reductions in primaquine efficacy against gametocytes were required to significantly alter the results. When the effectiveness of primaquine against gametocytes or the coverage of primaquine was halved, the time to elimination for ACT plus primaquine MDA and treatment increased from 4.2 to around 5.2 years and, when halved again, to 7.2 years.

Cessation before elimination. The model was used to predict what might happen when the study ended in 2007 if funds were not available to continue to provide ACT + single dose primaquine treatment at high coverage. To simulate this, primaquine was stopped and coverage with ACT treatment reduced from 78% to 19% at this time (**figure 3.3-7**). These coverages were derived from fitting the model to surveillance and trial data. The model predicted a steady increase in parasite prevalence over the following three years to a new equilibrium level. This would be the scenario if an elimination effort had to stop before total elimination had been achieved because of insufficient long-term funding or policy changes.

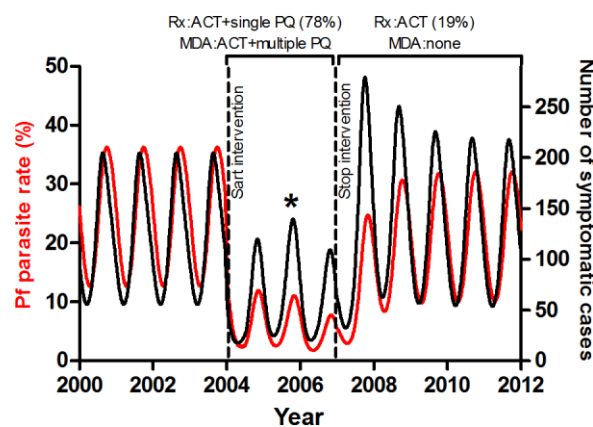


Figure 3.3-7. Cessation before elimination and population level immunity.

Predicted numbers of symptomatic cases and proportion with parasites in the blood before, during and after the trial. The red line is the percent of the population with blood stage parasites and the black line is the number of symptomatic cases. The * indicates the paradoxical increase in clinical cases despite a decrease in the proportion affected.

Population level immunity. The field study demonstrated a fall in the prevalence of *P. falciparum* parasitaemia over the three year study period but did not collect data on the numbers of symptomatic malaria cases. Similar reductions in parasite rates were found

for *P. vivax* and *P. malariae*, although these were not modelled. The model indicated that numbers of symptomatic cases do not mirror numbers of people with parasites because of changing levels of immunity which protect people from symptomatic infection. Rather, falling immunity leads to an increase in the proportion that is symptomatic. The model also predicted that with a successful elimination strategy, as numbers of parasitaemic people continue to fall, numbers of symptomatic cases can level off or even increase as population level immunity declines (**figure 3.3-7**). Following the end of the field study, the model predicted an initial large increase in symptomatic cases to numbers greater than before the study period despite malaria parasite prevalence being lower. This was because of the reduction in population level immunity during the intervention when there were fewer infections.

To assess the sensitivity of these results to the duration of immunity, its mean value was varied from 0.5-5 years. The longer lasting the immunity, the lower the number and proportion of symptomatic cases. In addition, a longer duration of immunity reduced the relative impact of increasing coverage with treatment but not the other interventions. This was because it increased the proportion of asymptomatic cases. Even with a duration of immunity of 5 years, however, a high coverage with ACT treatment was still the most effective strategy in the long-term. The other results reported above were robust to this changing duration of immunity. Increasing the percentage of immune patients who became symptomatic delayed the increase in both symptomatic and asymptomatic infections upon cessation of the intervention because their numbers fell further during the intervention as a higher proportion was treated.

Artemisinin resistance. In the model, the effectiveness of treatment and MDA with ACT on parasite prevalence was reduced by increasing the prevalence of artemisinin resistance (figure 3.3-8). In these simulations, the effectiveness of ACT waned over time as the resistant infections spread more quickly in the presence of continuing high coverages with ACT treatment. Once coverage with ACT treatment fell at the end of the trial, both artemisinin sensitive and resistant infections increased and the resistant proportion increased more slowly as the selection pressure was reduced. **Figure 3.3-8A** shows the effect of artemisinin resistance on the impact of treatment with ACT plus MDA with ACT and multiple rounds of primaquine. The addition of primaquine to each treatment (figure 3.3-8B) largely negated this effect.

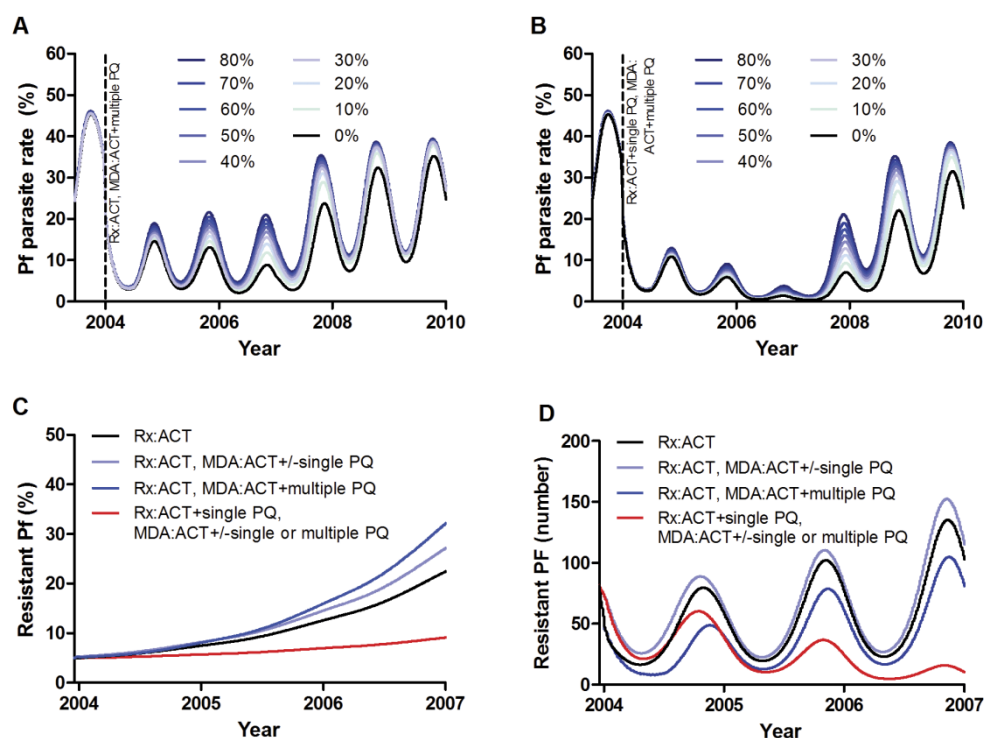


Figure 3.3-8. Artemisinin resistance and different elimination strategies.

Effect of an increasing prevalence of resistance (defined as the proportion of infections which are artemisinin resistant) from 0% to 80% on the success of MDA with ACT plus

multiple rounds of primaquine and treatment with **A** ACT or **B** ACT plus single dose primaquine from 2004-2007. **C-D** effects of different treatment and MDA regimes on the spread of artemisinin resistance (**C**: prevalence of resistant infections, **D**: number of resistant infections), presuming a starting prevalence of resistance of 5%. Interventions ceased in 2007.

Compared to before the trial when malaria treatment in the study area was thought to have comprised a variety of non-artemisinin antimalarials and low level artemisinin monotherapy, the proportion and number of artemisinin resistant infections increased much more quickly when ACT was introduced for treatment and only slightly more when MDA was added as well (**figure 3.3-8C and D**). Single dose primaquine added to ACT treatment greatly slowed this increase in artemisinin resistance. This was because it reduced transmission of resistant parasites sufficiently to prevent epidemic behaviour of the resistant subpopulation.

Multiple rounds of primaquine MDA greatly decreased the number of resistant infections whereas a single round of primaquine MDA did not (**figure 3.3-8D**). The proportion of resistant infections was unaffected by a single primaquine MDA but increased slightly when multiple rounds of primaquine MDA were added (**figure 3.3-8C**). This apparent paradoxical increase was because primaquine further reduced the number of infections and thus population level immunity. Infections could then spread more rapidly in the population, although only resistant infections increased in number as selection pressure from ACT continued to reduce the number of sensitive infections. In contrast, the effect of adding primaquine to treatment was cumulative over the course of the study and this

ongoing additional effect was sufficient to prevent the spread of both sensitive and resistant infections. This was robust to varying the duration of immunity between 0.5 and 5 years.

Optimization and design of future studies

The model was used to design optimal elimination strategies for testing in future field studies. This was done by varying timing of the different components, combining different interventions and investigating the effect of adding new interventions that were not in the field study, e.g. transmission blocking by insecticide treated bed nets.

Timing. There were no significant differences in the long-term rates of decline or times to elimination when interventions were introduced together at different times of year (**figure 3.3-9A**). Although combined strategies which included MDA caused a predicted greater initial decline in parasite prevalence if introduced when seasonal malaria was not at peak prevalence, this difference was not maintained. This is because the impact of the MDA was short-lived. There was thus no clear optimal time for simultaneous introduction of MDA plus treatment (as was done in the trial, **figure 3.3-10A**). When multiple interventions were introduced in the model at different times, however, the relative timing of each intervention became important, as outlined below.

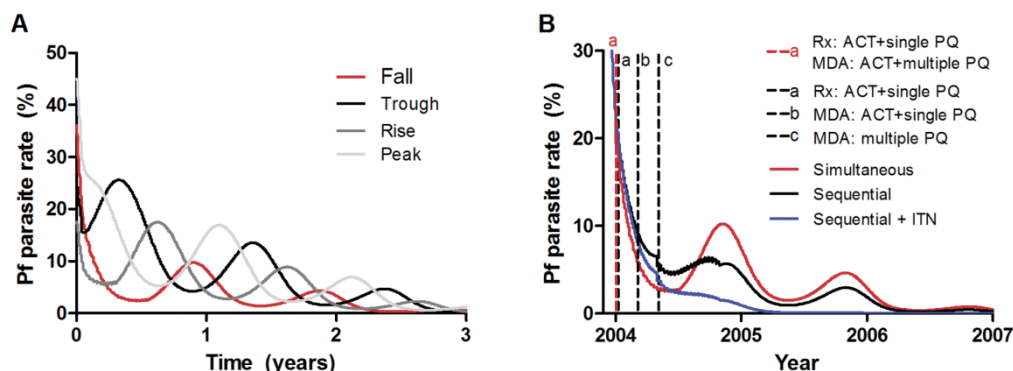


Figure 3.3-9. Effect of varying the timing of interventions on the prevalence of parasitaemia in the population.

A Varying the season in which interventions are implemented. MDA with ACT + multiple primaquine and Rx with ACT + single primaquine started together at different times during the malaria season: when parasite prevalence is falling, at trough, rising or at peak. **B** Simultaneous versus sequential interventions and the additional effect of bed nets. Simultaneous introduction of treatment with ACT + single primaquine *plus* MDA with ACT + multiple primaquine (red) versus optimally timed sequential interventions (black): a. treatment with ACT + single primaquine then b. MDA with ACT + primaquine at 9 weeks before the nadir and c. another MDA with multiple primaquine at the nadir and the same simultaneous interventions with the addition of long lasting insecticide treated bed nets with 30% coverage (blue).

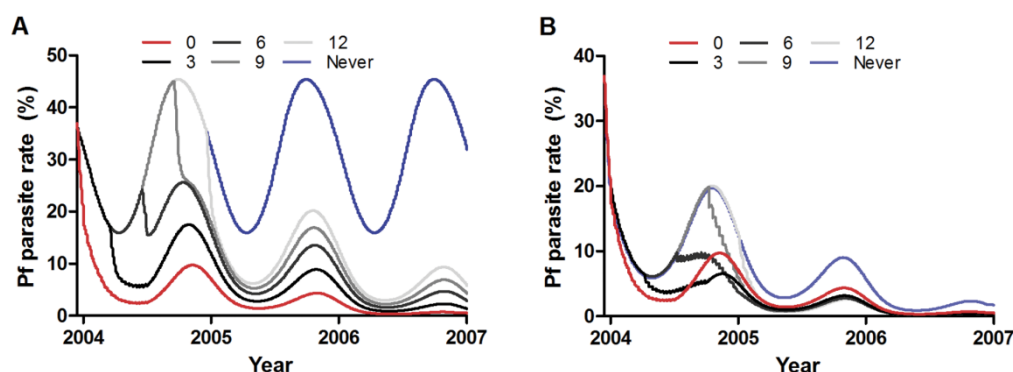


Figure 3.3-10. Effect of varying the timing of interventions used in the field study on the prevalence of parasitaemia in the simulated study population.

A MDA with ACT plus multiple primaquine and Rx with ACT plus single primaquine started together at different times after the start of 2004 (in months). **B** MDA with ACT plus multiple primaquine at different times after introducing Rx with ACT plus single primaquine in 2004 in months.

Combining Interventions. Combinations of interventions were predicted by the model to be much more effective than single interventions. This was particularly true when combining strategies which act at different parts of the parasite life cycle e.g. ACT against blood-borne parasites and transmission-blocking measures against mosquitoes, specifically long-lasting insecticide treated bed nets. The effect of multiple interventions using multiple different antimalarial drugs was greatest when they were introduced at different times (**figure 3.3-9B**). The optimal time for a single round of MDA was from 6 to 12 weeks before the nadir of seasonal parasite prevalence (**figure 3.3-10B**). In the field study, the MDA was approximately 14 weeks before the nadir. Where two or three rounds of MDA were used in the model, they had maximal impact if the final round of MDA was completed before the nadir e.g. one round each month for three months. When doing this, 3 rounds of ACT MDA over 3 months was found to be optimal and of comparable efficacy to the 19 rounds of primaquine MDA over 6 months used in the trial. MDA with multiple rounds of primaquine was most effective after a single round of ACT MDA when started at trough parasite prevalence. These optimal timings were because the effects of the different strategies overlapped and with shorter intervals the effects of the additional benefit from each strategy were reduced.

Transmission blocking. Insecticide treated bed nets were predicted to have a relatively large additional impact whenever they were introduced in addition to antimalarials. The optimal time was as early as possible, regardless of season (**figure 3.3-9B**). This was despite assuming only 30% efficacy in reducing transmission and was robust to changes in coverage from 50-100%. This large additional effect of bed nets was true for all strategies due to their transmission-blocking action at a different stage of the parasite life cycle from antimalarials. Bed nets alone, however, were never sufficient to eliminate malaria and combination with other interventions was always required to achieve this.

Optimal elimination strategy. From this model an optimum strategy for malaria elimination can be derived in this context. The most robust modelling results were that ACT treatment with high coverage is an essential component without which elimination cannot be achieved. If continued for long enough at high coverage, this alone may be sufficient. Adding adjunctive primaquine to ACT treatment accelerated elimination and adding other interventions (MDA and LLITN) further accelerated the process. To achieve maximal impact, MDA should be used as levels of infection fall in the low season and multiple rounds of MDA should be completed before the seasonal nadir in parasite prevalence. Three rounds of ACT MDA over three months were found to be optimal with little advantage from adding primaquine MDA.

3.3.3 Model 3

Model fitting & validation

Model 3 was able to closely reproduce government surveillance data on numbers of detected non-severe and severe malaria cases in Khagrachari, Bangladesh from 2001-2011 (**Section 3.1.2.1, figure 3.3-11A and 3.3-11B**) and Cambodia from 2004 to 2011 (**Section 3.1.1 and figure 3.3-11C**). The model could also reproduce the monthly numbers of severe malaria cases admitted to Chittagong Medical College Hospital (CMCH) in Chittagong, Bangladesh from 1999-2011¹⁶⁷ (**Section 3.1.2.2 and figure 3.3-11D**). The relative efficacies of quinine and artesunate for severe malaria⁷⁰ (**figure 3.3-11E**) and pre-referral rectal artesunate⁷⁴ (**figure 3.3-11F**) were accurately simulated by the model. The resulting model matched closely three independent data sets for parasite prevalence in Bangladesh and one for Cambodia.

Further details of the model fitting and validation are given in **Appendix 6.5.2** and derived parameter values in **Appendix 6.4.3, table 6.4-3**.

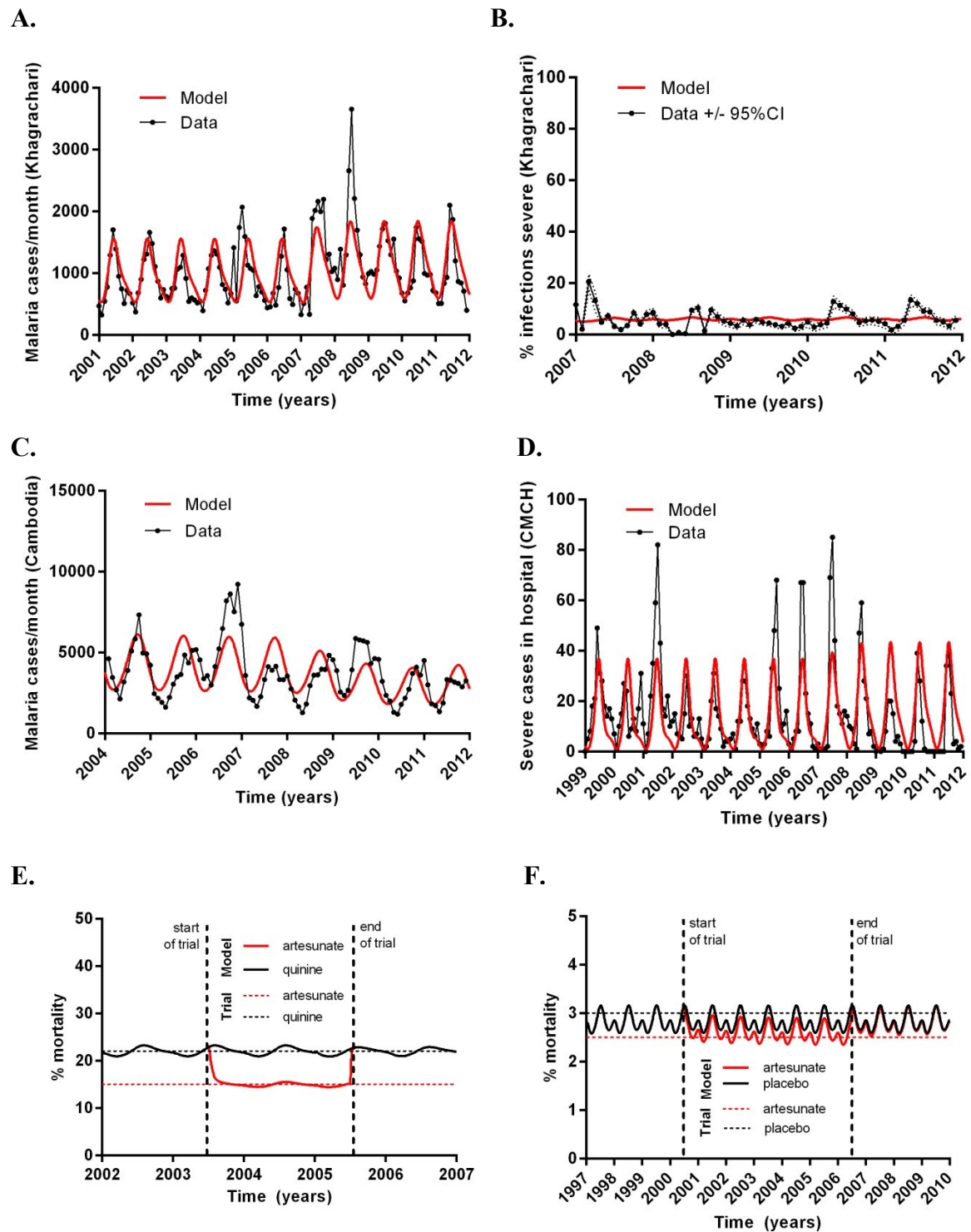


Figure 3.3-11. Model fitting and validation.

Fitting to government surveillance data on *P. falciparum* in Khagrachari District,

Bangladesh: **A** number of cases per month and **B** percent of infections which were severe

(mean with 95% CI). **C** fitting of model to numbers of severe cases admitted to CMCH and **D** numbers of cases in Cambodia. In **A-D** Model output is shown in red and data shown in black. Fitting of model to main mortality results of SEAQUAMAT study (**E**) and Study 13 (**F**). In **E** and **F**, solid lines are model output and dashed lines trial results; red is artesunate and black quinine.

Analysis

Malaria mortality in large populations. The model was used to simulate the effect of a wide scale introduction of intravenous artesunate with and without rectal artesunate for severe malaria in 2013. The model predicted a baseline annual total number of deaths from *P. falciparum* in Khagrachari of 1029 with 1865 severe cases and 31,109 total cases. The model predicted that a switch from intravenous quinine to intravenous artesunate for severe malaria would prevent 53/1029 (5%) deaths in the first year. Adding pre-referral rectal artesunate would prevent an additional 28/1029 (3%) deaths. This represented a total reduction of malaria mortality with both interventions of 8% (81/1029) (**figure 3.3-12**). When scaled up to the whole of Chittagong Division, the model predicted 468,767 cases, 26,409 severe cases and 15,989 deaths. With intravenous artesunate in place of quinine, the model predicted 817/15,989 (5%) of deaths would be prevented and an additional 390/15,989 (3%) deaths with rectal artesunate. Both interventions together would reduce mortality by 1207/15,989 (8%). In Cambodia, similar proportions were found. In the model, rectal artesunate had no discernible impact on mortality in severe cases that were treated in hospital but rather it reduced deaths in the community (**figure 3.3-12**).

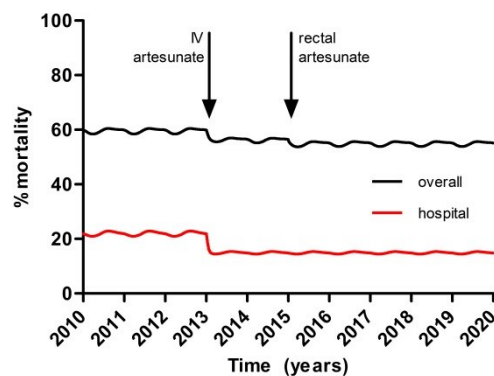


Figure 3.3-12. Mortality overall and in those treated in hospital following the introduction of intravenous artesunate in 2013 and prereferral rectal artesunate in 2015.

The majority (84%) of deaths in the model occurred outside of hospital. A sensitivity analysis showed changing the time taken to reach hospital had a large impact on mortality (**figure 3.3-13**). The mortality benefit from rectal artesunate decreased the shorter the time from administration to arrival in hospital. This mirrored Study 13 where there was no detectable decrease in mortality in those who received their dose less than 6 hours before admission and the benefit was greatest in those reaching hospital >15 hours later. In the model the maximum relative decrease in mortality due to rectal artesunate was in those who reached hospital around 0.75 days, i.e. 18 hours, after administration.

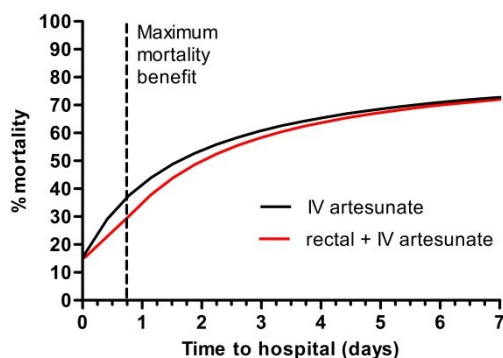


Figure 3.3-13. Impact of altering the time taken to reach hospital on mortality.

Lines are shown for IV artesunate alone and pre-referral rectal artesunate followed by IV artesunate.

Spread of artemisinin resistance. Relative to ACTs, both intravenous and rectal artesunate had a very small effect on the spread of artemisinin resistance in the model (**figure 3.3-14**). This was primarily because they contributed very little to the cumulative exposure of the infected population to artemisinins (**figure 3.3-15**). Also important were that many of those with severe malaria were either cured by treatment in hospital or died and there was thus no further transmission of their potentially resistant infection. There are occasional unverified reports of use of oral artesunate monotherapy for malaria in Bangladesh. When low usage rates for artesunate monotherapy were included in the model it had no noticeable additional impact on the spread of artemisinin resistance.

The overall rate of spread of resistance was highly dependent on the baseline prevalence of resistance. It is not known if there is any artemisinin resistance present currently in Bangladesh. Several alternative conservative scenarios were thus explored. When it was assumed that a very small number ($<0.1\%$ total) of artemisinin resistant infections were present when parenteral artemisinins were introduced, the spread of resistance was very slow, reaching 5% by 2030 (**figure 3.3-14A**). When this baseline prevalence was increased in the model to 1%, as may be the case in Cambodia, the spread of resistance was much quicker (**figure 3.3-14B**). This was because the increase in proportion of resistant infections was exponential with an increasingly steep curve with higher prevalences of resistance. The increase in resistance thus took many years from introduction to ‘take off’.

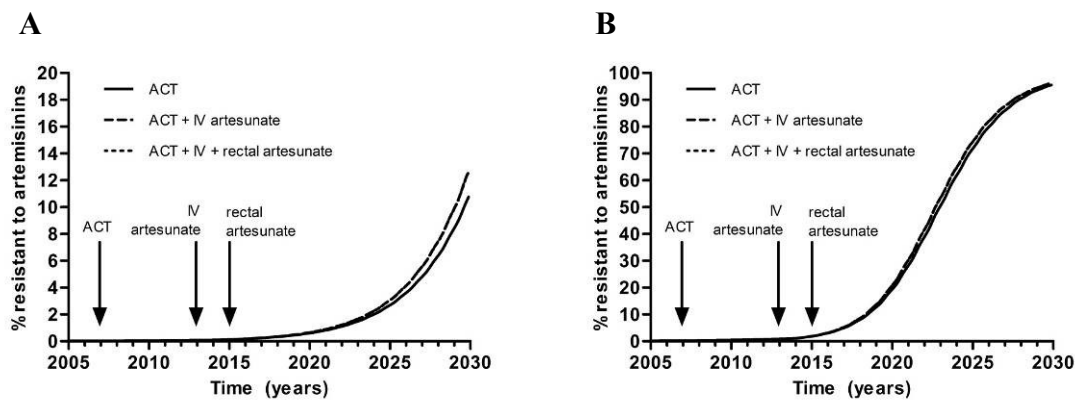


Figure 3.3-14. Effect of different artemisinin treatments on the proportion of *P. falciparum* infections resistant to artesunate over time.

Patients were treated in the model with different combinations of ACT for uncomplicated malaria (from 2007), pre-referral rectal artesunate (from 2015) and intravenous artesunate for severe malaria (from 2013) as shown. **A** shows the predicted spread from a small number of artemisinin resistant infections reaching around 12% of infections by 2030. **B** shows the predicted spread from a higher baseline prevalence of resistance.

Cumulative exposure to artemisinin antimalarials with each treatment regimen was compared. The exposure due to ACTs was far greater than that due to artemisinins for severe malaria (**figure 3.3-15A**). This was true even when using very high referral rates (81% as in Study 13) and treatment coverages (100%) for severe disease (**figure 3.3-15B**).

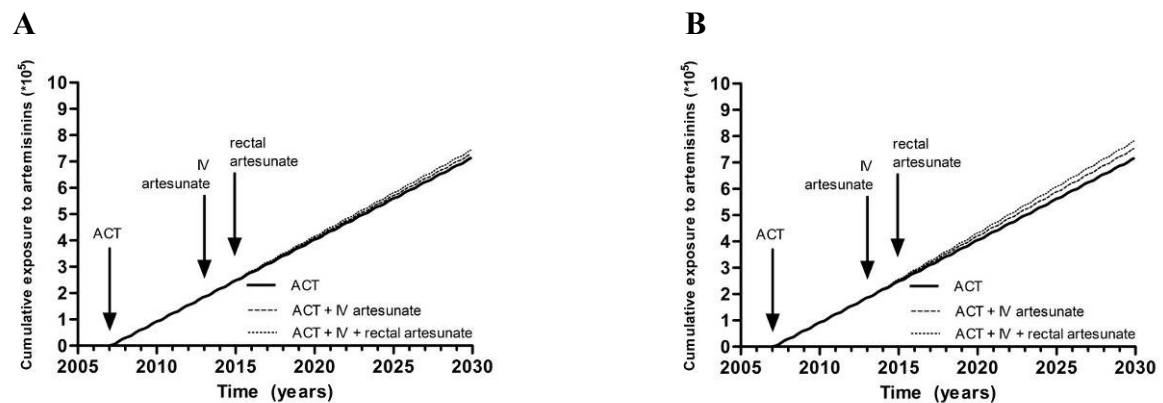


Figure 3.3-15. Cumulative exposure to artemisinin antimalarials.

A using true referral rates to hospital from prior to Study 13 (12%) and realistic values for coverage with rectal artesunate from Study 18 (16%); **B** high referral rates to hospital of 81% of severe cases (Study 13), high coverage of rectal and intravenous artesunate (95%).

The rate of increase in artemisinin resistance was also affected by the resistance phenotype. The current resistant phenotype in Cambodia is relatively mild – a near doubling of the parasite clearance half-life. In the model, a sensitivity analysis was performed for this. The phenotype was altered by multiplying the parasite clearance half-life for resistant infections by a factor – here called a ‘degree of resistance’. Values from 1-1000 were used to explore its impact, a value of 1 representing the current phenotype in Cambodia and 1000 complete resistance. The higher the degree of resistance the faster its spread (**figure 3.3-16**), with saturation occurring beyond a 100-fold increase in half-life.

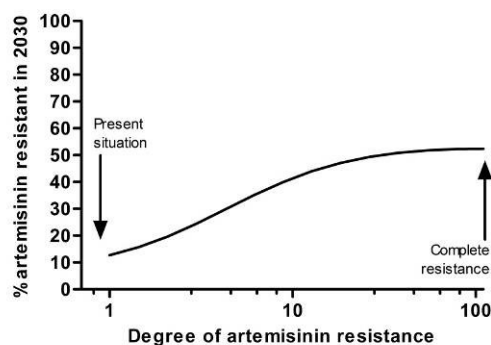


Figure 3.3-16. Relationship between degree of artemisinin resistance and proportion of infections resistant to artemisinins in 2030.

The degree of resistance is shown on a logarithmic scale.

Artemisinin resistance and efficacy. In the model there was very little effect of postulated current low levels of artemisinin resistance on the efficacy of artemisinins on in-hospital mortality from severe malaria (**figure 3.3-17A**). When the proportion of resistant infections approached 100%, the mortality increased to levels similar to those with IV quinine (**figure 3.3-17B**). Altering the artemisinin resistant phenotype by increasing the degree of resistance greatly increased in-hospital mortality (**figure 3.3-18**).

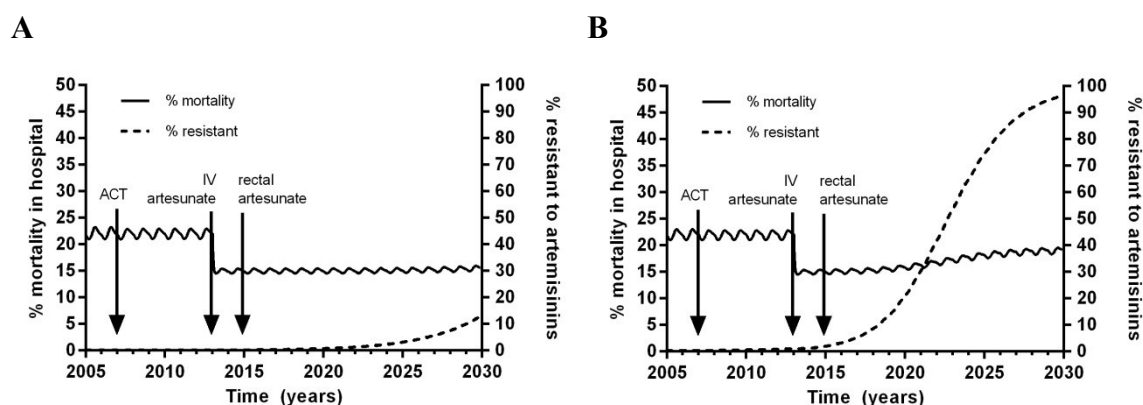


Figure 3.3-17. Predicted mortality in hospital with different prevalences of artemisinin resistance at baseline (2013).

In-hospital mortality following introduction of IV artesunate and **A** 0.1% and **B** 1% artemisinin resistance at baseline.

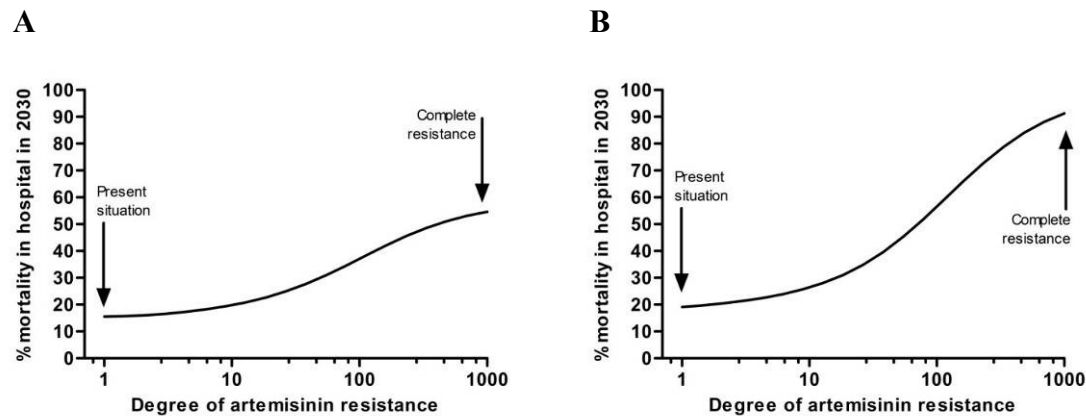


Figure 3.3-18. Predicted overall mortality in hospital by 2030 with different degrees of artemisinin resistance and two different baseline (2013) prevalences of resistance.

The degree of resistance is shown on a logarithmic scale. **A** 0.1% resistant at baseline and **B** 1% resistant at baseline.

Feasibility of malaria elimination. In the model, a combination of an increased coverage (up to 95%) with ACT and long-lasting insecticide treated bed nets (50% of population using LLITN) was introduced in 2013 to attempt elimination of *P. falciparum* in Khagrachari. Elimination was possible within a decade in the model with coverages of ACT of 64% and greater (**figure 3.3-19A**). When artemisinin resistance was added in the model with the current Cambodia phenotype at a prevalence of 0.1% in 2013, elimination was not possible even with higher coverages of ACT (**figure 3.3-19B**). This is because resistance spread faster than elimination could be achieved due to the high coverages of ACT. The small additional increase in the spread of resistance due to parenteral artemisinins made no noticeable difference to the effect of ACT on numbers of clinical cases over time. Very high coverages with ACT (>95%) together with increased coverage

with LLITN resulted in very low but stable transmission (**figure 3.3-19C**). To achieve elimination in the presence of artemisinin resistance in the model, additional strategies were required e.g. mass drug administration or alternative or additional non-artemisinin antimalarials (this will be explored in more detail in subsequent work outside of the scope of this thesis).

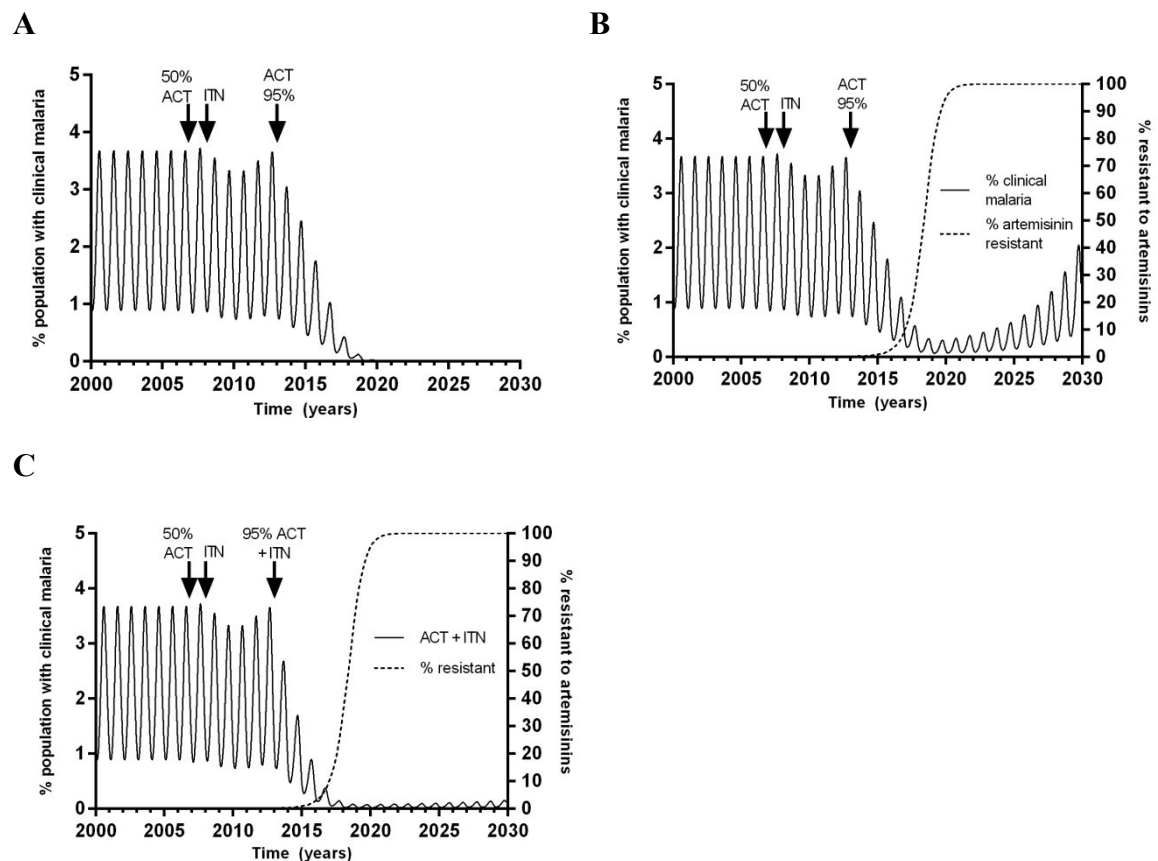


Figure 3.3-19. Effect of artemisinin resistance on elimination attempts using high coverage ACT.

Increased coverage with ACT to 95% without (**A**) and with (**B**) artemisinin resistance. **D** Increased coverage with ACT and LLITN to 95% in the presence of artemisinin resistance.

Strategies that successfully reduced the number of malaria infections also reduced the number of deaths from malaria. However, the case fatality rate increased (**figure 3.3-20A**) due to a decrease in population immunity (**figure 3.3-20B**).

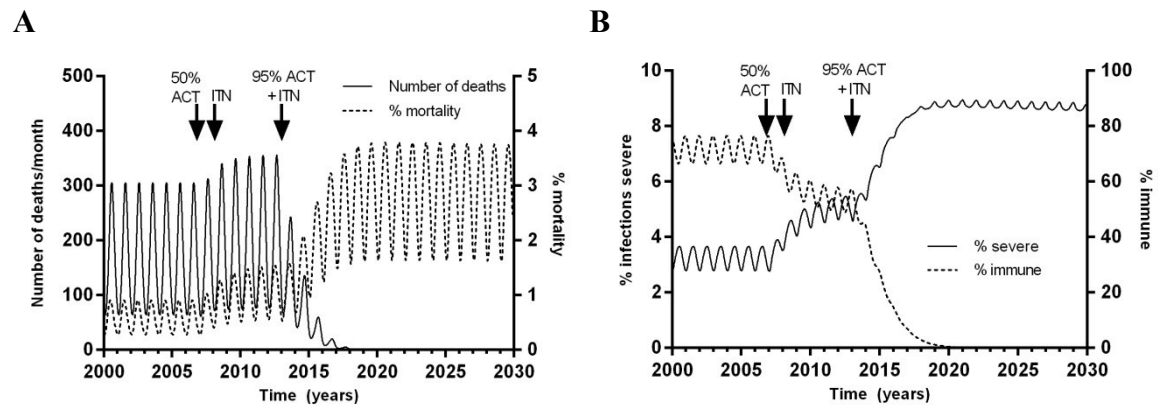


Figure 3.3-20. Effect of high coverage ACT and ITN on mortality.

A number of deaths and percent of overall cases which are fatal (case fatality rate). **B** % of infections which are severe and % of population with immunity.

In Cambodia, elimination could be achieved within a decade with coverages of ACT of $\geq 60\%$. With artemisinin resistance at a prevalence of 1% of infections, elimination was not possible with ACT. If LLITN were added, elimination was then possible with coverages of both of $\geq 95\%$. If lower coverages were used, additional strategies were required to achieve elimination in the presence of artemisinin resistance.

3.4 Summary of Results

The main results are summarised in **table 3.4-1**.

Table 3.4-1. Summary of the main results for each section.

3.1 Malaria epidemiology

3.1.1 Cambodia

1. Malaria is heterogeneously distributed across Cambodia
2. There has been no overall change in the incidence of *P. falciparum* although since 2007 it has decreased in western Cambodia and increased in the Northeast
3. The incidence of *P. vivax* has increased since 2008

3.1.2 Bangladesh

3.1.2.1 Khagrachari

1. The incidence of *P. falciparum* has remained steady
2. *P. vivax* has decreased since 2009

3.1.3.2 Chittagong

1. Most severe malaria cases do not receive essential high-level supportive care
2. There are many more cases of severe malaria than are formally reported in Bangladesh

3.2 Parasite clearance

3.2.1 Cambodia and Thailand

1. There is no clear relationship between recrudescence and slow parasite clearance in *P. falciparum* infections treated with artemisinin antimalarials
2. There is no clear dose-response relationship for oral artesunate
3. Recrudescence in artemisinin treated *P. falciparum* malaria is probably due to a

combination of factors including differences in antiparasite immunity, alterations in drug action (autoinduction) and parasite dormancy

3.2.2 Bangladesh

1. Parasite clearance half-lives due to intravenous artesunate vary greatly between individuals
2. There has been a small increase in parasite clearance half-lives in Bangladesh from 2004-2012

3.3 Mathematical models

3.3.1 Model 1

- 1 Discontinuing the use of artemisinin monotherapy and replacing it with high coverage with ACT can be sufficient to eradicate artemisinin-resistant malaria in Cambodia
- 2 To eliminate artemisinin-resistant malaria using strategies with ACT treatment requires elimination of *all P. falciparum* malaria as the last few infections to be cleared are almost all resistant

2.3.1 Model 2

- 1 MDA caused a large initial reduction in parasite prevalence but this was not sustained beyond 1 year
- 2 Sustained high coverage with ACT treatment was required for a long-term reduction in malaria
- 3 Primaquine added to ACT treatment reduced the increase in proportion of artemisinin resistant infections
- 4 A reduction in the total number of malaria infections was sometimes followed by an increase in the number of symptomatic individuals due to a fall in population level

immunity

- 5 The optimal strategy to achieve elimination of malaria was a combination of multiple interventions, of which sustained high coverage with ACT was an essential component. Elimination was accelerated by the addition of adjunctive treatment with primaquine, insecticide treated bed nets and multiple rounds of mass drug administration with ACT.

3.3.3 *Model 3*

- 1 Pre-referral treatment with rectal artesunate can reduce mortality from severe malaria in those who reach hospital quickly and receive further intravenous therapy
- 2 Using high coverage of rectal and intravenous artesunate for severe malaria has very little effect on the spread of artemisinin resistance
- 3 Increasing coverage with ACT decreases deaths from malaria but can increase mortality *rate* by reducing protective immunity at the population level
- 4 A policy change to rectal and intravenous artesunate in the context of pre-existing artemisinin resistance does not compromise the efficacy of ACT for malaria elimination

Chapter 4 : Discussion

4.1 Malaria epidemiology

4.1.1 Cambodia

The overall number of cases of *P. falciparum* malaria attending public sector facilities in Cambodia has remained relatively stable since 2004 whilst numbers of *P. vivax* cases have increased. This is despite substantial increases in the use of ACT and insecticide treated bed nets during that period. The recent fall in percent of screens for malaria which were positive with relatively high numbers screened in 2009-2011 suggests more effort has been put into screening to detect the same number of cases, particularly in the low season. This may be masking an actual decline in *P. falciparum* in 2010 and 2011. The marked increase in *P. vivax* in this study began a year too early for it to be related to the decrease in *P. falciparum*^{171, 172} Alternatively, it could be a result of introduction of pLDH RDT (CareStart) in 2009 in place of a Pf only HRP2 RDT (Paracheck) and improved microscopic differentiation of species on diagnosis and better sensitivity of screening with development of the malaria control programme and improvements in training of microscopists from 2009.¹⁷³ The decrease in mortality found in this study is likely to be due to improvement in healthcare provision during the study period. The VMW data suggest that VMWs may be contributing to this decreased mortality, presumably through earlier effective diagnosis and treatment at a village level as well as integrated preventive malaria measures.

The roll-out of VMWs across remote and high transmission areas of Cambodia has resulted in detection of a large previously unrecorded burden of disease. With VMWs in 25% of the villages in the higher transmission areas of Cambodia in the past two years,

the total number of detected cases of both species nationally has almost doubled. If VMWs were rolled out further, additional increases in the detected caseload would be expected. VMWs have the advantage of being accessible to villagers who may not otherwise attend public sector facilities with their malaria. In the absence of VMWs, a high proportion of these individuals are thought to purchase their medications from the private sector. This was estimated at 87% in a survey in 2002¹¹⁸ and 54% in 2011.¹⁷⁴ Despite the intensified malaria control efforts in and around Pailin, 43% were estimated to have received treatment from the private sector in 2011.¹⁷⁴ It is likely that many of those treated in the private sector will choose instead the free testing and treatment available from the VMWs, as had been shown previously¹¹⁸ and in a recent evaluation by CNM (unpublished). As data were not available on attendees at private pharmacies and clinics, the total burden of malaria in Cambodia remains unclear although it is evident from these data that it is grossly underestimated. This was suggested in a previous study which estimated this underestimate to be most prominent in remote areas of the country.¹⁴⁵ A conservative estimate from the present study would be over 100,000 cases of malaria per year. This figure does not include those who seek treatment in the private sector (roughly 50%) and it is not clear how many of these would attend a VMW if available. In 2011, VMWs were present in 25% of the villages in ODs with high malaria prevalence. If malaria prevalence across all villages in these ODs were similar, then the actual number of cases would be $1/0.25=4$ times the current number detected by VMWs. This would give a total of around 250,000 malaria cases per year in Cambodia. As of 2012, the private sector is no longer permitted to diagnose or treat malaria. A more accurate picture of malaria burden in Cambodia can be expected as a result.

By providing free testing and treatment, VMWs could be expected to increase the numbers of people being treated for malaria. This should lead to a decrease in transmission overall in areas with VMWs. This study confirmed this with a decrease in cases of *P. falciparum* in 2010-2011 in ODs with VMWs compared to ODs with no VMWs. Malaria mortality was also lower in ODs with VMWs highlighting the benefits of early effective treatment.

During the study period, the geographical distribution of malaria in Cambodia has changed. This is likely to be due to a combination of factors including changes in malaria control activities, population migration and deforestation. In some areas, particularly in parts of the west of Cambodia, *P. falciparum* decreased. Pailin Province had the greatest decrease in *P. falciparum* cases from 2007 onwards. It is not clear which factors caused this decrease although it coincided with the beginning of a period of intense focus on Pailin as the location of newly discovered artemisinin resistance.¹⁵⁶ This resulted in intensification of malaria control activities in the area. It was also around this time that long-lasting insecticide treated bed nets were introduced.¹⁷⁵ Pailin is also one of the areas of Cambodia that has experienced extensive deforestation, principally to create agricultural land. In Pailin much of this occurred prior to 2007.¹⁷⁶ Before 2007, Pailin was the OD with the highest malaria transmission in Cambodia (API/1000=244 in 2004). That the surrounding Province of Battambang experienced a coincident decline in malaria whilst other parts of Cambodia did not may reflect the decreasing influence of the Pailin malaria 'hot spot' on adjacent areas. As of 2012, every village in Pailin has a VMW and this is likely to result in both improved collection of malaria data and a further decrease in the burden of disease.

The northeast of Cambodia bucked the trend in the rest of the country with an increase in *P. falciparum* from 2007-2010. There was also an even larger proportional increase in *P. vivax* than that seen nationally since 2009. Reasons for this are not clear. Although the northeast is a relatively remote area with a high forest density, it has received similar levels of malaria control activity to many other parts of Cambodia,¹⁷⁷ with the exception of the intensified control programme in Pailin and adjacent areas with or suspected to have artemisinin resistance. Changing seasonal migration between the northeast and the highly populated south of the country may be one factor worth exploring further.¹⁷⁸

This study had several limitations. Malaria surveillance data are notoriously prone to selection bias. Self-treated and privately treated cases including those treated by private pharmacies and traditional healers (around 50%) were not captured by these data. Although it is likely that many of these underrepresented cases chose to attend village malaria workers where available, the true numbers of such cases are not known. Remote populations are also thought to have been underrepresented by the HIS data¹⁴⁵ although many of these communities are now included in the VMW project. Changes in efficiency of the systems used to collect the data are difficult to quantify and may introduce biases. The strength of these data is that several trends are apparent over time in the two independent datasets.

This study scratches the surface of analyses possible with the detailed epidemiological data on malaria collected in Cambodia. Further investigation of trends over time in individual ODs matched with details of malaria control interventions employed would be

highly informative for planning future malaria control activities. In particular, detailed data on ACT use have been collected in large national surveys and collection of information on the behaviours and malaria burden in migrant populations is ongoing. The VMW project collects more detailed data than there is space to present here including age, gender and breakdown by individual village. This growing dataset is an invaluable resource for the future for Cambodia. By combining these data with other studies currently being undertaken, for example, on population migration and the geographical distribution of artemisinin resistance, important new insights will be gained.

This study has identified several major trends in malaria caseload in Cambodia over time that warrant further investigation. Although malaria surveillance data are notoriously prone to collection bias, the use of data from two independent sources greatly increases the robustness of the findings.

4.1.2 Khagrachari

This study found that malaria in Khagrachari is mostly due to *P. falciparum* and the overall number of falciparum cases detected did not change from 2001 to 2011. The proportion of cases of *P. falciparum* in those < 1 year decreased whilst there was an increase in the proportion of malaria in adults. It is possible that the increase in adults was due to a decrease in transmission during the study period resulting in a reduction in population protective immunity. There was an increase in numbers tested and this may have masked a true overall decline in cases. It is unlikely that the increasing proportion of

positive cases in adults was due to a sampling artefact as the proportion tested in each age group did not change.

The total number of *P. vivax* cases decreased markedly from 2009 with an increasing proportion in adults. There were also two seasonal peaks in *P. vivax*, with only a single peak in *falciparum*. Similar findings were seen in numbers of malaria patients admitted to Chittagong Medical College Hospital in Bangladesh.¹⁶⁷ It was postulated that the double peak in *P. vivax* may be due to relapse of liver stage infections or long latency *vivax* from the previous seasons relapsing before the start of the next season.¹⁷⁹

Over half of cases of *P. falciparum* were in adults. This is consistent with the relatively low transmission (API of 25 per 1000 population) which would be insufficient to generate protective immunity during childhood. In areas with high transmission, such as much of sub-Saharan Africa, immunity from high transmission results in few adults having clinical disease.

Dighinala sub district had by far the highest number of cases, with a large increase since 2008. This is a largely rural and remote hilly area bordering India with high forest cover including large areas of reserve forest. A previous cluster sampling study of parasite prevalence in Khagrachari found Dighinala to have the highest parasite prevalence with 22% of people infected.¹⁶⁸ The main risk factors for high malaria prevalence in that study were proximity to forest, distance from water and house made with temporary construction materials. Malaria prevalence has previously been found to be particularly high in woodcutters in Bangladesh,¹⁸⁰ a common occupation in Dighinala. It is not clear why there was a large increase over the past three years and further study of malaria

epidemiology in this area is warranted as this is a potential malaria hotspot which could compromise control activities in adjacent areas.

There were several limitations for this study. The data used in this study do not reflect a complete picture of malaria cases in Khagrachari. That the proportion of tests which were positive did not change during the study, despite the number tested increasing, suggests that this study likely underestimated the total number of cases. The data did not include patients treated through private pharmacies and clinics, most of which do not test for malaria and treat presumptively. This lack of data from the private sector is a widespread problem across the tropics.^{181, 182} It is likely that healthcare provision in Khagrachari improved from 2001 to 2011 but no data were available to quantify this. In particular there has been expansion of training of doctors and microscopists in Bangladesh as whole and this should lead to an increase in the number of cases detected and treated. This may have improved diagnosis and increased the number of positive cases found or conversely decreased transmission through increased antimalarial treatment. There was also variation in the provision of healthcare by NGOs during the study period. Several large NGOs had programmes in the District including Médecins Sans Frontières (MSF), BRAC and United Nations Development Programme (UNDP). Data on NGO provision were not included in this study.

Malaria transmission in Khagrachari, Bangladesh, is low and geographically heterogeneous affecting equal proportions of children and adults. There has been no clear overall change in numbers of malaria cases in Khagrachari District from 2001-2011, although the true burden of disease was probably underestimated. This study highlights

the need for systems to capture malaria data from the private health sector for a more complete picture of malaria epidemiology in under-resourced settings.

4.1.3 Chittagong

This study strongly suggests that only a small proportion of severe cases reach tertiary care (0.2-1.6%), since a mean of 177 malaria patients per year were admitted to CMCH, the only tertiary care facility in the area, whereas the estimated total numbers of severe cases nationally is around 11,000-80,000 per year of which 80% reside in the CHT.¹³¹⁻¹³³

One of the explanations could be the financial cost of hospitalization, which is high in proportion to the average income in Bangladesh and can thus be a strong disincentive against admission of less severe patients. Many of the less severe cases were probably treated in peripheral Thana or District hospitals and many cases of severe malaria probably remained untreated in the community. Of the patients admitted to CMCH, 96% had *P. falciparum* and 4% of patients had *P. vivax*, whereas mortality was only associated with *P. falciparum*. Previous studies in uncomplicated malaria in this region show that 70% of malaria is caused by *P. falciparum* and 30% by *P. vivax*,^{125, 129, 132} which emphasizes the benign nature of *P. vivax* infections in this region. Malaria infection was commonest in young adult males in agreement with previous data from the south of Chittagong Division.¹²⁵ This may be related to greater occupational exposure of this group to forest malaria.¹⁸³ As the breadwinners, it could be postulated that they are also the family member most likely to be supported to attend a distant hospital for potentially expensive treatment,¹⁸⁴ hence their over-representation in those screened for malaria.

The number of hospitalized *P. falciparum* cases at CMCH decreased since 2007, despite an increase in the number of patients screened. This decrease was particularly dramatic from 2008–2010. During this period, there have been a number of changes in malaria programmes in the community and a greater than five-fold increase in funding for malaria control.^{127, 134} A large increase in the numbers of patients who receive early antimalarial treatment has occurred in the feeder hospitals and clinics referring to CMCH. In particular there has been a doubling of ACT usage from 2007 to 2008^{127, 134} and an increase in the availability of parenteral antimalarials. In addition, a new large-scale programme of free distribution of insecticide-treated bed nets^{127, 134} and introduction of rapid diagnostic tests in the community began in 2008.^{127, 134} The present study adds to the evidence that these strategies are having a significant impact. However, potential pockets of high transmission, which are mostly in remote areas¹⁸³ may not have been sufficiently covered by this study. Over the past two years there have been initiatives aiming at increased availability of early treatment in more remote areas, including early intravenous therapy in the District hospitals, and as a result it is possible a smaller proportion of cases are being referred to tertiary hospitals.

There was a strong and consistent seasonal pattern of *P. falciparum* incidence, with a large peak between May and September each year largely coinciding with the maximum rainfall during the monsoon season (June–August). This finding is in contrast with the pattern reported in two earlier reports^{127, 130} describing one transmission peak in March–May and one in September–November, with June–August being described as ‘off-peak months’.¹²⁷ However, in both these publications no monthly incidence data were presented. For the incidence of *P. vivax* an additional peak was observed in the months

from February to March, before arrival of the rains and transmission of *P. falciparum*, which uses the same vector system. The same seasonality has been described in vivax malaria in Hooghly District in West Bengal in India, and suggests that these are long latency relapse cases of vivax malaria.¹⁸⁵

By far the majority of individuals screened for malaria were from the south of Chittagong Division with relatively few from the CHT. All the malaria positive cases were from the south of Chittagong Division in the five endemic Districts: Chittagong, Cox's Bazar, Khagrachari, Rangamati and Bandarban. Over 80% of cases in Bangladesh are thought to be resident in the CHT,¹³¹⁻¹³³ although in this study only 12% of those referred to CMCH lived there. This is despite it being the main referral hospital for those needing more advanced care and local policy being to refer the sickest cases from all other government hospitals in the area to CMCH.

As expected from existing epidemiological data,¹³³ the probability of having malaria was highest in screened patients coming from the CHT, particularly the southern part: Lama and Alikadam Thana in Bandarban District but also adjacent Lohagara in Chittagong District. The high rate in Lohagara was mainly caused by an apparent focal epidemic of *P. falciparum* in 2010.

Population density in the CHT is much lower than elsewhere in Chittagong Division. The most densely inhabited areas are Chittagong City and Cox's Bazar and their surroundings and both these areas had very few malaria cases. The highest malaria positivity rates per population density in this study were mostly in those from areas of low population density

in a band from north to south through the centre of Chittagong Division. These were Fatikchari and Rangonia in Chittagong District, Kawkhali and Rangamati Sadar in Rangamati District and Bandarban Sadar, Lama, Alikadam, and Thanchi Thana in Bandarban District plus Chakaria in Cox's Bazar District. The high rates in Fatikchari, Rangonia and Chakaria were a particular surprise as these had not been previously identified as high risk areas, although they are near to the forest fringe. Few of the affected individuals in these three areas had visited the adjacent CHT. Even though very few cases of malaria seen at CMCH lived in the CHT, this study indicated almost three times this number, over a third of the total, are likely to have become infected there. Thus the CHT are an important source of malaria both for residents and travellers, although an important proportion of malaria transmission is outside this area, as has also been described in previous studies.¹²⁵ Albeit lower transmission outside the CHT, the much larger population in this area contributes significantly to the malaria case load. In this study two thirds of cases had not visited the CHT during the time in which they became infected. Malaria control efforts to date have been particularly focused on the CHT¹⁸⁶ but these data suggest a broader area should be targeted.

There were a limited number of cases from Khagrachari District, the northern third of the CHT, previously found to be the area with the highest transmission in Bangladesh.¹³³ The small District hospital in Khagrachari town is similar to those in Bandarban and Rangamati and lacks facilities for mechanical ventilation or renal dialysis, which are often needed for patients with severe falciparum malaria. The long travel time to Chittagong might discourage families and physicians from referral to CMCH.

Another underrepresented area known to be highly malarious was Rangamati District, particularly in the east near the border with India, although this area is also an endemic zone with relatively high transmission.¹³³ There are a number of possible reasons for this. A previous study showed that there is a strong preference among indigenous people in this area for seeking treatment from alternative practitioners in the first instance, although this may be different for the severely ill.¹⁸⁴ Transport from this area to Chittagong is also difficult. Between much of this area and Chittagong city is a large man-made lake, Kaptai Lake, and this can only be crossed by a long journey by boat. Road links to this area are poor, particularly in the wet (malaria) season, and from many areas the travel is long and arduous. These difficult travel conditions might encourage people to seek treatment locally. However, patients from other remote areas with similar difficulties during the wet season did reach CMCH.

Although there are many pharmacies and health centres in the CHT,¹⁸⁴ few of these can provide intravenous treatment and are very limited in their ability to provide more extended supportive care. There are larger and better equipped District hospitals in Khagrachari, Rangamati and Bandarban towns, but these cannot offer mechanical ventilation or renal dialysis, for which referral to a tertiary centre is necessary. The dearth of referrals from the CHT to CMCH thus indicates there is likely to be a large burden of patients receiving suboptimal medical treatment in the periphery, and the perceived and real risks of long transportation times to a tertiary treatment centre are likely part of the explanation. For very remote areas in the wet season, it may be that the risk of transport is just too high and the emphasis therefore has to be on improving care locally as much as possible. One recent example has been the introduction of pre-referral treatment with

rectal artesunate. This has the advantage that patients begin effective treatment earlier and could potentially ‘buy time’ to allow them to transfer more safely to a better equipped facility.⁷⁴ Expanding the availability of effective early antimalarial treatment in general, as has been occurring over the past few years,¹²⁹ will also mean fewer patients progressing to severe disease.

Although overall numbers were large, this study had several limitations. All data were from a single tertiary referral hospital. Data were only collected on those patients who had a malaria test by the on-site malaria diagnostic laboratory. There are several private laboratories in Chittagong who also provide malaria tests, although they charge a fee for this service. A small proportion of patients still undergo testing by these private laboratories although the vast majority of these are retested by the hospital laboratory. The study relies on the assumption that the quality of malaria diagnosis did not change significantly from 1999–2011. This is likely to be the case, as the same highly experienced staff were employed throughout and used the same techniques. It does, however also rely on the medical staff referring the same group of patients for testing during this period but data on this were not collected. Numbers of cases from the CHT and total numbers of *P. vivax* were small. Conclusions regarding *P. vivax* epidemiology are thus limited in their scope.

Since it has been reported in the most recent government report that in 2008 3.8% of *P. falciparum* cases in Bangladesh occurred in Chittagong District,¹³¹ and it was found that, in 2008, 130/165 CMCH admitted severe malaria cases were from that area, the total national number of severe malaria cases in Bangladesh was at least $130/0.038 = 3460$.

This figure ignores any patients with severe malaria admitted to the many other hospitals in Chittagong District, as well as those that did not reach healthcare and the actual total is thus likely to be much larger. Official data report a total number of 3591 severe cases for the whole country from mid-2008 to mid-2009, which is thus a severe underestimation¹³¹ and there is a clear need for more accurate and complete reporting. Current systems for collating this data are incomplete and many confirmed cases are missed from the official totals.¹²⁵⁻¹²⁸ Of particular interest would be the trends in numbers of cases, severe malaria and malaria deaths in the CHT which is essential information to assess the efficacy of malaria control measures as well as for the allocation of resources for patient care.

The lack of epidemiological data on severe and fatal malaria in Bangladesh makes it difficult to plan allocation of healthcare provision to achieve maximum impact on mortality with limited resources. This study demonstrates that a very small proportion of severe malaria cases receive essential high-level supportive care. Patients from the highest transmission zone, the CHT, are particularly underrepresented. These patients could signify an important hidden group with potentially preventable mortality. Despite the recent reductions in malaria incidence and mortality, optimizing care for those developing clinical disease and especially severe malaria remains a priority. Investment in improving early treatment and simple supportive care for severe malaria at peripheral sites should be prioritized together with streamlining referral pathways to higher level care facilities.

4.2 Parasite clearance

4.2.1 Parasite clearance rates in Cambodia and Thailand

Drug response dynamics and recrudescence in artemisinin-resistant *Plasmodium falciparum* malaria

Recrudescence and slow parasite clearance in this study were unrelated. There was no difference in parasite clearance rates between recrudescence and non-recrudescence infections. There was also no overall difference in recrudescence rates between Pailin and Wang Pha. This is despite the much lower clearance rates in Pailin, as also found in the original study of 40 patients at each site.¹⁰⁴ This suggests that artemisinin resistance does not increase the likelihood of recrudescence following treatment with artemisinins. The median clearance rate in Pailin was just over half that in Wang Pha. This was true for treatment with either artesunate monotherapy or artesunate-mefloquine and regardless of dose regimen used. With increasing doses of artesunate, there was no increase in clearance rate and thus no dose response relationship. This lack of a dose response relationship was also found in a recent clinical trial in western Cambodia.¹⁸⁷ The addition of mefloquine did not affect the clearance rate, thus supporting the assumption stated earlier that its' additional effect on top of that of artesunate is negligible.

The distribution of clearance rates in Pailin was continuous with there being no clear artemisinin resistant subgroup. This may be due to a multiple step process with gradual accumulation of a set of mutations producing increasing degrees of artemisinin resistance. Despite the clear difference, there was some overlap in parasite clearance rates in Pailin

and Wang Pha and clearance rates in both sites were widely spread. No clear cut-off for clearance rates in artemisinin resistance could be identified to separate the results from Pailin from those from Wang Pha. A small number of patients in Wang Pha had clearance rates similar to those of the majority in Pailin. It is possible that these infections in Wang Pha may also have been artemisinin resistant. A study using historical data from the same area has found that median clearance rates are slowly declining since 2002.¹⁴¹

Alternatively, they may be sensitive infections and the slow clearance due to the highly variable pharmacokinetics of artesunate causing low drug levels in this group. Measured artesunate and DHA levels, however, were similar in both groups.

Recrudescences occurred in patients from both study sites and with artesunate monotherapy and artesunate-mefloquine. That overall recrudescence rates in Pailin and Wang Pha were not different, was also true when comparing between treatment subgroups from the two sites. In Pailin, patients who received 2mg/kg artesunate had a higher recrudescence rate than those who received 6mg/kg/day although the numbers were small. This might suggest the higher dose may reduce the risk of recrudescence, although this dose-response effect did not apply to those who received artesunate-mefloquine and there was no difference in recrudescence rates in the 2mg/kg groups in Pailin and Wang Pha. Taken together, these results do not provide clear evidence for higher recrudescence rates in artemisinin resistant infections or with lower doses of artesunate treatment. A recent trial in western Cambodia also found there to be no difference in treatment failure rates with 2, 4 or 6mg/kg artesunate, although treatment failure rates in that study were very low.¹⁸⁷

The lack of difference in clearance rates between recrudescence and non-recrudescence infections was true at both study sites and for all treatment regimens used. If anything in Pailin, there was a trend towards higher clearance rates in recrudescence infections. This strongly suggests that the hypothesis of artemisinin resistance causing slow clearance and thus higher treatment failure rates is incorrect. Three alternative hypotheses were presented which may explain parasite dynamics below the limit of microscopic detection and the factors leading to recrudescence. These were differences in antiparasite immunity (immune threshold), alterations in drug action (autoinduction) and parasite dormancy. All three were plausible explanations for recrudescence in artesunate treated patients in this study. Although all of them remain poorly understood, there is a growing body of evidence for each and it seems likely that a combination of all three contributes to varying degrees to the likelihood of recrudescence in most patients. The patients in this study were all from relatively low transmission settings, thus antiparasite immunity is likely to be weak. In parts of Africa where transmission is much higher, immunity may be a more important factor. In the hypothesis presented above this would raise the threshold for parasite clearance by immunity and thus reduce the probability of recrudescence. This is testable by comparing data for the same treatment regimen from different sites with different transmission intensities. Changes in drug action have been studied very little. Future pharmacokinetic studies employing blood sampling throughout the course of treatment, possibly combined with enzyme assays, would help to clarify how much and in what situation this occurs. Dormancy has received much attention recently as a novel mechanism for treatment failure. All of the recrudescences in those treated with artesunate could be reproduced by dormancy in the present study. The derived estimates for duration of dormancy were also remarkably similar to those found in *in vitro* studies.

The rough modelled estimates presented above were dependent on a number of unverified assumptions. However, attempts were made to make these assumptions conservative and thus derive a broad range of possible values. Dormancy has not been demonstrated *in vivo* in humans, and if dormant parasites appear below the limit of parasite detection, this will remain a significant challenge. Taken together the results of the present study and modelling exercise support dormancy as a plausible mechanism for recrudescence in those treated with artesunate but not for artemisinin resistance. As discussed above, recrudescence and artemisinin resistance appear to be separate and unrelated phenomena, which is potentially good news for attempts at elimination. This may be because the current artemisinin resistant phenotype is relatively mild and future evolution to a more artemisinin resistant parasite will produce higher recrudescence rates.

Since the work presented in this Section was completed, there has been accumulating evidence of increased treatment failure rates with artesunate-mefloquine in western Thailand¹⁰⁸ and dihydroartemisinin-piperaquine in western Cambodia.¹⁸⁸ These are both areas with decreased sensitivity to artemisinins. It is not clear whether this is simply the result of a greater body of data or if it represents evolution of a more resistant phenotype. Detailed analysis of these data will be essential to inform future development of mathematical models of artemisinin resistance.

Attempts were made to derive parameters for a fitness cost of artemisinin resistance. Fitness cost of drug resistant compared to drug sensitive parasites can be an important factor when trying to predict the likely impact of control and elimination strategies, particularly by mathematical modelling.²³ A high fitness cost would increase the

likelihood of control or elimination. In this case, fitness cost was defined as a reduction in multiplication rates of resistant versus sensitive parasites. The scope of the data from the present study for accurately estimating fitness cost was very limited and only rough estimates could be derived. The results should thus be viewed with great caution. It can be seen in **table 3.2-2** that the estimates are highly dependent on the assumed normal multiplication rate of sensitive parasites. The commonly cited rate is an 8-fold increase per generation although this figure is for treatment naive parasites and comes primarily from in vitro studies. Assuming this normal multiplication rate gave a median estimate for fitness cost of 17%. It is possible that there is some damage to parasites at the end of treatment and the normal multiplication rate may be slower than this. This would give a lower value fitness cost. Curiously, the multiplication rate of the two recrudescent infections in Wang Pha was much greater than 8-fold and comparison with those in Pailin gave a fitness cost for artemisinin resistance of 37%. Thus no firm conclusions can be drawn from this data set about fitness cost and the derived estimates were not used in the modelling. Taken together, these data suggest that in general there is not a large fitness cost for artemisinin resistant parasites. A larger data set with more recrudescences at both sites may help to narrow down the estimate. Further in vitro studies comparing multiplication rates in treated and untreated parasites are needed.

The secondary aim of this study was to explore the feasibility of using modelling to predict the length of a treatment course required to cure a potentially recrudescent infection. This strategy was based on the assumption that recrudescent infections clear more slowly and thus may respond to longer treatment courses. As this assumption was incorrect, the strategy was deemed flawed and was not explored further.

This study has helped answer several questions important to mathematical modelling of artemisinin resistance and its control and elimination. It has provided numerical values for the range of parasite clearance rates in resistant and non-resistant infections including that there is no evidence for a dose response relationship with artesunate. It has clarified that there is no excess of recrudescences in artemisinin resistant infections and provided a range of possible durations for dormancy. However, it was unable to provide a usable estimate of fitness cost. To further clarify parasite dynamics below the limit of detection and estimate multiplication rates and relative fitness more precisely, *in vitro* studies have much to contribute. Current and on-going *in vitro* studies are exploring parasite dormancy and its possible role in recrudescence in more detail.

A clear understanding of the artemisinin resistant phenotype is essential to plan control and elimination strategies. It is also crucially important to develop mathematical models of artemisinin resistance with which to predict their impact. This study showed that recrudescence artemisinin-resistant infections cleared at the same rate as non-recrudescence infections and there was no evidence for artemisinin resistant infections having a higher recrudescence rate. However, new data indicate that treatment failure rates due to ACT are increased in areas with artemisinin resistance. Parasite clearance rates for artemisinin resistant *P. falciparum* were roughly half those of artemisinin sensitive *P. falciparum*, for both artesunate monotherapy and artesunate-mefloquine and for a range of doses of artesunate. There was no dose response relationship between artesunate dose and parasite clearance rates. Possible explanations for recrudescence included differences in antiparasite immunity, inhibition of drug action and parasite dormancy. The current

understanding of parasite population dynamics below the level of microscopic detection remains poor and there is much scope for further studies in this area.

4.2.2 Parasite clearance slope half-lives in severe malaria in Bangladesh

Evaluation of the *in vivo* responsiveness of *P. falciparum* to intravenous artesunate

The main findings were that *P. falciparum* parasite clearance half-lives due to intravenous artesunate in Bangladesh have increased from 2004-2012 and varied widely between individuals. Although there are other possible explanations, this may be the first evidence of decreasing sensitivity to artemisinins in Bangladesh. The change in half-lives has been small. In 2012, median clearance half-lives in Bangladesh were not significantly different from artemisinin sensitive *P. falciparum* in Mae Sot, Thailand, and were shorter than for Pailin.

The rate of change of artemisinin responsiveness over time in Bangladesh was similar to that found in a previous study in northwest Thailand over the same period.¹⁰⁸ In the Thai study, a change in parasite genetics was associated with the change in sensitivity to artemisinins. This was not assessed in Bangladesh. In Thailand, a cut-off of parasite clearance half-life of ≥ 6.2 hours was used to define 'slow clearers' and the proportion in this category increased from 0.6% to 20% over the period of the study. This cut-off was the geometric mean of clearance half-lives in a dataset from Pailin. In Bangladesh, very few infections cleared this slowly and there was no trend in the proportion of slow

clearers over time, the absolute numbers being small. In 2012, 13% of infections in Bangladesh were slow clearers by this definition.

The slow clearers in this study could be the tail of a distribution of parasite clearance half-lives rather than being due to artemisinin resistance per se. Preliminary results from an ongoing study to determine parasite clearance due to artemisinins in the south of Chittagong Division indicate that artemisinin resistance is not present there. However, the trend of increasing parasite clearance half-lives over time is concerning.

In addition to decreasing sensitivity of parasites to artemisinins, another factor that may contribute to the changing parasite clearance in Bangladesh is changing immunity. Over the period of this study, there has been up scaling of malaria control activities and a probable decrease in symptomatic infection rates in Bangladesh.¹⁶⁷ This could result in a reduction of antimalarial immunity in the population, potentially slowing parasite clearance. Currently there are no reliable methods to directly measure immunity in malaria and this was a retrospective study. However, there was a lack of association of parasite clearance half-lives with malaria transmission intensity and age in Bangladesh. This suggests that the contribution of antiparasite immunity to parasite clearance was negligible in this cohort compared to artesunate. However, even the highest transmission intensities in Bangladesh are far lower than those in parts of Africa where the contribution of immunity may be greater. There is as yet no evidence that immunity accelerates the already rapid parasite clearance due to artemisinins in any setting. Taken together these suggest changing immunity was not the explanation for the change in clearance half-lives, although it cannot be entirely excluded.

Parasite clearance half-lives due to intravenous artesunate monotherapy for severe malaria in Bangladesh were highly variable, similar to oral dosing. This is surprising as intravenous dosing based on body weight should theoretically produce more consistent plasma concentrations of drug between individuals than oral administration. The reason for this great variability found with intravenous artesunate is unknown and requires further investigation. For oral therapy with artesunate parasite clearance half-lives were also highly variable in the other studies in Cambodia and Thailand. However, this was thought to be due in part to variable rates of absorption of drug from the gut, a factor which does not apply to intravenous therapy. This absorption phase is also thought to contribute to the lag phase in parasite clearance.¹⁸⁹ However, the proportion of cases with a lag phase and the median duration of lag phase was the same in those treated with oral and intravenous artesunate in this study. This suggests a different mechanism may be causing the lag phase, possibly schizogony or parasites desequestration.¹⁹⁰ Perhaps reassuringly, the highly variable pharmacodynamics found in this study did not correlate with outcome i.e. mortality or coma recovery time.

Although a small number of patients (6%) in this study had detailed pharmacokinetic sampling, no relationships between drug levels in the blood and parasite clearance half-lives were found. To explore the contribution of variable pharmacokinetics to variability in parasite clearance for intravenous artesunate would require a much larger pharmacokinetic-pharmacodynamic (PK-PD) study. In the subgroup analysis parasite clearance half-lives in Bangladesh were a little higher than those due to 2mg/kg/24 hours in Thailand although not different to those due to 4mg/kg. This difference was barely

significant and the numbers of patients small. If real, it may indicate a dose response relationship in sensitive parasites, although this did not appear to be the case in Cambodia. This could also be explored further in a larger PK-PD study.

There was a weak negative correlation between parasite clearance half-lives and platelet count. This may have been a result of less sequestration and splenic trapping of platelets in some individuals with a higher proportion of parasites and platelets thus appearing in the peripheral blood.

There were several other limitations to this study. It relied on historical data from multiple previous studies. This greatly limited the clinical data with which the clearance half-lives could be compared and precluded genetic association studies which would have provided stronger evidence of emerging resistance. The analysis presented omitted many of those patients who died early in the course of illness. This is because there were insufficient parasite counts to calculate a clearance half-life. This may obscure a relationship between slow clearance and early mortality. However, it has been shown previously that most of the reduction in mortality due to intravenous artesunate occurs after the first 24-48 hours of treatment⁷⁰ and it is likely that in many of these the fatal pathology is already irreversible by the time they present to hospital.

These results indicate that parasite clearance half-lives due to intravenous artesunate in Bangladesh have increased from 2004-2012. This may indicate possible emerging artemisinin resistance in Bangladesh, although the contribution of changing immunity due to falling transmission intensity could not be excluded. Parasite clearance half-lives due

to intravenous artesunate were highly variable between individuals but there was no relationship between clearance half-life and outcome of severe malaria. Further detailed studies are required with larger numbers of patients and including parasite genetics. Ongoing surveillance of artemisinin sensitivity in this area is essential.

4.3 Mathematical models

4.3.1 Model 1

Using a relatively simple modelling framework, it was shown with **Model 1** that eliminating the use of artemisinin monotherapy and replacing it with ACT can be sufficient to eradicate artemisinin-resistant malaria in Cambodia. Short-term or pulsed interventions such as MSAT or MDA were not sufficiently effective to achieve eradication on their own. Their additional effect, when added to the elimination of monotherapy and replacement with ACT, was so small that it was questionable from this model whether they should be considered at all. AP was more effective than ACT when used in MSAT or MDA but the additional impact of MSAT or MDA, when used in addition to elimination of artemisinin monotherapy and switching treatment to ACT, was small. AP also had the disadvantages of slow clinical responses, high purchase cost, and a low threshold for high-grade resistance and was not therefore being considered for routine first-line treatment of symptomatic cases. In stark contrast, ITN greatly accelerated the impact of ACT, provided they were effective. Some vectors in western Cambodia bite very early in the evening and where these predominate, ITN would have marginal benefits. Assuming they were effective the ideal combination appeared to be long-lasting insecticide-treated bed nets and high coverage with ACT treatment.

If ACTs were used to eliminate artemisinin-resistant malaria then **Model 1** provided an important caveat. Elimination of artemisinin-resistant malaria required the elimination of all malaria as the last few infections to be cleared were almost all resistant. ACT provided a selective pressure for artemisinin resistance, especially in infections with concomitant

resistance to the partner drug (piperaquine). The result of this was that ACT produced an increase in the proportion of infections resistant to artemisinin every year that it was used. Monitoring studies would indicate an increase in resistance as incidence and prevalence fell. Any intervention aimed at achieving elimination must, therefore, be used continually through to elimination as discontinuing its use too early would allow the number of infections to increase again but this time a higher proportion of resistant infections would be present than if no intervention had been attempted.

Model 1 assumed that resistance to artemisinin monotherapy had already emerged on the Thai-Cambodian border. Early intervention had a greater chance of preventing its spread than delaying until higher levels of resistance develop (which would be more difficult to contain). Even if resistance was not present, eliminating artemisinin monotherapies and replacing them with an effective ACT with high coverage should remove the potential threat of emergence and spread of resistance, thus increasing the effective life span of this important class of anti-malarial drugs worldwide. **Model 1** provided some general guidelines and principles on elimination of artemisinin resistance using ACT, AP, primaquine and ITN. More detailed and specific conclusions would be possible when more epidemiological and clinical data become available. In summary, rapid, efficient and sustained action could combat the significant risk that artemisinin resistance poses to global public health. Attacking the last man standing is a bold strategy since failure could result in a far more resistant population than existed before the intervention. This strategy, therefore, requires a steadfast commitment from donors to ensure success.

4.3.2 Model 2

Model 2 used data from a recent field trial in Cambodia and showed that the major contributor to the large reductions in parasite prevalence seen over 3 years was use of high coverage with artemisinin-piperaquine ACT for treatment of fever cases. In contrast, MDA with this ACT produced a large initial drop in infected people but its effect was not sustained beyond 1 year. This was primarily because the MDA was only employed for a short period and the number of infections increased again after it ceased whereas artemisinin-piperaquine ACT treatment continued for the entire 3 year study period. Although the initial effect of MDA was greater than that of treatment, the cumulative effect of longer availability of ACT treatment was greater in the long-term.

From field studies, it was not clear to what extent 9mg of primaquine affects gametocytes and reduces malaria transmission. The effect of a higher single dose of adjunctive primaquine² carries a greater risk of haemolysis but its gametocytocidal effect may be greater. To achieve the best fits of the model to field data required the inclusion of an effect of a similar magnitude to 0.75mg/kg. When this was included in the model, the effect of ACT used for treatment was significantly enhanced by the addition of a single dose of primaquine, as had recently been found in a field study in Myanmar, using 0.75mg/kg.¹⁵¹ These findings provided support for large scale deployment of primaquine to help eliminate malaria but highlight the need for dose ranging studies. Primaquine was at the time being considered for country-wide use in Cambodia as an adjunct to ACT with the aim of elimination.¹⁹¹

For primaquine MDA, the modelling results were less encouraging. The modelling indicated that adding a single round of primaquine MDA had very little impact on numbers of infections, and the cumulative effect of multiple rounds would be required. Multiple repeated doses of primaquine given as MDA more than doubled the initial effect of the ACT MDA in the model. This would have been impractical and costly to implement on a large scale. A more pragmatic strategy would be to do several rounds of MDA, one every few weeks. Three rounds of MDA with ACT were found to be optimal in the model and most effective if completed in the 3 months before the nadir in parasite prevalence.

As well as uncertainties about the effective dose, there remained another important barrier to the rollout of primaquine. It can cause dangerous haemolysis in people with certain types of a common genetic abnormality, Glucose-6-phosphate dehydrogenase (G6PD) deficiency. This effect is thought to be dose related and administration of a single low dose, as was used in this trial, should minimize this. In the modelled field study, no cases of significant haemolysis were reported. Previous studies in Cambodia and Myanmar with 9 mg and 0.75 mg/kg base respectively did not encounter problems with haemolysis.^{150,}

¹⁵¹ It was not certain how prevalent G6PD deficiency is in Cambodia (estimates range from 15-20%, the most common variant being G6PD Viangchan (871G>A)^{192, 193}) and field studies are currently underway to investigate this.

The model was used to predict changes in immunity and symptomatic cases after the end of the trial. Immunity in malaria remains poorly understood; this is an important limitation for any model of malaria. A relatively simple structure was thus chosen for

immunity that had been previously validated for a range of transmissions settings. The parameters used for immunity were also varied within wide ranges to check the robustness of the results.

In the model, when on-going high coverage with ACT and single dose primaquine treatment was stopped, mimicking cessation of the project, a subsequent steady rise in parasite prevalence was predicted. In addition, due to a decrease in the population level immunity, the number of clinical cases initially increased to levels greater than before the intervention. This was despite continued availability of ACT at a lower rate of coverage. This would be the situation if a short-term elimination strategy were attempted and then funding withdrawn when numbers of infections were low. This, combined with the long-term decrease being primarily due to the ACT treatment illustrated the importance of ensuring sufficient long-term funding was available for elimination programmes. Even with a successful on-going malaria elimination strategy, in some situations, numbers of symptomatic cases in the model were predicted to increase. This was because as numbers of infected people fell, population immunity decreased and the proportion of people who were symptomatic increased. In the initial stages of an elimination strategy this can result in a levelling off or even increase in the number of symptomatic cases, although this will subsequently fall as numbers of infected people continue to decline.¹⁶⁶ This phenomenon has been observed in the field,^{194, 195} although it has not been possible to separate the role of declining immunity from other possible contributors. Regardless, the cumulative number of clinical cases following an intervention was always predicted by the model to be lower than if there had been no intervention. Where this paradoxical effect occurs, an intervention may appear falsely to be failing. Malaria surveillance systems typically rely

on numbers of reported cases in the absence of data on asymptomatic parasitaemia (passive surveillance) and this finding highlights the importance of regular population screening for overall parasite prevalence to capture both the symptomatic and asymptomatic cases,¹⁹⁶ to get a more accurate picture of the effectiveness or otherwise of elimination strategies. When the intervention was stopped in the model, numbers of symptomatic cases increased more rapidly than overall numbers of parasitaemic people due to a lack of immunity in the population following the period of low prevalence. In this case, although obviously undesirable, this rapid rise may be beneficial as an early warning sign of increasing parasite prevalence.

Model 2 showed that to have a realistic chance of eliminating malaria from an area, a combination of different strategies is required. MDA can significantly reduce the number of infections in the short term (in this model < 1 year), particularly when repeated in the low transmission season, but high coverage with ACT is also required for a prolonged period (4-7 years in the model). Elimination is greatly accelerated by the co-deployment of long-lasting insecticide treated bed nets and further enhanced by the transmission blocking effect of adjunctive primaquine treatment. All this requires significant investment including in longer-term initiatives such as training large numbers of village malaria workers to provide high coverage ACT in remote areas. This is the current strategy adopted by Cambodia which is embarking on a massive scale up of the village malaria worker scheme with funding from the Global Fund to Fight Aids, Tuberculosis and Malaria.

One potential spanner in the works that had not been considered in the models in this thesis is population migration. In-migration of infected individuals reintroduces infection which can make elimination significantly more challenging. There are little data on migration of different population groups in many malaria endemic countries, including Cambodia, and the degree of risk to any future elimination programme is uncertain. Attempts are being made in Cambodia to address this issue and studies are underway to quantify and characterize population migration. If in-migration of infected individuals is a significant contributor to malaria transmission in Cambodia these migrant groups must somehow be included in any elimination strategy, most likely using a similar combined approach to that outlined above.

Another potential limitation of these models was the assumption that only a single infection occurs at any one time in an individual. Although usually true in low transmission settings,¹⁹⁷ this is not the case where transmission is high. It is not known how such multiple clones would interact and affect the transmission dynamics thus it is difficult to predict how they may impact on the results presented. For this to be modelled realistically, further clinical and laboratory research is needed.

A reassuring finding from **Model 2** was that artemisinin resistance, in its current mild form,¹⁰⁴ does not appear to have a large impact on the effectiveness of the regimes used in the trial for elimination. This was true even with the current highest estimate of 10% of infections being resistant.¹⁹⁸ In the presence of hypothetical very high modelled prevalences of artemisinin resistance (70-80%), the effect of ACT was clearly diminished but the addition of primaquine to the ACT largely negated this reduction. As in a previous

model of artemisinin resistance,²³ ACT accelerated the increase in the number and proportion of artemisinin resistant infections, despite greatly decreasing the number of artemisinin sensitive infections. This was worsened by the addition of ACT MDA. This increase in resistance was significantly slowed by the addition of primaquine to ACT treatment as it had sufficient antimalarial action to decrease the overall numbers of both resistant and sensitive infections. These findings may be different if the current mildly resistant phenotype changes to be more resistant, although this was not included here as it is not known what form this phenotype may take.

Model 2 was used to explore a number of scenarios that were not included in the original field study. This was in order to explore possible means of optimizing the strategies used in order to assist with planning future field studies. Using modelling this way can be much more rapid and efficient than trialling multiple variants of strategies in the field. The modelling indicated that multiple combined interventions are more effective than single interventions and it is preferable to use different interventions which impact on the same part of the parasite life-cycle sequentially rather than simultaneously to maximize their long-term impact. This is presumably because there is a maximum effect for drugs with a similar mechanism of action and additional drugs will have no additional impact beyond this maximum. The addition of long-lasting insecticide treated bed nets to any strategy greatly enhanced its effectiveness, despite assuming low efficacy and coverage and a duration of action of only 2 years. There was no advantage to delaying their introduction as they act on an entirely different part of the parasite life cycle.

The results from **Model 2** can be summarized as five major policy implications (listed in **table 4.3-1**) that were presented to CNM. Although this model was developed specifically for Cambodia, these broad recommendations are also relevant to malaria elimination efforts worldwide.

Table 4.3-1. Main policy implications of modelling results.

Main policy implications:
1. High coverage with ACT treatment can produce a long-term reduction in malaria whereas the impact of MDA is generally only short-term 2. Primaquine enhances the effect of ACT in eliminating malaria and reduces the increase in proportion of artemisinin resistant infections 3. Parasite prevalence is a better surveillance measure for elimination programmes than numbers of symptomatic cases 4. Combinations of interventions are most effective 5. Sustained efforts are crucial for successful elimination.

In conclusion, from **Model 2** mathematical modelling when validated by good quality field data can combine information from diverse sources and be used as a tool for enhanced analysis to provide new insights into the results of clinical studies, to make predictions and to assist with planning future studies. **Model 2** provided predictions and a number of novel insights which will be of direct practical benefit to assist planning of future malaria elimination strategies, particularly in the context of the newly emerging artemisinin resistance.

4.3.3 Model 3

Rectal and intravenous artesunate have great potential to reduce mortality from severe malaria. **Model 3** demonstrated that their success is highly dependent on how they are implemented. The model predicted an overall reduction in mortality following introduction of these medications in Bangladesh of 8%. This was far lower than the predicted reduction in mortality from a change from intravenous quinine to intravenous artesunate in hospital.⁷⁰ That is because a large proportion of patients died before reaching hospital or reached hospital too late to benefit from the more effective antimalarial. Before Study 13, in southeast Bangladesh only 12% of patients needing admission reached hospital.¹⁹⁹ The time to reach hospital was a major determinant of mortality in the model, far more so than, for example, drug efficacy. Improvement in access to hospital care for patients with severe malaria has been highlighted previously as the factor likely to reduce mortality the most.¹⁶⁷

It was not known if overt artemisinin resistance has spread to or arisen in Bangladesh although sensitivity appears to be decreasing (**Section 3.2.2**). This model explored the effect of a recent introduction of artemisinin resistance on the efficacy of artemisinin antimalarials for treatment of severe malaria and elimination. The spread of artemisinin resistance in the model was driven mostly by ACT use and there was very little contribution from parenteral artemisinins. This was because only a small proportion of patients received parenteral artemisinins and those that did mostly either died or were cured. Spreading artemisinin resistance compromised the efficacy of rectal and intravenous artesunate for severe malaria and mortality was increased as a result. The resistance phenotype used was the current one in Cambodia. Being a relatively mild

resistance, in the model this had very little effect on mortality at low prevalences of resistance, as is likely to be the case in Bangladesh currently. At very high prevalences of resistance the efficacy of artesunate fell to approach that of quinine. Where the parasites in the model were made more resistant than the current phenotype, mortality from resistant infections was much higher and the spread of resistance faster.

In **Model 3**, it was possible to eliminate malaria in Khagrachari and Cambodia using high coverage of ACT and long-lasting insecticide treated bed nets, similar to what was found with **Models 1 and 2**. Where the Cambodia phenotype of artemisinin resistance was introduced in the model even at very low prevalences of resistance, elimination was no longer possible. This is because the resistance spread faster than the decrease in malaria so almost all infections became resistant before elimination. ACT and LLITN combined were sufficient for elimination only with near perfect coverage. Additional strategies were required to achieve elimination where artemisinin resistance was present e.g. mass drug administration or non-artemisinin antimalarials. The small increase in the rate of spread of artemisinin resistance due to parenteral artemisinins for pre-referral and severe malaria did not significantly affect the speed of elimination. High coverages of ACT reduced numbers of infected individuals in the population and thus population level immunity to malaria. This resulted in an increase in case fatality rate of severe malaria coincident with the decrease in number of deaths.

This model had several limitations. Although it fitted very well to government surveillance data from more than one source, and was validated with separate data, these data were far from perfect. Not all cases of malaria were captured by these data and

estimates of the proportions missed had to be made. The most detailed and reliable data available for model fitting were those from Khagrachari. The mean annual number of cases of malaria in the 2005-2011 government data there was 13,700 with 1670 severe cases compared to the model with 31,109 cases and 1865 severe. Numbers of deaths in the data from Khagrachari were incomplete and many are thought to occur before reaching medical care. Mortality rates thus had to be derived from separate sources. Data from Cambodia were thought to be less reliable and accurate numbers of severe cases and deaths were not available.

The malaria control interventions that had been used in Bangladesh and Cambodia were included in the model but the exact coverages used and precise dates of when they were implemented were not available. Estimates for these were thus used. For Bangladesh, the model was based primarily on detailed data from one District as this was of high quality and is what was available. Other parts of the highly malaria endemic region of Bangladesh are thought to have similar epidemiology to Khagrachari, as has much of Cambodia and other parts of malaria-endemic Asia. The broad results presented can thus be assumed to apply to a wide range of countries.

To assess the contribution of rectal artesunate to exposure of the infected population to artemisinins, and thus spread of artemisinin resistance, an estimate was required of how many individuals with malaria parasites would receive the drug. This was a combination of those with severe malaria, all of whom required parenteral therapy, those with uncomplicated malaria who could not take oral treatment and those with another febrile illness mimicking the first two groups but with incidental (i.e. asymptomatic)

parasitaemia. In Study 18, malaria status was not recorded. In Study 13, 79.3% of enrolled patients (i.e. eligible for and given rectal artesunate) progressed to severe malaria. However, it was not known how many would have progressed to severe malaria had they not received rectal artesunate. Thus a maximum of 20.7% of patients eligible for rectal artesunate in Study 13 had uncomplicated malaria. As it was the most conservative estimate, this was the figure used in the model. The third group is a potentially important one in high transmission settings where incidental parasitaemia can be common and it can be very difficult to determine whether the cause of a malaria positive patient's illness is malaria. In severe malaria, malaria retinopathy has been used for this purpose²⁰⁰ but this was not assessed in Study 13. Malaria retinopathy is a unique set of visible changes in the back of the eye that is present in most individuals with severe, particularly cerebral, malaria caused by *P. falciparum*. It can be used to distinguish severe disease due to falciparum malaria from severe disease due to other causes, e.g. sepsis or nonmalarial encephalopathy, in high transmission settings.²⁰¹ In low transmission settings, incidental parasitaemia is likely to be rare and in patients with parasites malaria is assumed to be the cause of their illness. This model was restricted to low transmission settings and thus only included the first two groups.

In conclusion, pre-referral treatment with rectal artesunate can reduce mortality from severe malaria in those who reach hospital quickly and receive further intravenous therapy. Using high coverage of rectal and intravenous artesunate for severe malaria has very little effect on the spread of artemisinin resistance. This is because only a small proportion of total cases receive them so drug pressure is low and almost all severe cases either die or are cured so do not go on to transmit resistant infection. Increasing coverage

with ACT decreases deaths from malaria but can increase mortality *rate* by reducing protective immunity at the population level. A policy change to rectal and intravenous artesunate in the context of pre-existing artemisinin resistance does not compromise the efficacy of ACT for malaria elimination.

4.4 General Discussion

The models presented in this thesis predict that it is possible to eliminate artemisinin resistant *P. falciparum* from Cambodia malaria using high coverage with ACTs, ideally combined with other strategies. In order to achieve this, the models predict that it is necessary to eliminate all falciparum malaria. This is because the proportion of infections which are artemisinin resistant increases as the overall number of infections decreases.

The key qualitative prediction is that, during a malaria elimination campaign using artemisinin antimalarials e.g. ACT, the number of artemisinin sensitive cases decreases more quickly than the resistant infections. This gives the resistant infections a competitive advantage within the decreasing numbers of cases leading to an increasing proportion of those cases being resistant. This phenomenon has been described in **Section 3.3.1** as the “last man standing” being the most resistant. The policy implication of this result is that an elimination campaign must be sustained until completion because early cessation is likely to result in an infectious population which is more resistant than at the beginning of the campaign. This early finding was incorporated into the plan for elimination of artemisinin resistance in western Cambodia and malaria elimination became the goal.

This model explored MDA and MSAT with ACT as strategies for elimination and found them to be relatively ineffective compared to treatment at the population level. With a lack of field data, piloting and scale-up of MDA and FEMSE (a strategy combining MDA

and treatment), were subsequently included in the Strategic Plan for Elimination of Malaria in Cambodia.¹⁹¹ They were consequently explored in more detail in the second model (see below).

For **Model 1**, the intention was to develop a simple model to provide broad conclusions rapidly. It was planned to further develop this model at a later date to address specific questions from policy makers in Cambodia and elsewhere. The goal was to gradually add complexity as data became available during the course of the project. This included increased understanding of artemisinin resistance and epidemiological data for fitting and validation of the model. In 2009, artemisinin resistance had been confirmed in only a single small study in Pailin, Cambodia and Wang Pha, Thailand. Conclusions about the behaviour of artemisinin resistant infections were very limited. Upon collection and analysis of much larger datasets from a series of on-going studies of artemisinin resistance in Cambodia, Thailand and Bangladesh for this thesis (**Sections 2.2.1, 2.2.2, 3.2.1 and 3.2.2**) the simple approximations used for artemisinin resistance and sensitivity in the first model were found to be valid. This new analysis showed no increase in recrudescence in artemisinin resistant infections so explicit modelling of this was omitted from subsequent models. There was also no dose response relationship thus potentially allowing the same model structure to be used for a range of drug doses. **Model 1** also included initial attempts to simulate the effects of MDA and MSAT incorporating primaquine and atovaquone-proguanil. Due to a lack of data and the need to produce urgent results, detailed modelling and analyses of these were left to later models.

The first detailed data that became available for model fitting and validation were the results of a large field study in Cambodia which aimed to eliminate malaria using ACT and primaquine.²⁷ This trial had been organized by a Chinese team in collaboration with CNM who were very interested in the potential of primaquine as an adjunctive gametocytocidal therapy to reduce *P. falciparum* malaria transmission. This has since been the topic of much discussion in the malaria community and several trials are at various stages of completion.²⁰² **Model 2** was developed to predict the potential efficacy of primaquine in contributing to elimination of *P. falciparum* malaria and its' possible impact on artemisinin resistance. For the second model, CNM provided additional unpublished details about their trial and independently collected government surveillance data for the trial area. This second model also included detailed modelling of antimalarial immunity. This allowed more accurate inclusion of treatment of symptomatic cases and asymptomatic infections, both of which had not been differentiated in **Model 1**.

Model 2 confirmed the main finding of the first, that high sustained coverage with ACT treatment can produce long-term reductions in malaria prevalence. Elimination was feasible, particularly when high coverage ACT treatment was combined with other strategies, particularly LLITN. This model also confirmed the finding from the **Model 1**, that MDA is relatively ineffective for elimination. The effect of MDA in the **Model 2** was short-term (under 1 year) and even multiple repeated rounds of MDA were insufficient for elimination. When MDA was combined with sustained high coverage with ACT treatment, elimination was much more readily achievable. The model showed that single dose primaquine could contribute to reducing malaria prevalence when added to ACT treatment and it helped prevent the spread of artemisinin resistance. This has contributed

to the current proposed treatment strategy for Cambodia of treatment with dihydroartemisinin-piperaquine plus single dose primaquine.¹⁹¹ MDA has been restricted to a pilot in the same plan. **Model 2** is currently being used to explore the efficacy of primaquine in a broad range of low transmission settings for WHO and will form the basis of an economic model to assess its' cost effectiveness.

For the **Model 3**, complexity was further increased by the addition of severe and fatal malaria. There was concern that spreading artemisinin resistance would compromise the efficacy of two important new therapies being rolled out for falciparum malaria.

Intravenous artesunate became recommended first line treatment for severe malaria and rectal artesunate for pre-referral treatment of malaria worldwide in 2010. In response to this, **Model 3** was developed to explore the possible impact of these interventions on the spread of artemisinin resistance and its' possible effect on their efficacy. The model was developed in close collaboration with the groups who performed the major trials of intravenous and rectal artesunate. At the time, it was not known whether *P. falciparum* remained fully sensitive to artemisinins in Bangladesh and this knowledge was necessary to parameterize the mathematical model. The study in **Section 2.2.2** was thus undertaken. The main finding, that artemisinin sensitivity in Bangladesh was slowly decreasing over time, was unexpected. It had also been discovered that the same was happening in northwest Thailand¹⁰⁸ over a similar period. Although not clearly indicating artemisinin resistance, these findings increased the urgency with which the modelling results were needed.

The main findings of **Model 3** were that a policy change to rectal and intravenous artesunate in the context of pre-existing artemisinin resistance did not compromise the

efficacy of ACT for malaria elimination in low transmission settings. There was also little impact of the current mild form of artemisinin resistance on their efficacy. This was because a minority of infected individuals received these therapies and most individuals who did receive them were either cured or died of their infection so did not go on to transmit artemisinin resistance. Roll-out of both strategies is continuing. The next step for this model is that it will form the basis of an economic model to assess the cost effectiveness of rectal artesunate in the context of artemisinin resistance.

This thesis illustrates the importance of quality data and detailed understanding of the underlying system for mathematical modelling. Because of the need to develop the initial models for this thesis in the absence of data, the conclusions that could be drawn from them were limited. As more data became available, the predictions were more directly relevant and useful to policymakers and as a result had a direct impact on planning and policy.

The modelling presented here was developed as an iterative process with interaction with other research disciplines and policymakers. This led to increasingly complex and refined models and movement from qualitative to quantitative predictions of more direct practical use.

The modelling and data collection and analysis in this thesis were carried out simultaneously. This was because there was an urgent need for preliminary modelling results to inform planning of interventions to tackle the WHO-declared emergency of artemisinin resistance. There was insufficient time and resources to plan and carry out

new studies and much of the data that had been collected were not readily available in a suitable format for analysis. For Cambodia, two separate sources, government HIS and VMW data were combined to gain a more complete picture. The HIS data were collated and entered into a computerized database and the VMW data separately, with final data entry not completed until autumn 2012. For Khagrachari, Bangladesh, government and NGO data were included. These were kept in paper records for which only a summary was available electronically. For this thesis, these paper records were entered into a database in the field for analysis. The epidemiology data from CMCH were also entered from paper records for this thesis. The parasite clearance data from Bangladesh were collected as part of clinical studies performed by myself and collaborators in the same research group. Because of this, it was possible to ensure high quality data entry and checking against source material. Combined with the use of data from multiple sources, these methods helped to ensure that the data used were of high quality.

The epidemiology data from Khagrachari and Cambodia were incomplete as they did not include information from the private sector, potentially a major contributor to malaria treatment in both countries. There is currently no system for collecting this information in either country (as is the case for most malaria endemic areas) so total numbers could only be derived from estimates and extrapolation. It is possible that the provision of private healthcare may have varied geographically and over time, and this may have contributed to the spatial and temporal patterns found in malaria distribution. In the absence of any information to the contrary, however, it was assumed for the purposes of the models that the patterns seen were representative of the whole picture.

To fully understand the patterns in the epidemiological data, details of the malaria control activities undertaken during the period of the study were also needed. On the whole, these details were vague. The timing of particular interventions and a rough idea of coverage were often the most that were available. Details were known for some areas, e.g. the elimination trial from 2004-2007 in Kampot and Kampong Speu in Cambodia²⁷ and VMW coverage in Cambodia but could not be obtained during the period of these studies for many others. These details may become available from separate sources in the future. With these additional details, further analyses of these data could be informative and this would help to build and parameterize more accurate and realistic mathematical models.

As outlined above and in **Section 5.3** further models of malaria elimination and artemisinin resistance are already under development, and yet more planned, to answer specific questions from policymakers in WHO, CNM and elsewhere. There is an on-going and urgent need to collect further detailed epidemiological and clinical data to inform development of these models. Continuing clinical and epidemiological data collection and new clinical studies are helping to meet this need.

Chapter 5 : Conclusion

5.1 Summary

Antimalarial resistance, particularly artemisinin resistance, is a major threat to *P. falciparum* malaria elimination efforts worldwide. Urgent intervention is required but field data on which to base planning of strategies are limited. This DPhil has contributed new data on artemisinin resistance and malaria epidemiology in Cambodia and Bangladesh. These include patterns over time and the identification of possible developing artemisinin resistance in Bangladesh. It has also provided the first of a series of mathematical models of malaria elimination and artemisinin resistance which are helping to design the optimal strategies for malaria elimination and treatment in the context of artemisinin resistance. These models have demonstrated that elimination of artemisinin resistant *P. falciparum* malaria would be achievable in Cambodia in the context of artemisinin resistance using high coverages with ACT treatment, ideally combined with LLITNs and adjunctive single dose primaquine. Sustained efforts would be necessary to achieve elimination and effective surveillance is essential, both to identify the baseline malaria burden and to monitor parasite prevalence as interventions are implemented. A modelled policy change to rectal and intravenous artesunate in the context of pre-existing artemisinin resistance would not compromise the efficacy of ACT for malaria elimination.

5.2 Policy implications and impact on strategy

This thesis is part of an on-going effort to collect data and develop mathematical models to help with efforts towards tackling artemisinin resistance. This includes strategies for the elimination of artemisinin resistant malaria and the design of optimal treatment strategies for severe malaria. The models presented in this thesis have already had a significant impact on strategies for Cambodia and have informed development of the strategic plan for the country. Through presentation in policy meetings and research conferences, as well as through publication they have contributed greatly to the discussions on artemisinin resistance in the wider research and policy communities. They are also forming the basis of dynamic economic models of malaria elimination and treatment which are currently under development and are contributing to the development of a spatial model of malaria elimination for Cambodia. The collection and analyses of detailed data on malaria epidemiology and sensitivity to artemisinins presented in this thesis have provided an invaluable resource, not only for development and parameterization of mathematical models but also through presentation and publication for the malaria research community as a whole.

5.3 Future Directions

Several models extending the work presented in this thesis are currently under development.

The coding and parameterisation have been completed for a model to compare atovaquone-proguanil treatment with ACT in the context of atovaquone and artemisinin

resistance. Preliminary runs of this model have already been used to help inform the decision of whether to adopt atovaquone-proguanil in Cambodia.

Model 2 is forming the basis of an economic model of the cost effectiveness of adjunctive single dose primaquine treatment for *P. falciparum* elimination.

Model 3 is being developed in collaboration with WHO into an economic model of the cost effectiveness of pre-referral rectal artesunate in the context of artemisinin resistance. As epidemiological data become available it is planned to extend this model to Africa.

A spatial model is also under development for Cambodia for the purpose of detailed strategy design. This will use the malaria incidence data from HIS and VMWs in Cambodia presented in **Section 3.1.1** to parameterize the model.

The epidemiological and parasite clearance data collections begun for this thesis in Cambodia and Bangladesh are on-going. Data from 2012 are currently being collated with a plan to publish the results in 2013. The parasite clearance data with intravenous artesunate in Chittagong to the end of 2012 (**Section 3.2.2**) will also be published as a separate manuscript. Beyond 2012, on-going collection of these data as part of on-going clinical studies there will help to monitor artemisinin sensitivity into the future as use of artemisinins in Bangladesh increases.

5.4 Conclusion

Artemisinin resistance is a new and urgent threat to malaria control and elimination efforts worldwide. Despite an initial lack of field data, by being developed rapidly in response to specific questions the models presented here are helping to inform planning efforts to combat artemisinin resistance. As further field data become available, their planned on-going development will produce increasingly realistic and informative models which can be expected to play a central role in planning efforts for years to come.

Chapter 6 : APPENDIX

6.1 Parasite clearance rates in Cambodia and Thailand

Hypotheses of drug response dynamics in response to artemisinins in *P. falciparum* malaria

Although measurable parasite clearance can be summarized with a log linear clearance slope, it can be seen from the above results that parasite clearance dynamics below the limit of detection in peripheral blood cannot always follow this pattern. Continuation of parasite clearance at the same log linear rate below the limit of detection for those in Wang Pha and Pailin would have resulted in much higher recrudescence rates after artesunate monotherapy than were found (6/20 (30%) versus 2/20 (10%) and 29/40 (73%) 6/40 (15%) respectively). In these patients, parasite clearance must have accelerated below the limit of detection to reach zero before 7 days. Conversely, most of those that did recrudescence on artesunate monotherapy (4/6 (67%) in Pailin and 1/2 (50%) in Wang Pha) would have cleared their infection before finishing treatment and thus not recrudesced. In these patients, parasite clearance must have slowed down or stopped below the limit of detection. Clearly another process or processes are contributing. Three possible interacting and potentially testable hypotheses that could explain this are outlined below.

1. Antiparasite immunity

The contribution of the immune response to parasite clearance in malaria is poorly understood. It has long been known that cure rates are higher in immune patients²⁰³ and more recently specifically in those with higher serum antimalarial antibody levels^{204, 205}. However, immunity does not increase parasite clearance rates in those receiving

artemisinins.²⁰⁵ This was also confirmed by a lack of association between patient age and parasite clearance rate in Pailin ($p=0.76$) using age as a surrogate for immunity. Thus the contribution of immunity to parasite clearance must be mostly below the limit of parasite detection and is therefore likely to be most important when numbers of parasites are small. Conceptually this can be thought of as a threshold of parasite biomass below which the immune system can clear parasites from the body (**figure 6.1-1A**). This threshold would move upwards or downwards (**figure 6.1-1B**) according to the strength of immunity in the individual. Thus an antimalarial drug would not have to reduce biomass to zero for an infection to be cleared (**figure 6.1-1A**), only to the postulated threshold for immune clearance. This would explain the low rates of recrudescence in slowly clearing infections in this study. If antiparasite immunity were weak, the host may not clear the small number of parasites remaining at the end of treatment and these could thus multiply again and recrudescence (**figure 6.1-1B**). This could explain the recrudescences in patients with fast parasite clearance. A major problem with this hypothesis for this study is that transmission intensity in Wang Pha and Pailin is low and thus levels of immunity are unlikely to vary much between most individuals. However, pockets of high transmission are known to exist²⁰⁶ so it could still be a factor in some. No data on transmission intensity or measurements of immunity were included in this analysis. There was no difference between median (IQR) age of those with recrudescence versus non-recrudescence infections (23 (17-28) years versus 21 (16.25-28.75) years, $p=0.74$) suggesting the impact of immunity is minor if any.

In this analysis, the median (IQR) log projected biomass at 7 days in patients in Pailin who cleared their parasites after artesunate monotherapy was 2.93 (1.09-4.04) i.e. around

3.5-6.5 logs below the limit of parasite detection. This suggests that patients with between 10 and 10^4 parasites in the body in Pailin were able to clear their parasites by immunity alone. These values were used as the postulated thresholds for immune clearance in **figure 6.1-1** to illustrate the concept. The contribution of different levels of immunity to parasite clearance and recrudescence could be further explored in future studies by comparing findings in different transmission settings and across age groups.

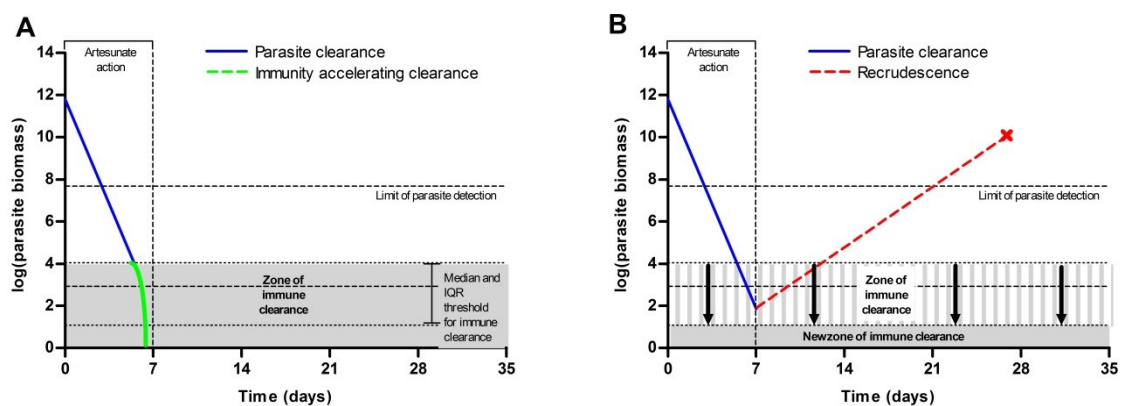


Figure 6.1-1. Hypothesis for the role of immunity in parasite clearance in *P. falciparum* malaria.

A parasite clearance due to artesunate (blue line) is accelerated (green line) by immunity when it falls below a certain threshold parasitaemia resulting in cure. **B** the level of immunity is low thus the parasite clearance does not reach the threshold for immune clearance before the end of treatment resulting in a recrudescence (red dotted line). The median and IQR thresholds for immune clearance in this example are those calculated from the projected log parasite biomass at 7 days in Pailin in those cured by artesunate monotherapy.

2. Alterations in drug action

Another possible explanation for recrudescence is that the rate of parasite killing by antimalarial drugs changes towards the end of treatment below the limit of parasite detection (**figure 6.1-2**). Slowing of drug action, for example by autoinduction of enzymatic drug metabolism,²⁰⁷ may allow infections that would otherwise clear to recrudescence. It is likely that the rate of drug metabolism and thus the strength of this effect vary greatly between individuals. It was not possible to assess this from the data for the present study as PK sampling was done only on enrolment. Such changes in drug metabolism over time may be detected in future studies by doing PK studies with sampling at the start and end, or ideally throughout, a treatment course. The patients in the present study received repeated doses of artesunate over 7 days and it is plausible that autoinduction of metabolism may have played a role in some of the recrudescences.

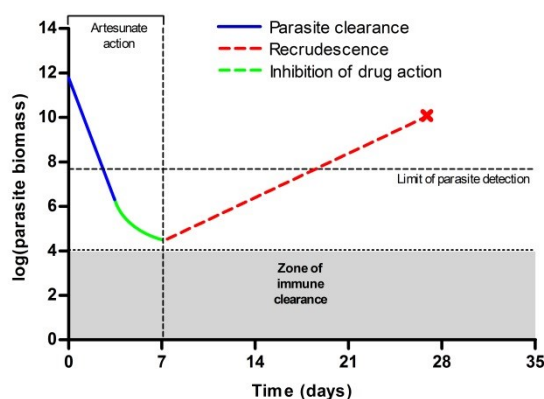


Figure 6.1-2. Recrudescence due to slowing of antimalarial drug action towards the end of treatment.

3. Dormancy

During treatment, a small proportion of parasites may enter a resting state and thus avoiding clearance by antimalarials or immunity. These parasites later begin multiplying

again resulting in a recrudescence (**figure 6.1-3**). This has been demonstrated to occur *in vitro* in falciparum malaria treated with artesunate²⁰⁸ and recently in *P. vinckei* in mice²⁰⁹ but has not yet been demonstrated in humans. *In vitro*, dormancy following treatment with a single dose of artemisinin lasts from 3 to 20 days, with 50% lasting 9 days, before parasites resume normal growth.²¹⁰ Dormancy has been proposed as a mechanism for both recrudescence²¹¹ and artemisinin resistance.²⁰⁸

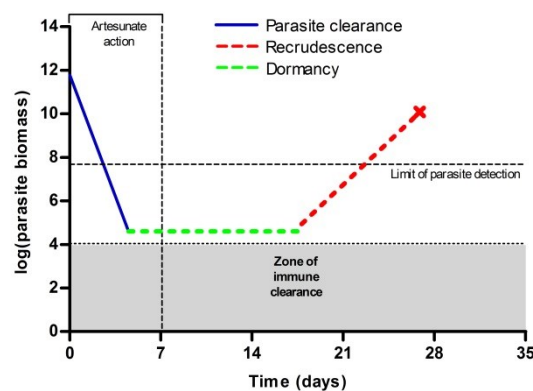


Figure 6.1-3. Dormancy as a postulated mechanism for recrudescence.

Parasites become dormant towards the end of treatment (green line) and later multiply to form a recrudescence (red line).

Dormancy has potential as a mechanism for recrudescence for any of the patients in the present study and could explain why recrudescence rates appear to be unrelated to parasite clearance rates. It has also been proposed that dormancy contributes to the slower parasite clearance rates found in artemisinin resistance.²⁰⁸ For dormancy to cause slower parasite clearance it would have to occur early and in a high proportion of parasites. Dormant parasites would thus be detectable above the limit of microscopic detection and this has not been described. If it is possible to differentiate dormant from non-dormant parasites morphologically,²¹⁰ future clinical studies on artemisinin resistance could add

this in to serial slide examination during treatment. If dormancy does occur early this would result in a higher rate of recrudescence in artemisinin resistant infections. The present study showed this not to be the case thus its role in artemisinin resistance is not supported.

For the patients in the present study, recrudescence was modelled using dormancy to explore it's feasibility as a mechanism and to generate a rough estimate for its' duration in those treated with artesunate monotherapy. To do this, a range of assumptions was employed to generate a broad range of possible values for each patient.

Several possibilities for the time that dormancy starts can be reasoned logically. 1) For dormancy not to slow parasite clearance, as discussed above, it must begin below the limit of parasite detection in the peripheral blood. Thus the earliest that dormancy can start is at just below this threshold. 2) The latest dormancy can start is when the parasite count is just above zero or at the end of treatment, whichever comes first. As it is not known if these dormant parasites would escape antiparasite immunity it is difficult to guess the number of dormant parasites required for the infection to survive. If dormant parasites are immunologically inert, it may be very small. 3) Or it may be that parasites become dormant above or near the threshold below which they are cleared by immunity (we have an estimate of this from the cleared infections of $\log \text{biomass} = 2.93$ (1.09-4.04)). 4) The final possibility to estimate the time that parasites first become dormant is to back extrapolate from the recrudescence point and assume the time taken to dormancy is the time taken to reach the parasitaemia of the recrudescence at day 7. This would have to assume a multiplication rate for the recrudescence infection which itself can take a range of

values (see below). Thus a range of up to 7 possible values for the time that dormancy starts can be derived by extrapolation of the log linear parasite clearance curve to various points.

The end point for a dormant period will depend on the subsequent rate of parasite multiplication to produce the recrudescence. Possible end-points for dormancy were thus identified by back extrapolation from the point of recrudescence at a 6, 8 or 10 –fold multiplication rate to the same biomasses as at the different start points. In addition, the median multiplication rate for recrudescences in Wang Pha was used. In this way, a range of possible values for the duration of dormancy in each individual were derived (**table 6.1-1**). Negative values were discarded. The individual patient data are shown in **figure 6.1-4**.

Table 6.1-1. Estimates for the duration of dormancy in 6 patients treated with artesunate monotherapy.

Each number is the median of four estimates which assumed parasite multiplication rate of 6, 8 or 10-fold or equal to recrudescence of artemisinin sensitive infections in Wang Pha. Low, medium and high indicate the duration of dormancy estimated at three possible parasite counts during the dormant period. Low is dormancy at parasite count=0 or that at 7 days, whichever was earlier; medium is parasite count=immune threshold; high is parasite count=just below the limit of detection.

Patient	Duration of dormancy (days)		
	Low	Medium	High
1	3.1	5.3	17.0
2	1.2	2.7	14.4
3	5.7	13.6	25.6
4	3.4	8.0	19.6
5	10.2	N/A	15.3
6	7.4	14.8	26.5
Median	4.6	8.0	18.3

It should be noted that these estimates assume there to be no fitness cost. If there were a fitness cost, then multiplication would be slower and dormancy shorter. This is thus a maximum duration of dormancy assuming parasites multiply normally at the end of dormancy.

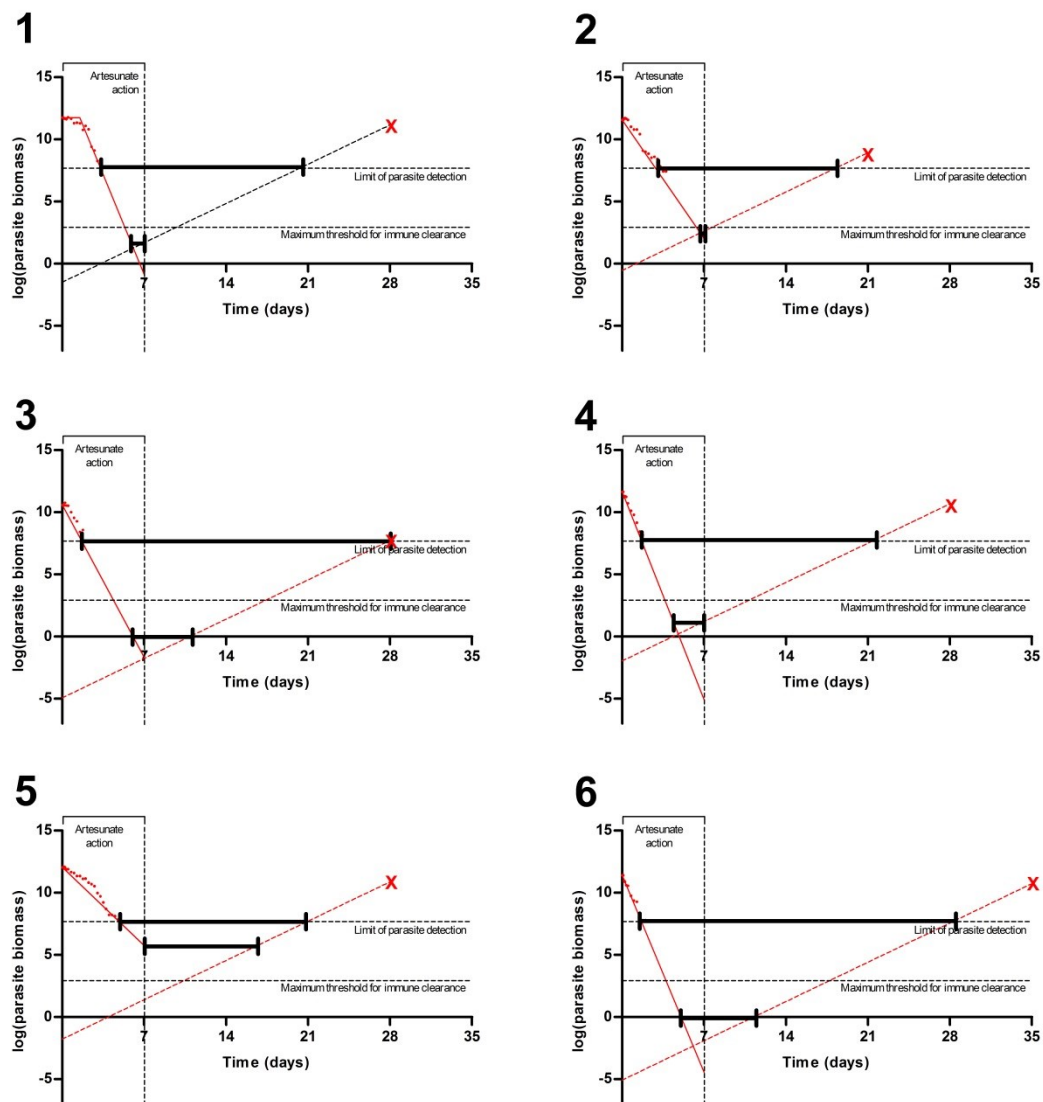


Figure 6.1-4. Individual parasite profiles of 6 patients from Pailin with recrudescent infections.

The red cross is the recrudescent and the small red dots the parasite biomass during clearance. The parasite clearance slope is shown with lag phase where present as a solid red line. The recrudescent with a presumed multiplication rate of 8-fold per 48 hours is shown as a red dotted line. For each, the horizontal black bars indicate the longest and shortest possible durations of dormancy.

6.2 Summary model equations

Each model is described by a set of nonlinear ordinary differential equations as follows:

6.2.1 Model 1

$$\begin{aligned}\dot{S}_{dg} &= b_d N - \frac{(1-\rho)\beta S_{dg} \sum_{g \in G} \sum_{d \in D} \sum_{r \in R} (1-c_r) I_{rdg}}{N} + \sum_{r \in R} (\nu_{L,rdg} L_{rdg} + \nu_{B,rdg} B_{rdg} + \nu_{I,rdg} I_{rdg}) - \mu S_{dg} + Y_{Sd} + f_S(\tau, S, L, B, I) \\ \dot{L}_{rdg} &= \frac{(1-\rho)\beta S_{dg} \sum_{g \in G} \sum_{d \in D} (1-c_r) I_{rdg}}{N} - (\gamma + \nu_{L,rdg} + \mu) L_{rdg} + Y_{Ld} + f_L(\tau, S, L, B, I) \\ \dot{B}_{rdg} &= \gamma L_{rdg} - (\sigma + \nu_{B,rdg} + \mu) B_{rdg} + Y_{Bd} + f_B(\tau, S, L, B, I) \\ \dot{I}_{rdg} &= \sigma B_{rdg} - (\nu_{I,rdg} + \mu) I_{rdg} + Y_{Id} + f_I(\tau, S, L, B, I)\end{aligned}$$

$$r \in R = \{none, a, b\}, \quad d \in D = \{none, a, b, ab\}, \quad g \in G = \{0, 1, 2, \dots\}$$

$$\mathbf{b} = (\mu \quad 0 \quad 0 \quad 0)$$

$$\mathbf{c} = (0 \quad c_a \quad c_b)$$

$$X_{none} = \begin{pmatrix} 0 & \frac{1}{x_a} & \frac{1}{x_b - x_a} & 0 \end{pmatrix}$$

$$X_a = \begin{pmatrix} 0 & -\frac{1}{x_a} & 0 & 0 \end{pmatrix}$$

$$X_b = \begin{pmatrix} 0 & 0 & -\frac{1}{x_b - x_a} & \frac{1}{x_a} \end{pmatrix},$$

$$X_{ab} = \begin{pmatrix} 0 & 0 & 0 & \frac{1}{x_a} \end{pmatrix}$$

$$\begin{aligned}\mathbf{S}_g &= (S_{none\ g} \quad S_{a\ g} \quad S_{b\ g} \quad S_{ab\ g}) \\ \mathbf{L}_{rg} &= (L_{r\ none\ g} \quad L_{r\ a\ g} \quad L_{r\ b\ g} \quad L_{r\ ab\ g}) \\ \mathbf{B}_{rg} &= (B_{r\ none\ g} \quad B_{r\ a\ g} \quad B_{r\ b\ g} \quad B_{r\ ab\ g}) \\ \mathbf{I}_{rg} &= (I_{r\ none\ g} \quad I_{r\ a\ g} \quad I_{r\ b\ g} \quad I_{r\ ab\ g})\end{aligned}$$

$$\mathbf{Y}_{Sd} = \mathbf{X}_d \bullet \mathbf{S}_g^T$$

$$\mathbf{Y}_{Ld} = \mathbf{X}_d \bullet \mathbf{L}_{rg}^T$$

$$\mathbf{Y}_{Bd} = \mathbf{X}_d \bullet \mathbf{B}_{rg}^T$$

$$\mathbf{Y}_{Id} = \mathbf{X}_d \bullet \mathbf{I}_{rg}^T$$

Where:

S susceptible

L liver stage infection

B non-infectious blood stage

I infectious blood stage infection

b = birth rate

μ = death rate

β = transmission parameter

γ = rate of going from liver stage to blood stage non-infectious

σ = rate of going from blood stage non-infectious to blood stage infectious

δ = recovery rate in nonimmune population

Artemisinin is represented as a and piperaquine as b with ACT being ab .

The sets R , D and G refer to the categories of resistance, drug activity and intervention strategy respectively. The arrays ν (recovery from infection under the action of the drugs i.e. recovery rates), τ (rate of drug acquisition i.e. treatment rates) and f (intervention treatment strategies) depend on the nature of the intervention strategies and combinations of drugs. The arrays $\mathbf{X}$ and $\mathbf{Y}$ define the dynamics of the sequential loss of drug effect where x_a is the duration of ACT treatment plus the time post treatment to sub therapeutic levels of artesunate and x_b is the duration of ACT treatment plus the time post treatment to sub therapeutic levels of the partner drug. T represents the transpose function on the associated vector.

This deterministic model was also rewritten as two stochastic models; one population based and another individual based. All three models used the same structure and parameters. The population dynamic stochastic model was a set of difference equations based on the Euler approximation of the corresponding differential equations as the means of a set of Poisson distributions from which the value of each variable was sampled at each time step. For the individual based model, a population of individuals was generated with a list of states which defined the variables of the corresponding deterministic model. The individuals change from one state to another with probabilities defined by the parameters of the corresponding deterministic model apart from the transmission parameter. In this instance, transition from uninfected to blood stage was modelled as the probability of a susceptible individual receiving an infectious bite from a mosquito which had previously bitten an infected individual chosen at random. These two

stochastic models produced very similar results and 200 runs of the population based model were used to generate the results given in this thesis.

6.2.2 Model 2

$$\dot{S} = bN - \frac{\beta S(I_S + I_R)}{N} + p_S(v_L L_S + v_B B_S + v_I I_S) - \mu S + \delta I_S + wR$$

$$\dot{L}_S = \frac{\beta S(I_S + I_R)}{N} - (\gamma + p_S v_L + \mu) L_S$$

$$\dot{B}_S = \gamma L_S - (\sigma + p_S v_B + \mu) B_S$$

$$\dot{I}_S = \sigma B_S - (\delta + p_S v_I + \mu) I_S - \gamma I_S$$

$$\dot{R} = -\frac{\beta R(I_S + I_R)}{N} + p_R(v_L L_R + v_B B_R + v_I I_R) - \mu R + d I_R + \gamma I_S - wR$$

$$\dot{L}_R = \frac{\beta R(I_S + I_R)}{N} - (\gamma + p_R v_L + \mu) L_R$$

$$\dot{B}_R = \gamma L_R - (\sigma + p_R v_B + \mu) B_R$$

$$\dot{I}_R = \sigma B_R - (d + p_R v_I + \mu) I_R$$

Where:

- S = susceptible, nonimmune
- L_S = liver stage, nonimmune
- B_S = blood stage non-infectious, nonimmune
- I_S = blood stage infectious, nonimmune
- R = susceptible, immune
- L_R = liver stage, immune
- B_R = blood stage non-infectious, immune
- I_R = blood stage infectious, immune
- d = recovery rate in immune population
- γ = rate of infectious blood stage in nonimmunes becoming immune
- w = rate of losing immunity
- p_S = proportion of nonimmunes who are symptomatic
- p_R = proportion of immunes who are symptomatic

6.2.3 Model 3*Uncomplicated malaria*

$$\dot{S} = bN - \frac{\beta S(I_S + I_R)}{N} + p_{Rxs}(v_L L_S + v_B B_S + v_I I_S) - \mu S + \delta I_S + wR$$

$$L_S^\cdot = \frac{\beta S(I_S + I_R)}{N} - (\gamma + p_{Rxs}v_L + \mu)L_S$$

$$B_S^\cdot = \gamma L_S - (\sigma + p_{Rxs}v_B + \mu)B_S - F_S B_S + rec_{FS} B_{FS}$$

$$I_S^\cdot = \sigma B_S - (\delta + p_{Rxs}v_I + \mu)I_S - \gamma I_S + rec_{FS} I_{FS}$$

$$\dot{R} = -\frac{\beta R(I_S + I_R)}{N} + p_{RxR}(v_L L_R + v_B B_R + v_I I_R) - \mu R + dI_R + \gamma I_S - wR$$

$$L_R^\cdot = \frac{\beta R(I_S + I_R)}{N} - (\gamma + p_{RxR}v_L + \mu)L_R$$

$$B_R^\cdot = \gamma L_R - (\sigma + p_{RxR}v_B + \mu)B_R - F_R B_R + rec_{FR} B_{FR}$$

$$I_R^\cdot = \sigma B_R - (d + p_{RxR}v_I + \mu)I_R + rec_{FR} I_{FR}$$

Severe malaria

$$\dot{S}_F = bN - \frac{\beta S_F(I_S + I_R)}{N} + p_{Rxs}(v_L L_{FS} + v_B B_{FS} + v_I I_{FS}) - \mu S_F + \delta I_{FS} + wR_{FS}$$

$$L_{FS}^\cdot = \frac{\beta S_F(I_S + I_R)}{N} - (\gamma + p_{Rxs}v_L + \mu)L_{FS}$$

$$B_{FS}^\cdot = \gamma L_{FS} - (\sigma + p_{Rxs}v_B + \mu)B_{FS} + F_S B_S - \mu_{mS} B_{FS} - rec_{FS} B_{FS}$$

$$I_{FS}^\cdot = \sigma B_{FS} - (\delta + p_{Rxs}v_I + \mu)I_{FS} - \gamma I_{FS} - \mu_{mS} I_{FS} - rec_{FS} I_{FS}$$

$$\dot{R}_F = -\frac{\beta R_F(I_S + I_R)}{N} + p_{RxR}(v_L L_{FR} + v_B B_{FR} + v_I I_{FR}) - \mu R_F + dI_{FR} + \gamma I_{FR} - wR_F$$

$$L_{FR}^\cdot = \frac{\beta R_F(I_S + I_R)}{N} - (\gamma + p_{RxR}v_L + \mu)L_{FR}$$

$$B_{FR}^\cdot = \gamma L_{FR} - (\sigma + p_{RxR}v_B + \mu)B_{FR} + F_R B_R - \mu_{mR} B_{FR} - rec_{FR} B_{FR}$$

$$\dot{I}_{FR} = \sigma B_{FR} - (d + p_{RxR}v_I + \mu)I_{FR} - \mu_{mR}B_{FR} - rec_{FR}I_{FR}$$

Where:

- μ_n = death rate from causes other than malaria
- μ_F = severe malaria
- p_{RxS} = proportion of nonimmunes who seek treatment
- p_{RxR} = proportion of immunes who seek treatment
- F_S = rate of developing severe malaria in nonimmunes
- F_R = rate of developing severe malaria in immunes
- μ_{mS} = death rate from severe malaria in nonimmunes
- μ_{mR} = death rate from severe malaria in immunes
- rec_{FR} = rate of spontaneous recovery from severe malaria in nonimmunes
- rec_{FR} = rate of spontaneous recovery from severe malaria in immunes

6.3 Model assumptions

6.3.1 Models 1, 2 and 3

Table 6.3-1. Assumptions common to all three models.

Assumption	Justification for making the assumption	Likely effect on modelled outcomes if incorrect
No or low (0 to 5%) survival disadvantage (fitness cost) for artesunate resistant parasites compared to drug sensitive parasites.	The relative viability of malaria parasites with artesunate resistant phenotypes is not known, although survival disadvantage, if it exists at all, is likely to be minimal.²¹² Regardless, it should have minimal impact on the effectiveness of interventions when resistance is rare or absent.	Would <i>decrease</i> efficacy of interventions using artemisinins. More robust resistant parasites are harder to eradicate. A greater fitness cost would reduce the rate of spread of artemisinin resistance and increases the effectiveness of an intervention against resistant parasites.²³

No treatment of nonmalarial fever with antimalarials.	Presumptive treatment of malaria is not standard practice in Cambodia and is discouraged worldwide.² There were no data available with which to parameterise such a model for Cambodia. To maintain simplicity.	Some people with incidental asymptomatic malaria infection would also receive treatment.
No pre-existing resistance to ACT, only to artesunate and piperaquine alone.	No evidence for pre-existing resistance to ACT has ever been found.	Likely to <i>increase</i> efficacy of interventions using artemisinins and/or ACT partner drug. Pre-existing resistance to both components of ACT would result in a higher treatment failure rate for uncomplicated malaria and a faster spread of artemisinin resistance.
Recombination	There is no strong evidence for	Likely to decrease efficacy

between drug resistant mutants not frequent enough to have a significant effect in the model timescale.	frequent recombination combining drug resistance mutations in malaria. The genetics of resistance to artemisinin and piperaquine are unknown therefore a model of this would be conjectural If the inheritance of resistance to either of these drugs is polygenic, e.g. acquired incrementally by the acquisition of a series of mutations, then recombination would decrease the strength and prevalence of resistance.	of interventions. Recombination has the potential to generate parasites resistant to both components of ACT.
No spatial heterogeneity i.e. transmission, coverage of interventions, access to health services, etc.	This is potentially important for planning interventions but it was not known to what degree and at what scale this exists in Cambodia or Bangladesh. In the absence of data about most spatially	Likely to <i>increase</i> efficacy of interventions . Infection in high transmission areas is harder to eradicate

	heterogeneous parameters (seasonal variation in parasite prevalence, coverage with interventions, geographical extent of each village, etc.) it was felt their incorporation at this stage was premature. The major questions for the models used single strategies applied to large areas for which a non-spatial model is appropriate. On average, the relative efficacy of different options applied across a range of areas should be similar to that in a non-spatial model.	therefore taking longer. Spatial heterogeneity in transmission would result in different levels of immunity and thus different proportions with severe and fatal disease in different areas.
No population migration.	To maintain simplicity. As the modelling was of containment strategies for the only area where artesunate	Would probably increase the effect of interventions but impact on spread of resistance is difficult to predict.

	resistance had been identified, in-migration of resistant infections was not relevant. Evidence for more widespread resistance was not discovered until after completion of Model 3. There are few or no data on population migration and how it might affect malaria transmission in the areas being considered.	With no migration, people do not continue to introduce new resistant parasites. In-migration of sensitive infections would accelerate the elimination of resistance. In-migration of resistant infections would slow it. Out-migration of resistant infections would mean control/elimination efforts would have to include these areas also in order to achieve elimination.
Single infecting clone in each individual	To maintain simplicity. A model of multiple clones within a host would be far more complex than that presented here.	The mixing of multiple clones within a host may aid in the selection of fitter parasites through

	Most infections in Cambodia are with a single clone,¹⁹⁸ although multiple clones are common in high transmission settings. Bangladesh is thought to be similar. It is not understood how multiple clones interact within a host and how this affects the transmission dynamics.	competition for resources. This would reduce the likelihood of elimination. Alternatively, interbreeding between clones may dilute any drug resistance mutations in the population and thus increase the likelihood of elimination.
Recombination between drug resistant mutants not frequent enough to have a significant effect in the model timescale. Thus no resistance to both components of ACT in the same individual.	There is no strong evidence for frequent recombination combining drug resistance mutations in malaria. To date, no co-existing resistance to both components of any ACT has been confirmed and there is thus no data on which to base a model.	Would <i>increase</i> the effect of interventions. Resistance to both components of ACT in the same parasite would result in a higher rate of treatment failure for those receiving ACT, slower elimination, more severe disease and greater

	The genetics of resistance to artemisinin and piperazine are unknown therefore a model of this would be conjectural If the inheritance of resistance to either of these drugs is polygenic, e.g. acquired incrementally by the acquisition of a series of mutations, then recombination would decrease the strength and prevalence of resistance.	mortality overall. A higher proportion of severe malaria infections would have artemisinin resistance and this would reduce the efficacy of rectal artesunate.
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6.3.2 Model 1

Table 6.3-2. Additional assumptions for Model 1.

Assumption	Justification for making the assumption	Likely effect on time to eradication if true and reasoning
No immunity to malaria i.e. transmission rate is low.	Transmission rates in western Cambodia are generally much lower than in sub-Saharan Africa, for example. ¹⁴⁵ There are small focal areas with higher rates and it is planned to explore this with a spatially heterogeneous model when sufficient data becomes available.	Likely to <i>increase</i> time to eradication of artesunate resistance Infection more likely to result in symptoms therefore more people seeking and receiving treatment.
No mortality due to malaria.	In reality the proportion of malaria infections which are fatal in this region is low, around 0.6%. ²¹³	Likely to <i>increase</i> time to eradication of artesunate resistance Those people with resistant infections are less likely to respond to treatment and therefore more likely to die, thus

		removing them from the transmitting population.
Artesunate is the only available effective treatment before 2009.	Although co-blistered artesunate and mefloquine has been the official first-line drug since 2000, in reality a wide range of treatments is available over the counter in Cambodia. The majority receive artesunate monotherapy whereas most of the other treatments are inadequate (resistant parasites/wrong dose/wrong duration) to cure infection. ⁹⁵	Likely to <i>increase</i> time to eradication of artesunate resistance Artesunate would be less likely to cure artemisinin-resistant infections than non-artemisinin drugs, if available. If significant amounts of ACT were available, this would decrease the baseline parasite prevalence.
Rate of resistance to artesunate is increasing exponentially at the time of intervention.	Expert opinion in the absence of historical data.	If the rate of resistance was stable or decreasing then infection would be easier to eradicate.
Rate of resistance to piperaquine is stable at the time of	Expert opinion in the absence of historical data.	Likely to <i>increase</i> time to eradication of artesunate resistance

intervention.		If the rate of piperaquine resistance was increasing then piperaquine would take longer to eradicate infection.
No resistance to atovaquone, proguanil or primaquine.	Rates of resistance to each of these drugs are thought to be low in this region.	Likely to <i>decrease</i> time to eradication of artesunate resistance Interventions using these drugs will have maximal effectiveness if there is no resistance.

6.3.3 Model 2

Table 6.3-3. Additional assumptions for Model 2.

Assumption	Justification for making the assumption	Likely effect on efficacy of interventions if assumption is true and reasoning
No mortality due to malaria.	The proportion of malaria infections which are fatal in this region is around 0.6%, ²¹³ a small proportion of overall infections.	Likely to <i>decrease</i> efficacy of interventions Those people with resistant infections are less likely to respond to treatment and therefore more likely to die, thus removing them from the transmitting population.
Artesunate monotherapy was the only available effective treatment before 2004.	As for Model 1.	Likely to <i>decrease</i> efficacy of interventions. As for Model 1.
Proportion of	Expert opinion in the absence of	Likely to <i>increase</i> efficacy

infections resistant to artesunate is very low or absent at the time of the trial.	data.	of interventions. If the proportion resistant was high then interventions including ACT would be less effective. This is explored in Section 3.3.2 .
No resistance primaquine.	No primaquine resistance has been identified in Cambodia.	Likely to <i>increase</i> efficacy of interventions. If resistance was present then interventions including primaquine would be less effective.
No stochasticity	A deterministic framework was chosen to maintain flexibility and efficiency to rapidly explore a wide range of scenarios in accordance with the aims for this model.	Likely to <i>increase</i> efficacy of interventions. Infections more likely to spontaneously eliminate when numbers are small.

6.3.4 Model 3

Table 6.3-4. Additional assumptions for Model 3.

Assumption	Justifications for making the assumption	Likely effect on outcomes if incorrect and reasoning
The effects of antimalarial immunity in a population are lower rates of: • Symptomatic infection • Uncomplicated malaria becoming severe • Mortality in those with severe malaria	This is based on current understanding of malaria infection.	Antimalarial immunity not having some or all of these effects would result in each of these rates being higher in the population and higher rates of treatment. Higher use of artemisinin-containing regimens would result in faster spread of artemisinin resistance and thus reduced efficacy of rectal artesunate in the population.
ACT and oral or rectal artesunate monotherapy are the only treatments available for uncomplicated malaria. There is only one	To maintain simplicity. ACT is the mainstay of treatment of uncomplicated malaria in the areas being	If use of non-ACT antimalarials were widespread and those drugs were effective (correct dose, correct duration,

ACT available.	considered and in each country most patients who receive it are given the same drug. In Bangladesh only artemether-lumefantrine is available. In Cambodia, only dihydroartemisinin-piperaquine is available. Data on effective use of non-ACT antimalarials are very sparse. Oral artesunate monotherapy is only relevant to Cambodia where it is still in use. Rectal artesunate	sensitive parasites), this would reduce transmission intensity, decreasing population level immunity and increasing mortality in all patients with severe disease. Otherwise, the effect would be minimal. Use of other ACTs in addition to those modelled would accelerate the spread of artemisinin resistance, reducing the efficacy of rectal artesunate in the population.
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	monotherapy for uncomplicated malaria reflects practice in the trial and intended practice in the field where it is used for patients who cannot take oral antimalarials, regardless of severity.	
Intravenous or intramuscular quinine and intravenous and/or rectal artesunate are the only treatments available for severe malaria.	Quinine remains the mainstay of treatment for severe disease in the areas being considered. Intravenous artesunate is expected to replace quinine in the near future. The only other treatment option for severe malaria is intramuscular artemether but this is not widely used due to unpredictable absorption.²¹⁴	If intramuscular artemether were used then mortality would be similar to that in those receiving quinine.²¹⁵

6.4 Parameter values

These were based largely on expert opinion of the co-authors and were derived from published data, where available, as stated below. For those parameters for which a range of values is given, this reflects uncertainty of their true value. For these parameters, the underlined values were used to generate the plots and results stated in the text and the ranges were used in the sensitivity analysis.

6.4.1 Models 1, 2 and 3

Table 6.4-1. Parameters common to all three models.

Symbol	Description	Value	Source
Natural history of malaria infection			
δ	Natural recovery rate from infection in nonimmune subjects	1/200 - <u>1/60</u> days ⁻¹	216-221
γ	Rate of liver stage becoming blood stage	1/5 days ⁻¹	216-218
σ	Rate of blood stage becoming gametocytes	1/15 days ⁻¹	222, 223
Rates of initiation and proportions of population receiving drug treatment			
start _a	Artemisinin monotherapy		
	Year of introduction of artemisinin monotherapy in Cambodia	1975	Expert opinion

Drug pharmacodynamics			
X_{ao}	Duration of efficacy against sensitive parasites (X)		
	Full course of artemisinin monotherapy	7 days	224
X_b	Piperaquine	<u>20</u> -30 days	225
C_{Broda}	Rates of clearance of drug sensitive infection (ν) by treatment		
	Artemisinin vs. non-infectious blood stage	$1/7 \text{ days}^{-1}$	226
	Artemisinin vs. infectious blood stage	$1/4 \text{ days}^{-1}$	Unpublished data from Lee S
	Piperaquine vs. non-infectious blood stage	$1/3 \text{ days}^{-1}$	227
	Piperaquine vs. infectious blood stage	$1/21 \text{ days}^{-1}$	227
	ACT vs. any stage	$1/7 \text{ days}^{-1}$ (no synergy assumed) – $1/3 \text{ days}^{-1}$ (synergy assumed)	226, 228, 229
cost	Fitness cost of drug resistance This was modelled by multiplying the transmission parameter β by (1-cost)		

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cost _a	²³⁰ for each drug:		
	Artemisinin	<u>0</u> - 0.1	²¹²
cost _b	Piperaquine	<u>0</u> - 0.1	²¹²
Effectiveness of bed nets			
ρ	Degree of transmission reduction (the product of coverage and efficacy)	0.3	^{231, 232}

6.4.2 Model 1

Table 6.4-2. Additional parameters for Model 1.

Symbol	Description	Value	Source
Population demographics			
N_0	Total population size	3.2×10^6	²³³
μ	Birth rate = death rate	15/1000/year	²³⁴
Prevalence of malaria in population			
p_{BI}	Proportion of population with slide positive malaria infection in high transmission season in 2009	0.074	¹⁴⁵
p_{inf}	Proportion of population with infectious blood stage infection at time=0	0.16	(the value required to give $p_{BI} \sim 0.074$)
p_a	Proportion of malaria infections that are resistant to artesunate in 2008	0.1	Expert opinion
p_b	Proportion of malaria infections that are resistant to piperaquine in 2009	0.05	Expert opinion
Natural history of malaria infection			
amp	Amplitude of seasonal variation of transmission	0.67	¹⁷⁵

Rates of initiation and proportions of population receiving drug treatment			
$\tau = \tau_{ai1}$ $= \tau_{ai2}$ propRx_{am} prop_a adh_a propRx_a	Artemisinin monotherapy		
	Rate of starting artemisinin monotherapy	1/16 infected people per day	⁹⁵
	Proportion of infected population who receive antimalarials	0.63	⁹⁵
	Proportion of antimalarials constituting artemisinin monotherapy before an intervention	0.4	⁹⁵
	Proportion of infected population that take full 7 day course of artemisinin monotherapy	0.2	⁹⁵
	Proportion of infected population that take effective artemisinin monotherapy = $\text{propRx}_{am} * \text{prop}_a * \text{adh}_a$	0.05	$= \text{propRx}_{am} * \text{prop}_a * \text{adh}_a$
$\tau_{ab} = \tau_{ab1}$ $= \tau_{ab2}$	Interventions		
	Rate of starting ACT for treatment	16 infected people per day	⁹⁵

$\tau_1 = \tau_2 = \tau_3$	Rate of reaching maximum coverage for MDA or MSAT	$1/0.25 \text{ years}^{-1}$	Expert opinion
$\text{cov}_{i1} =$ $\text{cov}_{i2} =$ cov_{i3}	Maximum coverage of MDA or MSAT	0.8	Expert opinion
cov_{ab}	Maximum coverage with ACT after replacement of artemisinin monotherapy	0.6	Expert opinion
p_{sab}	Proportion of vendors selling modern drugs that could sell ACT	0.85	95
adh_{ab} $= \text{adh}_{vg}$	Adherence to 3 day course of ACT = Adherence to 3 days of atovaquone/ proguanil	0.77	95
propRx_{i1} $= \text{propRx}_{i2}$ $= \text{propRx}_{i3}$	Proportion that receive full 3 day course of MDA/MSAT	0.616	$= \text{cov}_{i1} * \text{adh}_{ab}$ or $\text{cov}_{i2} * \text{adh}_{vg}$ or $\text{cov}_{i3} * \text{adh}_{ab}$
p_{ab}	Proportion that receive full 3 day course of ACT after switch	0.3927	$= \text{cov}_{ab} * p_{sab} * \text{adh}_{ab}$
Duration of intervention and drug availability			
$\text{dur}_1 = \text{dur}_2$ $= \text{dur}_3$	Total duration of MDA or MSAT	0 years – long term	Expert opinion

n_{i2}	Number of times per year MSAT with atovaquone/proguanil is carried out	$\underline{1}$ -4 years ⁻¹	Expert opinion
$dur_{\tau1}$ = $dur_{\tau2}$ = $dur_{\tau3}$	Duration of each pulse of MDA or MSAT	0.25 years	Expert opinion
dur_a	Duration of availability of artemisinin monotherapy	0 years or long-term	Expert opinion
dur_{ab}	Duration of availability of ACT	0 years or long-term	Expert opinion
dur_{bn}	Duration of effectiveness of bed nets	$\underline{0}$ or 4 years	Expert opinion
Drug pharmacodynamics			
	Duration of efficacy against sensitive parasites (X)		
X_v	Atovaquone (as 3 days atovaquone/proguanil)	10- <u>15</u> days	²³⁵
X_g	Proguanil (as 3 days atovaquone/proguanil)	4 days	²³⁶
X_p	Primaquine (1 day course)	1 day	²³⁷
	Rates of clearance of drug sensitive infection (ν) by treatment		
c_{Ldv}	Atovaquone/proguanil vs. liver stage	1/3 days ⁻¹	²³⁸
c_{Bdv}	Atovaquone/proguanil vs. non-	1/3 days ⁻¹	²³⁵

	infectious blood stage		
$c_{Idvg} = c_{Idv}$	Atovaquone/proguanil vs. infectious blood stage= atovaquone vs. infectious blood stage	$1/(4.5) \text{ days}^{-1}$	Unpublished data from Lee S
c_{Ldv}	Atovaquone vs. liver stage	$1/6 \text{ days}^{-1}$	238
c_{Bdv}	Atovaquone vs. non-infectious blood stage infection	$1/3 \text{ days}^{-1}$	235
c_{Ldp}	Primaquine vs. liver stage infection	$1/7 \text{ days}^{-1}$	235
c_{Idp}	Primaquine vs. infectious blood stage infection	$1/1 \text{ days}^{-1}$	237, 239
	Effect of drug resistance on pharmacodynamics As this is unknown, it was a modelled by multiplying the clearance rate for each drug by its relative effectiveness against resistant infections, ϵ , such that $0 \leq \epsilon \leq 1$.		
pct_{roda}	Parasite clearance time for artemisinin vs. sensitive infections	30 hours	240
pct_{rada}	Parasite clearance time for artemisinin vs. resistant infections	83 hours	104
p_{recra}	Proportion of infections resistant to artemisinin that recrudesce after treatment with artemisinin monotherapy	0.35	104

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ϵ_{rada}	Relative effectiveness of artemisinin against artemisinin resistant parasites	0.27	$= \text{pct}_{roda} / \text{pct}_{rada} * (1$ $- \text{p}_{recra})$
ϵ_{rddb}	Relative effectiveness of piperaquine against resistant parasites	0.8	²²⁷

6.4.3 Model 2

Table 6.4-3. Parameters for Model 2.

Where possible, these were taken directly from the published field study data²⁷ ('Field study') or by fitting model output to results of the field study (**figure 3.3-4** and **Appendix 6.5.1**: 'Fitting'). Other sources were unpublished interim reports for the field study ('Report'), unpublished surveillance data from CNM or discussion with the staff who ran the field study at CNM ('Verbal'). Parameters not specific to the field study were based largely on expert opinion of the co-authors and were derived from published data, where available, as stated below. For those parameters for which a range of values is given, this reflects uncertainty of their true value. For these parameters, the underlined values were used to generate the plots and results stated in the text and the ranges were used in the sensitivity analyses. For the efficacy of drug resistance on pharmacodynamics, as this is unknown, it was modelled by multiplying the clearance rate for each drug by its relative effectiveness against resistant infections, ε , such that $0 \leq \varepsilon \leq 1$.

Symbol	Description	Value	Source
Population demographics			
N_0	Population of Kampot OD	122,330	²³⁴
	Overall study population	3653	²⁷
μ	Birth rate = death rate	15/1000/year	^{233, 234}
Prevalence of malaria in population			
p_{inf}	Proportion of population with blood stage infection when interventions start	0.5585	Fitting

p_a	Proportion of malaria infections that were resistant to artesunate in 2004	0.5, 10-80%	Expert opinion
p_b	Proportion of malaria infections that were resistant to piperaquine in 2004	0.05	Expert opinion
Natural history of malaria infection			
δ_R	Recovery rate from untreated infection (in immune subjects)	$1/200 - 1/30$ days ⁻¹	216-221
amp	Amplitude of seasonal variation of transmission	0.55	Fitting
phi	Peak of seasonal variation of transmission	0.62	Fitting
Ω	Duration of immunity to malaria	0.5, 1-5 years	40
propRx _R	Proportion of infected cases with immunity who are treated	0-0.1-1.0	40
propRx _N	Proportion of infected cases without immunity who are treated	0.9	40
Artemisinin monotherapy			
$\tau = \tau_{ai1}$ $= \tau_{ai2}$	Rate of starting artemisinin monotherapy	1/16 infected people per day	118
propRx _{am}	Proportion of infected population who receive antimalarials	0.63	118
prop _a	Proportion of antimalarials constituting artemisinin monotherapy before an intervention	0.4	118
adh _a	Proportion of infected population that take full 7 day course of artemisinin monotherapy	0.2	118

propRx_a	Proportion of infected population that take effective artemisinin monotherapy = $\text{propRx}_{am} * \text{prop}_a * \text{adh}_a$	0.05	$= \text{PropRx}_{am} * \text{prop}_a * \text{adh}_a$
Interventions			
$\tau_{ab} = \tau_{ab1}$ $= \tau_{ab2}$	Rate of reaching maximum coverage with ACT treatment	$1/14 \text{ days}^{-1}$	118
τ_{MDA1} $= \tau_{MDA2}$	Rate of reaching maximum coverage with MDA	$1/14 \text{ days}^{-1}$	Verbal/Report
cov_{MDA1}	Maximum coverage of first MDA	0, 0.51 or <u>0.95</u>	Fitting
cov_{MDA2}	Maximum coverage of second MDA	0 or <u>0.95</u>	Fitting
T_{MDA2}	Time to start of second MDA	42 or 365 days	Field study
$\text{dur}_{i1} = \text{dur}_{i2}$	Duration of single round of MDA	14 days	Verbal
dur_{pp}	Duration of multiple rounds of primaquine MDA	6 months	Field study
freq_{pp}	Frequency of multiple rounds of primaquine MDA	$1/10 \text{ days}^{-1}$	Field study
cov_{ab}	Coverage with ACT treatment during trial	0.78 or 0.95	Fitting
cov_{ab2}	Coverage with ACT treatment after trial	0.19	Fitting
p_{sab}	Proportion of vendors selling modern drugs that could sell ACT	0.85	118
adh_{ab}	Adherence to 2 day course of ACT	0.77	118

propRx_{i1} $= \text{propRx}_{i2}$	Proportion that receive full 2 day course of MDA	0.73	$= \text{cov}_{i1} * \text{adh}_{ab}$ or $\text{cov}_{i2} * \text{adh}_{vg}$
p_{ab}	Proportion that actually receive full 2 day course of ACT during field study	0.62	$= \text{cov}_{ab} * p_{sab} * \text{adh}_{ab}$
sens_{Dx}	Sensitivity of diagnostic test used to decide whether to treat febrile cases	0.8	Expert opinion
$\text{dur}_1 = \text{dur}_2$	Total duration of MDA	0 or 6 months	Field study
dur_a	Duration of availability of artemisinin monotherapy	0 years or long-term	Expert opinion
dur_{ab}	Duration of availability of ACT	0 years or long-term	Expert opinion
Duration of efficacy against sensitive parasites (X)			
X_{ai}	Artemisinin as part of ACT (2 day course)	2 days	224
X_p	Primaquine (1 day course)	1 day	237
Rates of clearance of drug sensitive infection (ν) by treatment			
C_{Irodab}, C_{Brodab}	Artemisinin-piperaquine vs. infectious or non-infectious blood stage	$1/3 \text{ days}^{-1}$	226, 228, 229
C_{Ldp}	Primaquine vs. liver stage infection (9mg)	$1/14 - 1/7 \text{ days}^{-1}$	237
C_{Idp}	Primaquine vs. infectious blood stage infection (9mg)	$1/4, 1/2, 1/1 \text{ days}^{-1}$	151, 237, 239
Effect of drug resistance on pharmacodynamics			
pct_{roda}	Parasite clearance time for artemisinin vs. sensitive infections	30 hours	104

pct_{rada}	Parasite clearance time for artemisinin vs. resistant infections	83 hours	104
p_{recra}	Proportion of infections resistant to artemisinin that recrudesce after treatment with artemisinin monotherapy	0.35	104
ϵ_{rada}	Relative effectiveness of artemisinin against artemisinin resistant parasites	0.27	$= pct_{roda}/pct_{rada} * (1 - p_{recra})$
ϵ_{rddb}	Relative effectiveness of piperaquine against resistant parasites	0.8	227
Bed nets			
τ_{bn}	Time to introduce bed nets	1 month	Expert opinion
cov_{bn}	Coverage with insecticide treated bed nets	<u>0</u> to 0.75	Expert opinion
dur_{bn}	Duration of effectiveness of bed nets	0 or <u>2</u> years	Expert opinion

6.4.4 Model 3

Table 6.4-4. Parameters for Model 3.

Parameters were taken directly from AQUAMAT, SEAQUAMAT, Study 13 or Study 18, from other published data and from analyses presented elsewhere in this thesis, as indicated. Other sources were fitting to data (**figure 3.3-11** and **Appendix 6.5.2** ‘fitting’) and expert opinion of the co-authors (‘Expert opinion’). For those parameters for which a range of values is given, this reflects uncertainty of their true value. For these parameters, the underlined values were used to generate the plots and main results and the ranges were used in the sensitivity analyses.

Symbol	Description	Value	Source
Population demographics			
N_0	Population in Khagrachari	588,132	²⁴¹
	Population in Chittagong Division, Bangladesh	11,504,573	¹²⁰
	Population of Cambodia	13,395,682	¹¹⁷
	Population in Study 18 area	58,915	²⁴²
	Population in Study 13 area	30,000	Est. ⁷⁴
	Population in SEAQUAMAT area	1461	⁷⁰
μ	Birth rate = death rate/1000/year		
	Bangladesh	23.0	²⁴³
	Cambodia	25.2	²⁴⁴
Prevalence of malaria and drug resistance in population			

p_{inf}	Proportion of general population with asexual blood stage infection in peak season i.e. point prevalence of <i>P. falciparum</i> malaria		
	Khagrachari, Bangladesh	0.15 (RDT+) * 0.72 (specificity) = 0.11 (September - just after peak)	133, 168, 183, 245
	Chittagong Division, Bangladesh	0.07 (RDT+) * 0.72 (specificity) = 0.050 (July-September approx.)	169, 245
	Cambodia	0.016, 0.033, <u>0.049</u> , 0.099 in peak season	145
p_a	Proportion of malaria infections that are resistant to artemisinins		
	Bangladesh	<u>0</u> , 0.001, 0.01	Expert opinion
	Cambodia	<u>0.01</u> , 0.10	opinion
start_a	Year that artemisinin resistance first arises		

p_b	Cambodia	1980	Expert opinion
	Bangladesh	1980-2010 (This was varied in the model)	Expert opinion
	Proportion of malaria infections that are resistant to partner drug		
	Lumefantrine	0	Expert opinion
	Piperaquine	0, <u>0.05</u>	Expert opinion
Natural history of malaria infection			
δ_R	Recovery rate from untreated infection (immune)	1/200 - <u>1/30</u> days ⁻¹	216-221
amp	Amplitude of seasonal variation of transmission		
	Khagrachari	0.60	Fitting
	Chittagong Division	0.97	Fitting
	Cambodia	0.34	Fitting
phi	Peak of seasonal variation of transmission (0=1 st January, 1=31 st December)		

Ω	Khagrachari	0.54	Fitting
	Chittagong Division	0.54	Fitting
	Cambodia	0.61	Fitting
	Immunity		
	Duration of immunity to malaria	0.5, <u>1</u> -5 years	40
	t_R Time to become immune	14 days	
	$propR_{x_R}$ Proportion of infected cases with immunity who seek treatment	0- <u>0.1</u> -1.0	40
	$propR_{x_N}$ Proportion of infected cases without immunity who seek treatment	0.9	40
	$prot_{RF}$ Proportion of immune patients vs. nonimmune patients who become severe	0.167	$=p_{WHOsevere_{high}}/p_{WHOsevere_{low}}$
	$prot_{RFmort}$ Proportion of immune patients vs. nonimmune patients who die	0.64	$=p_{WHOfatal_{d_{high}}}/p_{WHOfatal_{d_{low}}}$
p_{WHO_F}	Severe malaria		
	WHO criteria		
	Proportion of patients in Study 13 who met WHO severity criteria	0.793	74

pWHOsevere	Proportion of untreated malaria		
pWHOsevere	which progresses to severe disease		
pWHOsevere _R	Nonimmune	0.05-0.18-0.25	242
	Immune	0.01- <u>0.03</u> -0.05	242
pWHOfatal_{do}	Proportion of severe without		
	treatment which are fatal		
pWHOfatal _{do}	Nonimmune	0.50- <u>0.70</u> -0.8-	242
		0.9	& Expert
pWHOfatal _{doR}	Immune	0-0.30- <u>0.45</u> -	opinion
		0.71	
pWHOrec_F	Proportion of untreated severe		
	disease who recover		
	spontaneously		
pWHOrec _F	Nonimmune (=1-pWHOfatal _{do})	0.20- <u>0.30</u> -0.50	
pWHOrec _{FR}	Immune (=1-pWHOfatal _{doR})	0.29- <u>0.55</u> -0.70	
pWHOfatal_{dq}	Proportion of those treated with	0.22	70
	full course IV/IM quinine which		
	are fatal		
pWHOfatal_{da}	Proportion of those treated with	0.15	70
	full course IV artesunate which are		
	fatal		

pWHOfatal_{draq}	Proportion of those pre-treated with IV/IM quinine then artesunate which are fatal	0.11	70, 74
timefatal	Time from developing severe disease to death without antimalarial treatment	2 days	74
psevere	Proportion of untreated malaria which progresses to severe disease (=pWHOsevere*pWHO _F)		Expert opinion
psevere	Nonimmune	0.040-0.15- 0.20	
psevere _R	Immune	0.008-0.024- 0.040	
pWHOsev_{hosp}	Proportion of severe who reach hospital:		
pWHOsev _{hosp_{dr}} as	a) those that receive rectal artesunate	0.89	242
pWHOsev _{hosp_d} o	b) those who don't receive rectal artesunate	0.198	242
timerec_F	Time from onset of untreated	56 days	216-221

	severe disease to clearance of parasites in those who recover spontaneously i.e. without antimalarial treatment (=1/ δ - timesevere)		
	Study 13 & 18 entry criteria (NPO status)		
psevere	Proportion of untreated malaria which progresses to severe disease (=pWHOsevere*pWHO _F)		
psevere	Nonimmune	0.040- <u>0.15</u> - 0.20	246
psevere _R	Immune	0.008- <u>0.024</u> - 0.040	246
ratiofatal	Ratio mortality WHO severe vs. enrolled study patients	0.856	74, 242
pfatal_{do}	Proportion of NPO severe without treatment which are fatal (=pWHOfataldo*ratiofatal)		
pfatal _{do}	Nonimmune	0.43- <u>0.60</u> -0.69	242, 246
pfatal _{doR}	Immune	0.26- <u>0.39</u> -0.61	242, 246
prec_F	Proportion of untreated severe disease who recover		

	spontaneously (=1-pfataldo)		
$prec_F$	Nonimmune	0.32- <u>0.40</u> -0.57	1-pfataldo
$prec_{FR}$	Immune	0.39- <u>0.61</u> -0.74	1-pfataldo
pfatal_{dq}	Proportion of those treated with full course IV/IM quinine which are fatal (=pWHOfataldq*ratiofatal)	0.188	70
pfatal_{da}	Proportion of those treated with full course IV artesunate which are fatal (=pWHOfatalda*ratiofatal)	0.128	70
pfatal_{draq}	Proportion of those pre-treated with IV/IM quinine then artesunate which are fatal (=pWHOfataldraq*ratiofatal)	0.094	70
timefatal	Time from developing severe disease to death without antimalarial treatment	2 days	74
timesevere	Time from non-infectious blood stage to severe disease	4 days	Expert opinion
psevhosp	Proportion of severe who reach hospital:		

$p_{sevhospd_{as}}$	a) those that receive rectal artesunate	0.809	242
$p_{sevhosp_{do}}$	b) those who don't receive rectal artesunate	0.191	242
$p_{sevCMCH}$	Proportion of severe who are admitted to CMCH	0.002-0.016	167
Treatment			
cov_q	Coverage with quinine for severe disease in those reaching hospital	0.9	242
$start_{as}$	Year that rectal artesunate first introduced	2000, <u>2013</u>	Expert opinion, ⁷⁴
$stop_{as}$	Time rectal artesunate no longer available	2006, <u>2030*</u>	Expert opinion
$time_{das}$	Time from infection becoming severe to receiving artesunate suppository	1 day	242
cov_{as}	Maximum coverage with artesunate suppository in population	<u>0.162</u> -0.95*	242
p_{as}	Proportion that actually use artesunate suppository	1	74
k_{as}	Time to distribute and start administering artesunate	30 days to 1 year	Expert opinion

pasclin	suppository after it first becomes available in the country		
	Proportion who go to clinic after receiving rectal artesunate	0.81	242
pasdo	Proportion who receive no treatment after rectal artesunate	0.082	242
Uncomplicated malaria			
propRx_{am}	Proportion of infected population who receive antimalarials	0.63	118
start_{ab}	Artemisinin combination therapy		
	Year of introduction of ACT		
	Bangladesh	2004, coverage increased 2007	173, 247
	Cambodia	2000	173
	Components of ACT (artemisinin and partner drug) used in each country		
	Bangladesh	Artemether-lumefantrine	247
ab	Cambodia	DHA-piperaquine	173
$\tau = \tau_{abi1}$ $= \tau_{abi2}$	Rate of starting ACT	1/14 infected people per day	118

cov_{ab}	Proportion of infected population who take ACT in 2007		Sections:
	Khagrachari	0.38	3.1.2.1
	Chittagong Division	0.128	3.1.2.2, ⁷⁴
	Cambodia	propRx _{ab}	3.1.1
prop_{ab}	Proportion of antimalarials constituting ACT in 2007		
	Bangladesh	<u>0.85</u>	Expert opinion
	Cambodia	<u>0.72</u>	248
adh_{ab}	Proportion of infected population who are given ACT that take full 3 day course	0.93	249
propRx_{ab}	Proportion of infected population that take effective ACT in 2007		
	Khagrachari = cov _{ab} *adh _{ab}	0.23	
	Chittagong Division = cov _{ab} *adh _{ab}	0.078	
	Cambodia= PropRx _{am} *prop _{ab} *adh _{ab}	0- <u>0.42</u>	
t_{ab}	Time to introduce ACT from when it first becomes available	<u>14-30</u> days to 1 year	Expert opinion
Artemisinin monotherapy			

start_a	Year of introduction of artemisinin monotherapy Bangladesh	1975-2007	Expert opinion
$\tau = \tau_{ai1}$ $= \tau_{ai2}$	Rate of starting artemisinin monotherapy	1/16 infected people per day	118
prop_a	Proportion of antimalarials constituting artemisinin monotherapy before 2007 Bangladesh	<u>0.0</u> -0.1	Expert opinion
	Cambodia	<u>0.4</u>	118
adh_a	Adherence: proportion of infected population that take full 7 day course of artemisinin monotherapy as prescribed	0.2	118
propRx_a	Proportion of infected population that take effective artemisinin monotherapy $= \text{propRxam} * \text{propa} * \text{adha}$ Bangladesh	<u>0.0</u> -0.01	
	Cambodia	0.05	
Elimination Interventions			

τ_{ab}	Rate of reaching maximum coverage with ACT treatment	$1/14 \text{ days}^{-1}$	[4]
cov_{ab2}	Increased coverage with ACT treatment	<u>0-0.64-0.95</u> (This was varied in the model)	Expert opinion
p_{sab}	Proportion of vendors selling modern drugs that could sell ACT	0.85	Expert opinion, ¹¹⁸
sens_{Dx}	Sensitivity of diagnostic test used to decide whether to treat febrile cases	0.95	Expert opinion, ^{250, 251}
dur_a	Duration of availability of artemisinin monotherapy	0 years or long-term	Expert opinion
dur_{ab}	Duration of availability of ACT	0 years or long-term	Expert opinion
Drug pharmacodynamics			
X_{ai}	Duration of efficacy against sensitive parasites (X)		
	Artemisinin as part of ACT (3 day course)	3 days	²²⁴

X_{bl}	Lumefantrine	10.5 days	252
Rates of clearance of drug sensitive infection (ν) by treatment			
C_{Irodabl}	Artemether-lumefantrine vs. infectious	1/3 days ⁻¹	226
C_{Brodabl}	DHA-piperaquine vs. non-infectious blood stage	1/3 days ⁻¹	226, 228, 229
C_{Brodbl}	Lumefantrine vs. non-infectious blood stage	1/10 days ⁻¹	252
C_{Irodbl}	Lumefantrine vs. infectious blood stage	0 days ⁻¹	253
Effect of drug resistance on pharmacodynamics As this is unknown, it was modelled by multiplying the clearance rate for each drug by its relative effectiveness against resistant infections, ϵ , such that $0 \leq \epsilon \leq 1$.			
K_{wangpha}	Parasite clearance half-life for artemisinin vs. sensitive infections	3.1 hours	Section 3.2
K_{pailin}	Parasite clearance half-life for artemisinin vs. resistant infections	5.7 hours	Section 3.2
P_{precra}	Proportion of infections resistant to artemisinin that recrudesce after treatment with artemisinin	0.12	Section 3.2

ϵ_{rada}	monotherapy Relative effectiveness of artemisinin against artemisinin resistant parasites	0.54	= Kwangpha/K pailin
ϵ_{rdbp}	Relative effectiveness of piperaquine against resistant parasites	0.8	²²⁷
ϵ_{rdbpl}	Relative effectiveness of lumefantrine against resistant parasites	N/A	Expert opinion
Getting to Hospital			
$timetohospital$	Time to get to hospital after onset of severe disease	0.25- <u>2.3</u> -7 days	²⁴²
$timetohospitalas$	Time to hospital after receiving rectal artesunate	0.25- <u>1.75</u> -7 days*	²⁴²
Bed nets			
$start_{bn}$	Year of scale-up of long-lasting insecticide treated bed nets Bangladesh Cambodia	 2008 2007-2009	 ²⁵⁴ ¹⁷³
τ_{bn}	Time to introduce bed nets	1 month	Expert

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cov_{bn}	Coverage with insecticide treated bed nets	0 to <u>0.75</u>	opinion 255
	Bangladesh Cambodia	0 to <u>0.9</u>	173
dur_{bn}	Median duration of effectiveness of bed nets – modelled as linear decay in ρ	<u>2</u> years	Expert opinion

6.5 Model fitting and validation: additional details

6.5.1 Model 2

Surveillance data

1. Fitting to National surveillance data (2002-2004)

In order to determine values for the timing and amplitude (amp and phi respectively) of seasonal variation in malaria prevalence in the study area, the model was fitted to baseline national surveillance data.¹⁷⁵ There was found to be a single annual peak in prevalence in mid-September and amplitude of variation of 0.55 of the peak prevalence. Root mean squared deviation (RMSD) for this fit was 7.38%.

2. Fitting to Results of field study

Although most details of the strategies used in the field study were recorded (²⁷ and unpublished reports), it was not possible to measure the exact coverage for each component. The model was thus fitted to a range of field data from the field study to derive these coverages. Proportions of the population with detected asexual blood stage parasites and gametocytes were fitted simultaneously for strategies where these data were available.

The model was fitted to the results of three strategies investigated in the field study for which detailed results were available. The values for coverages for each component of these strategies were varied within realistic ranges (50-95%) set by discussion with the trial team until RMSD was minimised. Details of the strategies and results are shown in **figure 3.3-4B-E** and **table 6.5-1**.

Table 6.5-1. Results of fitting the model to field data to derive coverages of the different strategies employed in the field study.

One or both of prevalences of detected asexual parasitaemia and gametocytes were fitted as indicated. Strategy A was employed in 17 villages in Kampong Speu OD, B in 3 villages in Kampot OD and C in 4 villages in Kampong Speu OD.

Strategy	Components	Derived coverage (%)	Fitted output	RMSD (%)
A	1. MDA ACT+PP	94.99	Asexual	0.70
	2. Rx ACT+P	78.08	Gametocytes	0.29
			Asexual+gametocytes	1.16
B	1. MDA ACT+PP	94.95	Asexual	5.97
	2. Rx ACT+P	92.45		
	3. MDA2 ACT+P at 42 days	95.0		
C	1. MDA ACT+PP	51.03	Asexual	7.12
	2. Rx ACT+P	94.99		
	3. MDA2 ACT+P at 1 year	94.9		

3. Validation with OD surveillance data (2004-2010)

The number of clinical cases from the model was validated against surveillance data from CNM for Kampot OD (**Section 3.1.1**) using an OD population (N0) of 122,330²³⁴ and trial population of 2387.²⁷ During the trial period, the study team provided treatment with ACT plus primaquine to a much larger number of malaria cases in Kampot than were

included in the trial (actual number not recorded). MDA was only used in the trial population. The high coverage with ACT plus single dose primaquine treatment likely resulted in the decrease in malaria cases seen throughout the OD during the trial period. The subsequent decreasing coverage following the departure of the study team probably resulted in the increase in cases from 2008 onwards. The model was able to reproduce the data using realistic values for coverage with the strategies employed in the field. The best fit was for a coverage with ACT plus single dose primaquine treatment of 60% of symptomatic cases during the trial, falling to 19% by 2008. RMSD for the best fit was 37.48 with the result shown in **figure 3.3-4A**.

6.5.2 Model 3

Determining seasonality of malaria transmission at baseline

To reproduce the seasonal pattern in malaria prevalence in each country, the model was fitted to available malaria prevalence and incidence data. This was done by varying the prevalence of malaria infection at time=0, the timing and amplitude of seasonal variation, the efficacy of the government surveillance programme and the proportion of cases which are severe within estimated ranges until a minimum root mean squared deviation was obtained. For each country, the timing and estimated coverages of ACT for treatment of clinical disease and insecticide treated bed nets were input into the model.

The data used for this procedure were as follows:

Bangladesh:

Khagrachari

Government reported cases in Kagrachari District from 2001 to 2011 (**Section 3.1.2.1**). Kagrachari is one of three Districts in Bangladesh comprising the Chittagong Hill Tracts. This is an inland forested area with a large proportion of cases and the highest malaria endemicity in Bangladesh.^{133, 169} Malaria transmission is highly seasonal with a peak in May to August. Malaria prevalence data are thought to be particularly reliable from this area. The model was fitted simultaneously to monthly numbers of total malaria cases and severe malaria cases in Khagrachari.

The model for Khagrachari was validated against two field studies which estimated the prevalence of malaria parasites in the population in Khagrachari in 2007.^{133, 168, 169, 183} Both studies found a prevalence of 15% in September-November 2007 using a PfHRP2 based rapid test. In the model the median (range) parasite prevalence during the period of that survey was 13.8%-17.1% during the same period (**figure 6.5-1**), assuming a specificity of 72% for active infections.²⁴⁵

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The model was used to reproduce the major results of these two studies^{70, 74} to determine parameter values for the relative efficacies of intravenous artesunate and quinine and rectal artesunate preventing mortality. The model was set to match the enrolment conditions of the trials and fitted to the trial results. The values derived for relative efficacy in preventing mortality were: 0.87 for intravenous quinine, 1.48 for intravenous artesunate and 0.09 for rectal artesunate.

Chittagong Division

Hospitalised cases in Chittagong Medical College Hospital (CMCH), Chittagong, Bangladesh from 1999-2011.¹⁶⁷ An estimated parameter for the proportion of cases admitted to CMCH was included, taken from the analysis in **Section 3.1.2.2**.¹⁶⁷ This is the main tertiary referral hospital for severe malaria in Chittagong Division and receives cases from across the Division. Chittagong Division was chosen in preference to the entire country as almost all malaria cases in Bangladesh occur in Chittagong Division.^{133,}
¹⁶⁹ Much of the malaria transmission occurs in forests and forest fringe areas.

The model was validated against a study which determined the point prevalence of *P. falciparum* RDT positive individuals in Chittagong Division in 2007¹⁶⁹ (**figure 6.5-1**). In Chittagong, the point prevalence by RDT in 2007 was estimated at 7.0%¹⁶⁹ and in the model it was 5.5-7.7% during the same period (**figure 6.5-1**).

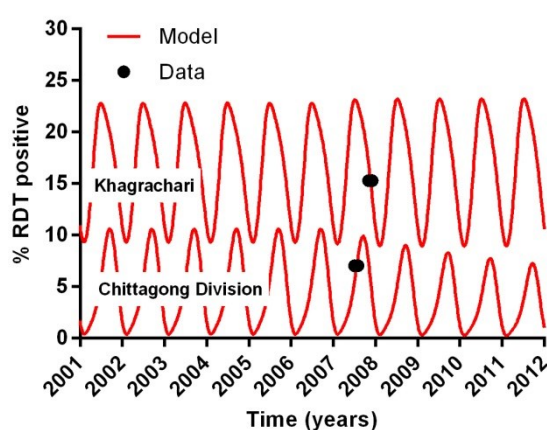


Figure 6.5-1. Model validation with percent of population RDT positive in Khagrachari and Chittagong Division.

Cambodia:

Government reported cases in Cambodia from 2001-2011 (**Section 3.1.1**). Malaria is widespread across Cambodia with highly seasonal transmission. The seasonal peak is from November to March and much of the transmission occurs in forests and forest fringe. The model was fitted to monthly numbers of malaria cases recorded by the Cambodian Government Health Information System.

The model was validated against a study which determined the prevalence of malaria parasites by microscopy.¹⁴⁵ In this study, the peak point prevalence of asexual parasitaemia in Cambodia by microscopy in 2001-2003 was 4.9%.¹⁴⁵ The model gave a peak prevalence of 5.1% (4.8-5.5) for the same period.

The derived parameter estimates from fitting the model to data are shown in **Table 6.5-2**.

Table 6.5-2. Derived parameter estimates from fitting model to data.

Parameter	Definition	Khagrachari		CMCH	Cambodia
		Total cases	% severe	Severe cases	Total cases
Number of runs		127	17	39	321
RMSD		353.53	3.77	12.51	1170.25
amp	Amplitude of seasonal variation	0.60		0.97	0.34
phi	Timing of seasonal peak in transmission	0.54			0.61
pinf0	Proportion of population infected at time=0	0.52			0.30
psevere	Proportion of infections in nonimmune individuals which become severe		0.03		
pRsevere	Proportion of infections in immune individuals which become severe		0.0000		
pcmch	Proportion of P. falciparum malaria cases that attend CMCH			0.0012	

6.6 Berkeley Madonna code for Model 1

Full Model Code

The models in this thesis were run using a software package called Berkeley Madonna™.

(Berkeley Madonna™ can be downloaded as a free trial version at

<http://www.berkeleymadonna.com/download.html>)

Below is the full Berkeley Madonna™ code for **Model 1**.

In order to run the model, the following steps should be followed:

- 1) Replace the terms β , γ , δ , μ , π , ρ , σ and τ with their names e.g. beta, gamma, etc.
- 2) Cut and paste the entire code into the Equations window of Berkeley Madonna™
- 3) Run the code with T_{BI} (total number of blood stage infections), N_{ra} (number of infections resistant to artesunate) and $perc_{ra}$ (percentage of infections resistant to artesunate) displayed in the Graph window
- 4) Use the parameter sliders to alter parameter values to explore their effects on the results

STARTTIME = 1960

STOPTIME=2050

DT = 0.0027

;Model to examine the effect of elimination interventions on artesunate resistance in western Cambodia

;All times are in years

;Artesunate monotherapy begins in 1975

;Artesunate resistance first arises in 1980

;Interventions begin in 2009

;NOTATION

;N = total population size ($N = S+L+B+I$)

;t = time for drug to clear sensitive infection

;c = clearance rate of infection by drug according to drug sensitivity

;S = susceptible people

;L = liver stage infections

;B = noninfectious blood stage infections

;T = total

;I = infectious blood stage infections

;r = resistant to...

;d = drug given

;o = no drug

;a = artesunate

;ai = artesunate as part of ACT

;b = piperazine

;ab = ACT (dihydroartemisinin plus piperazine)

;p = primaquine

;e.g.1 t_{Bda} = time for artesunate to clear noninfectious blood stage infection

;e.g.2 c_{Bradab} = clearance rate (c) of noninfectious blood stage infections (B) resistant to artesunate (ra) by ACT (dab)

;e.g.3 $c_{Irod p}$ = clearance rate (c) of infectious blood stage infections (I) resistant to no drugs i.e. sensitive to all (ro) by primaquine (dp)

;i₀ = no intervention

;i₁ = mass drug administration (MDA) (Intervention 1)

;i_{1a} = treatment of symptomatic patients who have already received MDA

;i₂ = mass screen and treat (MSAT) with atovaquone/proguanil (Intervention 2)

;i₃ = MSAT with ACT (Intervention 3)

;e.g.4 d_{ai1a} = treatment of symptomatic patients with artesunate monotherapy (d_a) who have already received MDA

;e.g.5 d_{abi1a} = treatment of symptomatic patients with ACT who have already received MDA

;X = effective duration of drug action

;e.g.6 X_{ai} = effective duration of artesunate as part of ACT

;e = relative effectiveness of drug against drug resistant infections vs drug sensitive infections

;bn = bed nets

;dur = duration

;PARAMETERS

;Population parameters

$\mu = 0.015$; i.e. birth rate & death rate

$N_0 = 3200000$; total population at time=0

$I_0 = N_0 * p_{inf}$; total infected at time=0

;Natural history of infection

$\gamma = 365/5$; rate of liver stage becoming noninfectious blood stage

$\sigma = 365/15$; rate of developing gametocytes

$\delta = 365/60$; natural recovery rate of infectious

;Drug action

;Time for drug to clear drug sensitive infection in days

$t_{Bda} = 7$; time for artesunate to clear B

$t_{Ida} = 4$; time for artesunate to clear I

$t_{Bdb} = 3$; time for clearance of noninfectious blood stage by piperaquine

$t_{Idb} = 21$; time for clearance of infectious blood stage by piperaquine

$t_{Bdab} = 3$; time for ACT to clear B = 3 days if synergy or 7 days if none

$t_{Idab} = 3$; time for ACT to clear I = 3 days if synergy or 4 days if none

$t_{Ldvg} = 3$; time for clearance of liver stage by atovaquone/proguanil

$t_{Bdvg} = 3$; time for clearance of noninfectious blood stage by atovaquone/proguanil

$t_{Idvg} = 4.5$; time for clearance of infectious blood stage by atovaquone/proguanil

$t_{Ldv} = 6$; time for clearance of liver stage by atovaquone

$t_{Bdv} = 3$; time for clearance of noninfectious blood stage by atovaquone

$t_{Idv} = 4.5$; time for clearance of infectious blood stage by atovaquone

$t_{Ldp} = 7$; time for clearance of liver stage by primaquine

$t_{Idp} = 1$; time for clearance of infectious blood stage by primaquine

;rate of clearance of infection by drug according to drug sensitivity

$c_{Brodo} = 0$; rate of clearance of sensitive noninfectious blood stage by no drug

$c_{Brado} = 0$; rate of clearance of artesunate resistant noninfectious blood stage by no drug

$c_{Brbdo} = 0$; rate of clearance of piperaquine resistant noninfectious blood stage by no drug

$c_{Irodo} = 0$; rate of clearance of sensitive infectious blood stage by no drug

$c_{Irado} = 0$; rate of clearance of artesunate resistant infectious blood stage by no drug

$c_{Irbdo} = 0$; rate of clearance of piperazine resistant infectious blood stage by no drug
 $c_{Broda} = 365/t_{Bda}$; rate of clearance of blood stage resistant to none treated with artesunate
 $c_{Brada} = e_{rada} * c_{Broda}$; rate of clearance of blood stage resistant to artesunate treated with artesunate
 $c_{Brbda} = 365/t_{Bda}$; rate of clearance of blood stage resistant to piperazine treated with artesunate
 $c_{Iroda} = 365/t_{Ida}$; rate of clearance of I resistant to none treated with artesunate
 $c_{Irada} = e_{rada} * c_{Iroda}$; rate of clearance of I resistant to artesunate treated with artesunate
 $c_{Irbda} = 365/t_{Ida}$; rate of clearance of I resistant to piperazine treated with artesunate
 $c_{Brodb} = 365/t_{Bdb}$; rate of clearance of B resistant to none treated by ACT
 $c_{Bradab} = e_{rada} * 365/t_{Bdb} + (1 - e_{rada}) * 365/t_{Bdb}$; rate of clearance of artesunate resistant noninfectious blood stage by ACT
 $c_{Brbdb} = e_{rddb} * 365/t_{Bdb} + (1 - e_{rddb}) * 365/t_{Bda}$; rate of clearance of piperazine resistant noninfectious blood stage by ACT
 $c_{Irodab} = 365/t_{Idab}$; rate of clearance of sensitive infectious blood stage by ACT
 $c_{Iradab} = e_{rada} * 365/t_{Idab} + (1 - e_{rada}) * 365/t_{Idb}$; rate of clearance of artesunate resistant infectious blood stage by ACT
 $c_{Irbdb} = e_{rddb} * 365/t_{Idab} + (1 - e_{rddb}) * 365/t_{Ida}$; rate of clearance of piperazine resistant infectious blood stage by ACT
 $c_{Brodb} = 365/t_{Bdb}$; rate of clearance of sensitive noninfectious blood stage treated with piperazine (after artesunate i.e. minus 3 days)
 $c_{Bradb} = 365/t_{Bdb}$; rate of clearance of artesunate resistant noninfectious blood stage treated with piperazine
 $c_{Brbdb} = e_{rddb} * 365/t_{Bdb}$; rate of clearance of piperazine resistant noninfectious blood stage treated with piperazine
 $c_{Irodb} = 365/t_{Idb}$; rate of clearance of sensitive infectious blood stage treated with piperazine
 $c_{Iradb} = 365/t_{Idb}$; rate of clearance of artesunate resistant infectious blood stage treated with piperazine
 $c_{Irbdb} = e_{rddb} * 365/t_{Idb}$; rate of clearance of piperazine resistant infectious blood stage treated with piperazine
 $c_{Lp} = p_p * 365/t_{Ldp}$; rate of clearance of liver stage treated with primaquine
 $c_{Ip} = p_p * 365/t_{Idp}$; rate of clearance of infectious blood stage treated with primaquine
 $c_{Ldvg} = 365/t_{Ldvg}$; rate of clearance of liver stage treated with atovaquone/proguanil
 $c_{Bdvg} = 365/t_{Bdvg}$; rate of clearance of noninfectious blood stage treated with atovaquone/proguanil
 $c_{Idvg} = 365/t_{Idvg}$; rate of clearance of infectious blood stage treated with atovaquone/proguanil
 $c_{Ldv} = 365/t_{Ldv}$; rate of clearance of liver stage treated with atovaquone after atovaquone/proguanil
 $c_{Bdv} = 365/t_{Bdv}$; rate of clearance of noninfectious blood stage treated with atovaquone after atovaquone/proguanil
 $c_{Idv} = 365/t_{Idv}$; rate of clearance of infectious blood stage treated with atovaquone after atovaquone/proguanil

;Effective duration of drug action

$X_{ao} = 7/365$; effective duration of artesunate monotherapy

$X_{ai} = 3/365$; effective duration of artesunate as part of ACT
 $X_b = 20/365$; effective duration of piperazine
 $X_g = 4/365$; effective duration of proguanil as part of atovaquone/proguanil
 $X_v = 15/365$; effective duration of atovaquone
 $X_p = 1/365$; effective duration of primaquine

;Drug resistance
 $e_{rada} = (1 - prec_{ra}) * pct_{roda}/pct_{rada}$; relative effectiveness of artesunate against artesunate resistant infections (0-1)
 $pct_{roda} = 30$; parasite clearance time for artesunate vs sensitive infections
 $pct_{rada} = 83$; parasite clearance time for artesunate vs resistant infections
 $prec_{ra} = 0.35$; proportion recrudescences in resistant infections
 $e_{rddb} = 0.8$; relative effectiveness of piperazine in piperazine resistant infections (0-1)

;Rates of receiving treatments
;Artesunate monotherapy
 $\tau = 365/16 * propRx_a * SQUAREPULSE(start_a, dur_a)$; rate of starting artesunate in infected patients
 $dur_a = stop_a - start_a$; duration availability of artesunate
 $start_a = 1975$; time that artesunate first introduced
 $stop_a = 2009$; time from when artesunate no longer available

;Treat symptomatic infection with ACT
 $\tau_{ab} = \tau_{mag_{ab}} * propRx_{ab} * SQUAREPULSE(start_{ab}, dur_{ab})$
 $\tau_{mag_{ab}} = 365/16$
 $dur_{ab} = 41$; 'long-term'
 $start_{ab} = 2009$;

;Intervention 1: MDA with ACT
 $\tau_{pulse_1} = \text{if } mod(time - dur_{\tau_1}, 1) \geq 1 - dur_{\tau_1} \text{ then } propRx_{i1} * \tau_{mag_{i1}} \text{ else } 0$
 $\tau_1 = \tau_{pulse_1} * SQUAREPULSE(2009, dur_{i1})$
 $\tau_{mag_{i1}} = 4$; rate of starting intervention 1 in 1/years
 $dur_{i1} = 0$; total duration of intervention 1 in years
 $dur_{\tau_1} = 0.25$; duration of each round of intervention 1 in years

;Intervention 2: MSAT with atovaquone/proguanil
 $\tau_{pulse_2} = \text{if } mod(time - dur_{\tau_2}, fr_{i2}) \geq fr_{i2} - dur_{\tau_2} \text{ then } propRx_{i2} * \tau_{mag_{i2}} \text{ else } 0$
 $\tau_2 = \tau_{pulse_2} * SQUAREPULSE(2009, dur_{i2})$
 $\tau_{mag_{i2}} = 4$; rate of starting intervention 2 in 1/years
 $dur_{i2} = 0$; total duration of intervention 2 in years
 $dur_{\tau_2} = 0.25$; duration of each round of intervention 2 in years
 $fr_{i2} = 1/n_{i2}$; 1/frequency of intervention 2 per year
 $n_{i2} = 1$; frequency of intervention 2 per year

;Intervention 3: MSAT with ACT.
;this uses the same section of code as switching treatment to ACT

```

; to make this run, do the following:
;1) add a ';' to the beginning of the first line of the 'treat symptomatic infection with ACT'
section (above) to give “;τab = τmagab*propRxab*SQUAREPULSE(startab, durab)”
τpulse3 = if mod(time-durτ3,1)>=1-durτ3 then propRxi3*τmagi3 else 0
;2) remove the ‘;’ from the beginning of the line “;τab= τpulse3*SQUAREPULSE(2009,
duri3)” (below)
τpulse3 = if mod(time-durτ3,1)>=1-durτ3 then propRxi3*τmagi3 else 0
;τab= τpulse3*SQUAREPULSE(2009, duri3)
τmagi3 = 4 ; rate of starting intervention 3 in 1/years
duri3 = 0 ; total duration of intervention 3 in years
durτ3 = 0.25 ; duration of each round of intervention 3 in years

;ACT for symptomatic infection during intervention 1
τabi1 = τmagabi1*propRxab*SQUAREPULSE(startab, durab); rate of starting ACT in
infected people during intervention 1
τmagabi1 = 365/16

;Artesunate monotherapy for symptomatic infection during intervention 1
τai1 = τmagai1*propRxa*SQUAREPULSE(2009, dura); rate of starting artesunate in
infected people during intervention 1
τmagai1 = 365/16

;ACT for symptomatic infection during intervention 2
τabi2 = τmagabi2*propRxab*SQUAREPULSE(2009, durab); rate of starting ACT during
intervention 2
τmagabi2 = 365/16

;Artesunate monotherapy for symptomatic infection during intervention 2
τai2 = τmagai2*propRxa*SQUAREPULSE(2009, dura); rate of starting artesunate in
infected people during intervention 2
τmagai2 = 365/16

;Bed nets
bn=bnmag*SQUAREPULSE(2009, durbn)
bnmag=0.3; transmission reduction due to bed nets
durbn=0; 0 or 4 years

;Initial conditions
pa = 0 ; proportion resistant to artesunate in 1960
pb = 0.05 ; proportion resistant to piperazine in 1960 (and 2009)
po = 1-pa-pb ; proportion sensitive to artesunate and piperazine in 1960
pBI = 0.0743 ; proportion infected with detectable blood stage infection in 2009 (data is
from 2006)
pinf = 0.16 ; proportion any malaria infection in 1960

```

$p_p = 0$; proportion given primaquine = 0-1

$st = 1980$; time that artesunate resistance first arises - this is set relatively early to maximise pa in 2009 so near 10%

$mst = 350$;

$propRx_a = propRx_{am} * prop_a * adh_a$; proportion that get effective artesunate treatment for symptomatic infection = 0.052

$propRx_{am} = 0.63$; proportion receiving antimalarials

$prop_a = 0.4$; proportion of antimalarials constituting artesunate monotherapy

$adh_a = 0.2$; proportion that take full 7 day course artesunate monotherapy

$propRx_{ab} = \text{IF TIME} < 2009 \text{ THEN } 0 \text{ ELSE } p_{ab} * (cov_{ab} - cov_{ab} * \exp(-k * (TIME - 2009)))$; proportion that get effective ACT treatment for symptomatic infection

$cov_{ab} = 0.6$; maximum coverage with ACT for treatment

$p_{sab} = 0.85$; proportion of shops that sell modern drugs

$adh_{ab} = 0.77$; adherence to 3 day regime of ACT

$adh_{vg} = 0.77$; adherence to 3 day regime of atovaquone/proguanil

$p_{ab} = cov_{ab} * p_{sab} * adh_{ab}$; proportion that actually take 3 day course of ACT

$k = 30$; speed of introduction of intervention

$propRx_{i1} = cov_{i1} * adh_{ab}$; proportion that complete a 3 day course of MDA with ACT

$propRx_{i2} = cov_{i2} * adh_{vg}$; proportion that complete a 3 day course of atovaquone/proguanil during MSAT

$propRx_{i3} = cov_{i3} * adh_{ab}$; proportion that complete a 3 day course of ACT during MSAT

$cov_{i1} = 0.8$; coverage with intervention 1

$cov_{i2} = 0.8$; coverage with intervention 2

$cov_{i3} = 0.8$; coverage with intervention 3

;Calculate intial conditions

$ROOTI \beta_n = \mu * N_0 - \beta_n * (p_{inf} * N_0) * S_{rodoio1} / N_0 + \delta * (p_{inf} * N_0) - \mu * S_{rodoio1}$

$ROOTI S_{rodoio1} = \beta_n * (p_{inf} * N_0) * S_{rodoio1} / N_0 - (\gamma + \mu) * L_{rodoio1}$

$ROOTI L_{rodoio1} = \gamma * L_{rodoio1} - (\sigma + \mu) * B_{rodoio1}$

$ROOTI B_{rodoio1} = \sigma * B_{rodoio1} - (\delta + \mu) * (p_{inf} * N_0)$

GUESS $\beta_n = 10$

LIMIT $\beta_n \geq 0$

LIMIT $\beta_n \leq 1000$

GUESS $S_{rodoio1} = 2.1e+6$

LIMIT $S_{rodoio1} \geq 0$

LIMIT $S_{rodoio1} \leq N_0$

GUESS $L_{\text{rodoio1}} = 7e+4$

LIMIT $L_{\text{rodoio1}} \geq 0$

LIMIT $L_{\text{rodoio1}} \leq N_0$

GUESS $B_{\text{rodoio1}} = 2e+5$

LIMIT $B_{\text{rodoio1}} \geq 0$

LIMIT $B_{\text{rodoio1}} \leq N_0$

;MODEL

;Treatment with artesunate monotherapy

;N_{doio} and N_{daio}

;Box N_{doio}

init $S_{\text{doio}} = S_{\text{rodoio1}}$

$I_{\text{doio0}} = N_0 * p_{\text{inf}}$

$L_{\text{doio}} = L_{\text{rodoio}} + L_{\text{radoio}} + L_{\text{rbdoio}}$

$B_{\text{doio}} = B_{\text{rodoio}} + B_{\text{radoio}} + B_{\text{rbdoio}}$

$I_{\text{doio}} = I_{\text{rodoio}} + I_{\text{radoio}} + I_{\text{rbdoio}}$

init $L_{\text{rodoio}} = L_{\text{rodoio1}}$

init $L_{\text{radoio}} = 0$

init $L_{\text{rbdoio}} = 0$

init $B_{\text{rodoio}} = B_{\text{rodoio1}}$

init $B_{\text{radoio}} = 0$

init $B_{\text{rbdoio}} = 0$

init $I_{\text{rodoio}} = p_o * I_{\text{doio0}}$

init $I_{\text{radoio}} = p_a * I_{\text{doio0}}$

init $I_{\text{rbdoio}} = p_b * I_{\text{doio0}}$

;S_{doio}

; Example of code for stochasticity $S_{\text{doio}}(t+dt) = \max(\text{poisson}(S_{\text{doio}} + (\mu * N_0 - \mu * S_{\text{doio}} - \beta * S_{\text{doio}} * T_{\text{Iro}}/N_0 + \delta * I_{\text{rodoio}} + c_{\text{Brodo}} * B_{\text{rodoio}} + c_{\text{Irodo}} * I_{\text{rodoio}} - \beta * S_{\text{doio}} * T_{\text{Ira}}/N_0 + \delta * I_{\text{radoio}} + c_{\text{Brado}} * B_{\text{radoio}} + c_{\text{Irado}} * I_{\text{radoio}} - \beta * S_{\text{doio}} * T_{\text{Irb}}/N_0 + \delta * I_{\text{rbdoio}} + c_{\text{Brbdo}} * B_{\text{rbdoio}} + c_{\text{Irbdo}} * I_{\text{rbdoio}} + (1/X_{\text{ao}}) * S_{\text{daio}} - \tau_1 * S_{\text{doio}} + (1/(X_b - X_{\text{ai}})) * S_{\text{dbio}}) * dt), 0)$

; this form of code was substituted for every differential equation in the model to achieve stochasticity

$$\begin{aligned} d/dt(S_{\text{doio}}) = & \mu * N_0 - \mu * S_{\text{doio}} - \beta * S_{\text{doio}} * T_{\text{Iro}}/N_0 + \delta * I_{\text{rodoio}} + c_{\text{Brodo}} * B_{\text{rodoio}} + c_{\text{Irodo}} * I_{\text{rodoio}} - \\ & \beta * S_{\text{doio}} * T_{\text{Ira}}/N_0 + \delta * I_{\text{radoio}} + c_{\text{Brado}} * B_{\text{radoio}} + c_{\text{Irado}} * I_{\text{radoio}} - \beta * S_{\text{doio}} * T_{\text{Irb}}/N_0 + \delta * I_{\text{rbdoio}} + \\ & c_{\text{Brbdo}} * B_{\text{rbdoio}} + c_{\text{Irbdo}} * I_{\text{rbdoio}} + (1/X_{\text{ao}}) * S_{\text{daio}} - \tau_1 * S_{\text{doio}} + (1/(X_b - X_{\text{ai}})) * S_{\text{dbio}} \end{aligned}$$

```
;rodoio
```

$$d/dt(L_{rodoio}) = \beta * S_{doio} * T_{Iro}/N_0 - (\mu + \gamma) * L_{rodoio} + (1/X_{ao}) * L_{rodaio} - \tau_1 * L_{rodoio} + (1/(X_b - X_{ai})) * L_{rodbio}$$

$$d/dt(B_{rodoio}) = \gamma * L_{rodoio} - (\mu + \sigma) * B_{rodoio} - c_{Brdo} * B_{rodoio} - \tau * B_{rodoio} + (1/X_{ao}) * B_{rodaio} - \tau_1 * B_{rodoio} - \tau_2 * B_{rodoio} - \tau_{ab} * B_{rodoio} + (1/(X_b - X_{ai})) * B_{rodbio}$$

$$d/dt(I_{rodoio}) = \sigma * B_{rodoio} - (\mu + \delta) * I_{rodoio} - c_{Irodo} * I_{rodoio} - \tau * I_{rodoio} + (1/X_{ao}) * I_{rodaio} - \tau_1 * I_{rodoio} - \tau_2 * I_{rodoio} - \tau_{ab} * I_{rodoio} + (1/(X_b - X_{ai})) * I_{rodbio}$$

```
;radoio
```

$$d/dt(L_{radoio}) = \beta * S_{doio} * T_{Ira}/N_0 - (\mu + \gamma) * L_{radoio} + (1/X_{ao}) * L_{radaio} - \tau_1 * L_{radoio} + (1/(X_b - X_{ai})) * L_{radbio}$$

$$d/dt(B_{radoio}) = \gamma * L_{radoio} - (\mu + \sigma) * B_{radoio} - c_{Brdo} * B_{radoio} - \tau * B_{radoio} + (1/X_{ao}) * B_{radaio} - \tau_1 * B_{radoio} - \tau_2 * B_{radoio} - \tau_{ab} * B_{radoio} + (1/(X_b - X_{ai})) * B_{radbio}$$

$$d/dt(I_{radoio}) = \sigma * B_{radoio} - (\mu + \delta) * I_{radoio} - c_{Irado} * I_{radoio} - \tau * I_{radoio} + (1/X_{ao}) * I_{radaio} - \tau_1 * I_{radoio} - \tau_2 * I_{radoio} - \tau_{ab} * I_{radoio} + (1/(X_b - X_{ai})) * I_{radbio} + \text{SQUAREPULSE}(st, 1/365) * mst$$

```
;rbdoio
```

$$d/dt(L_{rbdoio}) = \beta * S_{doio} * T_{Irb}/N_0 - (\mu + \gamma) * L_{rbdoio} + (1/X_{ao}) * L_{rbdaio} - \tau_1 * L_{rbdoio} + (1/(X_b - X_{ai})) * L_{rdbbio}$$

$$d/dt(B_{rbdoio}) = \gamma * L_{rbdoio} - (\mu + \sigma) * B_{rbdoio} - c_{Brbdo} * B_{rbdoio} - \tau * B_{rbdoio} + (1/X_{ao}) * B_{rbdaio} - \tau_1 * B_{rbdoio} - \tau_2 * B_{rbdoio} - \tau_{ab} * B_{rbdoio} + (1/(X_b - X_{ai})) * B_{rdbbio}$$

$$d/dt(I_{rbdoio}) = \sigma * B_{rbdoio} - (\mu + \delta) * I_{rbdoio} - c_{Irbdo} * I_{rbdoio} - \tau * I_{rbdoio} + (1/X_{ao}) * I_{rbdaio} - \tau_1 * I_{rbdoio} - \tau_2 * I_{rbdoio} - \tau_{ab} * I_{rbdoio} + (1/(X_b - X_{ai})) * I_{rdbbio}$$

```
;Box Ndaio
```

$$\text{init } S_{daio} = 0$$

$$\text{;init } B_{daio} = B_{daio0}$$

$$\text{;init } L_{daio} = L_{daio0}$$

$$\text{;init } I_{daio} = I_{daio0}$$

$$L_{daio} = L_{rodaio} + L_{radaio} + L_{rbdaio}$$

$$B_{daio} = B_{rodaio} + B_{radaio} + B_{rbdaio}$$

$$I_{daio} = I_{rodaio} + I_{radaio} + I_{rbdaio}$$

$$\text{init } L_{rodaio} = 0$$

$$\text{init } L_{radaio} = 0$$

$$\text{init } L_{rbdaio} = 0$$

$$\text{init } B_{rodaio} = 0$$

$$\text{init } B_{radaio} = 0$$

$$\text{init } B_{rbdaio} = 0$$

$$\text{init } I_{rodaio} = 0$$

$$\text{init } I_{radaio} = 0$$

$$\text{init } I_{rbdaio} = 0$$

$$\begin{aligned} d/dt(S_{daio}) = & -\mu * S_{daio} - \beta * S_{daio} * T_{Iro}/N_0 + \delta * I_{rodaio} + c_{Broda} * B_{rodaio} + c_{Iroda} * I_{rodaio} - \\ & \beta * S_{daio} * T_{Ira}/N_0 + \delta * I_{radaio} + c_{Brada} * B_{radaio} + c_{Irada} * I_{radaio} - \beta * S_{daio} * T_{Irb}/N_0 + \delta * I_{rbdaio} + \\ & c_{Brbda} * B_{rbdaio} + c_{Irbda} * I_{rbdaio} - (1/X_{ao}) * S_{daio} - \tau_1 * S_{daio} \end{aligned}$$

;rodaio

$$\begin{aligned} d/dt(L_{rodaio}) = & \beta * S_{daio} * T_{Iro}/N_0 - (\mu + \gamma) * L_{rodaio} - (1/X_{ao}) * L_{rodaio} - \tau_1 * L_{rodaio} \\ d/dt(B_{rodaio}) = & \gamma * L_{rodaio} - (\mu + \sigma) * B_{rodaio} - c_{Broda} * B_{rodaio} + \tau * B_{rodaio} - (1/X_{ao}) * B_{rodaio} - \\ & \tau_1 * B_{rodaio} - \tau_2 * B_{rodaio} \\ d/dt(I_{rodaio}) = & \sigma * B_{rodaio} - (\mu + \delta) * I_{rodaio} - c_{Iroda} * I_{rodaio} + \tau * I_{rodaio} - (1/X_{ao}) * I_{rodaio} - \tau_1 * I_{rodaio} - \\ & \tau_2 * I_{rodaio} \end{aligned}$$

;radaio

$$\begin{aligned} d/dt(L_{radaio}) = & \beta * S_{daio} * T_{Ira}/N_0 - (\mu + \gamma) * L_{radaio} - (1/X_{ao}) * L_{radaio} - \tau_1 * L_{radaio} \\ d/dt(B_{radaio}) = & \gamma * L_{radaio} - (\mu + \sigma) * B_{radaio} - c_{Brada} * B_{radaio} + \tau * B_{radaio} - (1/X_{ao}) * B_{radaio} - \tau_1 * B_{radaio} - \\ & \tau_2 * B_{radaio} \\ d/dt(I_{radaio}) = & \sigma * B_{radaio} - (\mu + \delta) * I_{radaio} - c_{Irada} * I_{radaio} + \tau * I_{radaio} - (1/X_{ao}) * I_{radaio} - \tau_1 * I_{radaio} - \\ & \tau_2 * I_{radaio} \end{aligned}$$

;rbdaio

$$\begin{aligned} d/dt(L_{rbdaio}) = & \beta * S_{daio} * T_{Irb}/N_0 - (\mu + \gamma) * L_{rbdaio} - (1/X_{ao}) * L_{rbdaio} - \tau_1 * L_{rbdaio} \\ d/dt(B_{rbdaio}) = & \gamma * L_{rbdaio} - (\mu + \sigma) * B_{rbdaio} - c_{Brbda} * B_{rbdaio} + \tau * B_{rbdaio} - (1/X_{ao}) * B_{rbdaio} - \\ & \tau_1 * B_{rbdaio} - \tau_2 * B_{rbdaio} \\ d/dt(I_{rbdaio}) = & \sigma * B_{rbdaio} - (\mu + \delta) * I_{rbdaio} - c_{Irbda} * I_{rbdaio} + \tau * I_{rbdaio} - (1/X_{ao}) * I_{rbdaio} - \tau_1 * I_{rbdaio} - \\ & \tau_2 * I_{rbdaio} \end{aligned}$$

$$\begin{aligned} N_{doio} = & S_{doio} + L_{rodoio} + B_{rodoio} + I_{rodoio} + L_{radoio} + B_{radoio} + I_{radoio} + L_{rbdoio} + B_{rbdoio} + I_{rbdoio} \\ N_{daio} = & S_{daio} + L_{rodaio} + B_{rodaio} + I_{rodaio} + L_{radaio} + B_{radaio} + I_{radaio} + L_{rbdaio} + B_{rbdaio} + I_{rbdaio} \end{aligned}$$

;Intervention 1

;MDA using artesunate/piperaquine +/- primaquine

;N_{dabil}, N_{dbil}, N_{doi}

;Box N_{dabil}

init S_{dabil} = 0

;init B_{dabil} = B_{dabil}0

;init L_{dabil} = L_{dabil}0

;init I_{dabil} = I_{dabil}0

L_{dabil} = L_{rodabil} + L_{radabil} + L_{rbdabil}

B_{dabil} = B_{rodabil} + B_{radabil} + B_{rbdabil}

I_{dabil} = I_{rodabil} + I_{radabil} + I_{rbdabil}

$\text{init } L_{\text{rodabil}} = 0$
 $\text{init } L_{\text{radabil}} = 0$
 $\text{init } L_{\text{rbdabil}} = 0$
 $\text{init } B_{\text{rodabil}} = 0$
 $\text{init } B_{\text{radabil}} = 0$
 $\text{init } B_{\text{rbdabil}} = 0$
 $\text{init } I_{\text{rodabil}} = 0$
 $\text{init } I_{\text{radabil}} = 0$
 $\text{init } I_{\text{rbdabil}} = 0$

$$\begin{aligned} d/dt(S_{\text{dabil}}) = & -\mu * S_{\text{dabil}} - \beta * S_{\text{dabil}} * T_{\text{Iro}}/N_0 + \delta * I_{\text{rodabil}} + c_{\text{Brodab}} * B_{\text{rodabil}} + c_{\text{Irodab}} * I_{\text{rodabil}} - \\ & \beta * S_{\text{dabil}} * T_{\text{Ira}}/N_0 + \delta * I_{\text{radabil}} + c_{\text{Bradab}} * B_{\text{radabil}} + c_{\text{Iradab}} * I_{\text{radabil}} - \beta * S_{\text{dabil}} * T_{\text{Irb}}/N_0 + \delta * I_{\text{rbdabil}} + \\ & c_{\text{Brbdab}} * B_{\text{rbdabil}} + c_{\text{Irbdbab}} * I_{\text{rbdabil}} + \tau_1 * S_{\text{doio}} + \tau_1 * S_{\text{daio}} - (1/X_{\text{ai}}) * S_{\text{dabil}} + c_{\text{Lp}} * L_{\text{rodabil}} + \\ & c_{\text{Ip}} * I_{\text{rodabil}} + c_{\text{Lp}} * L_{\text{radabil}} + c_{\text{Ip}} * I_{\text{radabil}} + c_{\text{Lp}} * L_{\text{rbdabil}} + c_{\text{Ip}} * I_{\text{rbdabil}} \end{aligned}$$

;rodabil

$$\begin{aligned} d/dt(L_{\text{rodabil}}) = & \beta * S_{\text{dabil}} * T_{\text{Iro}}/N_0 - (\mu + \gamma) * L_{\text{rodabil}} + \tau_1 * L_{\text{rodoio}} + \tau_1 * L_{\text{rodaio}} - (1/X_{\text{ai}}) * L_{\text{rodabil}} - \\ & c_{\text{Lp}} * L_{\text{rodabil}} \\ d/dt(B_{\text{rodabil}}) = & \gamma * L_{\text{rodabil}} - (\mu + \sigma) * B_{\text{rodabil}} - c_{\text{Brodab}} * B_{\text{rodabil}} + \tau_1 * B_{\text{rodoio}} + \tau_1 * B_{\text{rodaio}} - \\ & (1/X_{\text{ai}}) * B_{\text{rodabil}} + \tau_{\text{abil}} * B_{\text{rodoi1}} + \tau_1 * B_{\text{rodoi1}} \\ d/dt(I_{\text{rodabil}}) = & \sigma * B_{\text{rodabil}} - (\mu + \delta) * I_{\text{rodabil}} - c_{\text{Irodab}} * I_{\text{rodabil}} + \tau_1 * I_{\text{rodoio}} + \tau_1 * I_{\text{rodaio}} - \\ & (1/X_{\text{ai}}) * I_{\text{rodabil}} + \tau_{\text{abil}} * I_{\text{rodoi1}} - c_{\text{Ip}} * I_{\text{rodabil}} + \tau_1 * I_{\text{rodoi1}} \end{aligned}$$

;radabil

$$\begin{aligned} d/dt(L_{\text{radabil}}) = & \beta * S_{\text{dabil}} * T_{\text{Ira}}/N_0 - (\mu + \gamma) * L_{\text{radabil}} + \tau_1 * L_{\text{radoio}} + \tau_1 * L_{\text{radaio}} - (1/X_{\text{ai}}) * L_{\text{radabil}} - \\ & c_{\text{Lp}} * L_{\text{radabil}} \\ d/dt(B_{\text{radabil}}) = & \gamma * L_{\text{radabil}} - (\mu + \sigma) * B_{\text{radabil}} - c_{\text{Bradab}} * B_{\text{radabil}} + \tau_1 * B_{\text{radoio}} + \tau_1 * B_{\text{radaio}} - \\ & (1/X_{\text{ai}}) * B_{\text{radabil}} + \tau_{\text{abil}} * B_{\text{radoi1}} + \tau_1 * B_{\text{radoi1}} \\ d/dt(I_{\text{radabil}}) = & \sigma * B_{\text{radabil}} - (\mu + \delta) * I_{\text{radabil}} - c_{\text{Iradab}} * I_{\text{radabil}} + \tau_1 * I_{\text{radoio}} + \tau_1 * I_{\text{radaio}} - \\ & (1/X_{\text{ai}}) * I_{\text{radabil}} + \tau_{\text{abil}} * I_{\text{radoi1}} - c_{\text{Ip}} * I_{\text{radabil}} + \tau_1 * I_{\text{radoi1}} \end{aligned}$$

;rbdabil

$$\begin{aligned} d/dt(L_{\text{rbdabil}}) = & \beta * S_{\text{dabil}} * T_{\text{Irb}}/N_0 - (\mu + \gamma) * L_{\text{rbdabil}} + \tau_1 * L_{\text{rbdoio}} + \tau_1 * L_{\text{rbdaiio}} - (1/X_{\text{ai}}) * L_{\text{rbdabil}} - \\ & c_{\text{Lp}} * L_{\text{rbdabil}} \\ d/dt(B_{\text{rbdabil}}) = & \gamma * L_{\text{rbdabil}} - (\mu + \sigma) * B_{\text{rbdabil}} - c_{\text{Brbdab}} * B_{\text{rbdabil}} + \tau_1 * B_{\text{rbdoio}} + \tau_1 * B_{\text{rbdaiio}} - \\ & (1/X_{\text{ai}}) * B_{\text{rbdabil}} + \tau_{\text{abil}} * B_{\text{rbdoi1}} + \tau_{\text{abil}} * B_{\text{rbdbi1}} + \tau_1 * B_{\text{rbdoi1}} \\ d/dt(I_{\text{rbdabil}}) = & \sigma * B_{\text{rbdabil}} - (\mu + \delta) * I_{\text{rbdabil}} - c_{\text{Irbdbab}} * I_{\text{rbdabil}} + \tau_1 * I_{\text{rbdoio}} + \tau_1 * I_{\text{rbdaiio}} - \\ & (1/X_{\text{ai}}) * I_{\text{rbdabil}} + \tau_{\text{abil}} * I_{\text{rbdoi1}} + \tau_{\text{abil}} * I_{\text{rbdbi1}} - c_{\text{Ip}} * I_{\text{rbdabil}} + \tau_1 * I_{\text{rbdoi1}} \end{aligned}$$

$$N_{\text{dabil}} = S_{\text{dabil}} + L_{\text{rodabil}} + B_{\text{rodabil}} + I_{\text{rodabil}} + L_{\text{radabil}} + B_{\text{radabil}} + I_{\text{radabil}} + L_{\text{rbdabil}} + B_{\text{rbdabil}} + I_{\text{rbdabil}}$$

;Box N_{dbi1}

init S_{dbi1} = 0

;init B_{dbi1} = B_{dbi1}0

;init L_{dbi1} = L_{dbi1}0

;init I_{dbi1} = I_{dbi1}0

L_{dbi1} = L_{rodbi1}+L_{radbi1}+L_{rdbi1}

B_{dbi1} = B_{rodbi1}+B_{radbi1}+B_{rdbi1}

I_{dbi1} = I_{rodbi1}+I_{radbi1}+I_{rdbi1}

init L_{rodbi1} = 0

init L_{radbi1} = 0

init L_{rdbi1} = 0

init B_{rodbi1} = 0

init B_{radbi1} = 0

init B_{rdbi1} = 0

init I_{rodbi1} = 0

init I_{radbi1} = 0

init I_{rdbi1} = 0

$$\begin{aligned} d/dt(S_{dbi1}) = & -\mu*S_{dbi1} - \beta*S_{dbi1}*T_{Iro}/N_0 + \delta*I_{rodbi1} + c_{Brodb}*B_{rodbi1} + c_{Irodb}*I_{rodbi1} - \\ & \beta*S_{dbi1}*T_{Ira}/N_0 + \delta*I_{radbi1} + c_{Bradb}*B_{radbi1} + c_{Iradb}*I_{radbi1} - \beta*S_{dbi1}*T_{Irb}/N_0 + \delta*I_{rdbi1} + \\ & c_{Brbdb}*B_{rdbi1} + c_{Irbdb}*I_{rdbi1} + (1/X_{ai})*S_{dabi1} - (1/(X_b-X_{ai}))*S_{dbi1} \end{aligned}$$

;rodbi1

$$d/dt(L_{rodbi1}) = \beta*S_{dbi1}*T_{Iro}/N_0 - (\mu+\gamma)*L_{rodbi1} + (1/X_{ai})*L_{rodabi1} - (1/(X_b-X_{ai}))*L_{rodbi1}$$

$$d/dt(B_{rodbi1}) = \gamma*L_{rodbi1} - (\mu+\sigma)*B_{rodbi1} - c_{Brodb}*B_{rodbi1} + (1/X_{ai})*B_{rodabi1} - (1/(X_b-X_{ai}))*B_{rodbi1}$$

$$d/dt(I_{rodbi1}) = \sigma*B_{rodbi1} - (\mu+\delta)*I_{rodbi1} - c_{Irodb}*I_{rodbi1} + (1/X_{ai})*I_{rodabi1} - (1/(X_b-X_{ai}))*I_{rodbi1}$$

;radbi1

$$d/dt(L_{radbi1}) = \beta*S_{dbi1}*T_{Ira}/N_0 - (\mu+\gamma)*L_{radbi1} + (1/X_{ai})*L_{radabi1} - (1/(X_b-X_{ai}))*L_{radbi1}$$

$$d/dt(B_{radbi1}) = \gamma*L_{radbi1} - (\mu+\sigma)*B_{radbi1} - c_{Bradb}*B_{radbi1} + (1/X_{ai})*B_{radabi1} - (1/(X_b-X_{ai}))*B_{radbi1}$$

$$d/dt(I_{radbi1}) = \sigma*B_{radbi1} - (\mu+\delta)*I_{radbi1} - c_{Iradb}*I_{radbi1} + (1/X_{ai})*I_{radabi1} - (1/(X_b-X_{ai}))*I_{radbi1}$$

;rdbi1

$$d/dt(L_{rdbi1}) = \beta*S_{dbi1}*T_{Irb}/N_0 - (\mu+\gamma)*L_{rdbi1} + (1/X_{ai})*L_{rdbabi1} - (1/(X_b-X_{ai}))*L_{rdbi1}$$

$$d/dt(B_{rdbi1}) = \gamma*L_{rdbi1} - (\mu+\sigma)*B_{rdbi1} - c_{Brbdb}*B_{rdbi1} + (1/X_{ai})*B_{rdbabi1} - (1/(X_b-X_{ai}))*B_{rdbi1} - \tau_{abi1}*B_{rdbi1} - \tau_{ai1}*B_{rdbi1}$$

$$d/dt(I_{rdbi1}) = \sigma*B_{rdbi1} - (\mu+\delta)*I_{rdbi1} - c_{Irbdb}*I_{rdbi1} + (1/X_{ai})*I_{rdbabi1} - (1/(X_b-X_{ai}))*I_{rdbi1} - \tau_{abi1}*I_{rdbi1} - \tau_{ai1}*I_{rdbi1}$$

$$N_{dbil} = S_{dbil} + L_{rodbil} + B_{rodbil} + I_{rodbil} + L_{radbil} + B_{radbil} + I_{radbil} + L_{rdbil} + B_{rdbil} + I_{rdbil}$$

;Box N_{doil}

init S_{doil} = 0;N₀-I_{doil}0

;init B_{doil} = B_{doil}0

;init L_{doil} = L_{doil}0

I_{doil}0 = 0;N₀*p_{inf}

L_{doil} = L_{rodoil}+L_{radoil}+L_{rbdil}

B_{doil} = B_{rodoil}+B_{radoil}+B_{rbdil}

I_{doil} = I_{rodoil}+I_{radoil}+I_{rbdil}

init L_{rodoil} = 0

init L_{radoil} = 0

init L_{rbdil} = 0

init B_{rodoil} = 0

init B_{radoil} = 0

init B_{rbdil} = 0

init I_{rodoil} = 0;p_o*I_{doil}0

init I_{radoil} = 0;p_a*I_{doil}0

init I_{rbdil} = 0;p_b*I_{doil}0

$$\begin{aligned} d/dt(S_{doil}) = & -\mu*S_{doil} - \beta*S_{doil}*T_{Iro}/N_0 + \delta*I_{rodoil} + c_{Brodo}*B_{rodoil} + c_{Irodo}*I_{rodoil} - \\ & \beta*S_{doil}*T_{Ira}/N_0 + \delta*I_{radoil} + c_{Brado}*B_{radoil} + c_{Irado}*I_{radoil} - \beta*S_{doil}*T_{Irb}/N_0 + \delta*I_{rbdil} + \\ & c_{Brbdo}*B_{rbdil} + c_{Irbdo}*I_{rbdil} + (1/(X_b-X_{ai}))*S_{dbil} + 1/(X_b-X_{ai}-X_{ao})*S_{dbila} + (1/X_{ao})*S_{daila} \end{aligned}$$

;rodoil

$$\begin{aligned} d/dt(L_{rodoil}) = & \beta*S_{doil}*T_{Iro}/N_0 - (\mu+\gamma)*L_{rodoil} + (1/(X_b-X_{ai}))*L_{rodbil} + 1/(X_b-X_{ai}-X_{ao})*L_{rodbila} \\ & + (1/X_{ao})*L_{rodaila} \end{aligned}$$

$$\begin{aligned} d/dt(B_{rodoil}) = & \gamma*L_{rodoil} - (\mu+\sigma)*B_{rodoil} - c_{Brodo}*B_{rodoil} + (1/(X_b-X_{ai}))*B_{rodbil} - \tau_{abil}*B_{rodoil} + \\ & 1/(X_b-X_{ai}-X_{ao})*B_{rodbila} - \tau_{ail}*B_{rodoil} + (1/X_{ao})*B_{rodaila} - \tau_l*B_{rodoil} \end{aligned}$$

$$\begin{aligned} d/dt(I_{rodoil}) = & \sigma*B_{rodoil} - (\mu+\delta)*I_{rodoil} - c_{Irodo}*I_{rodoil} + (1/(X_b-X_{ai}))*I_{rodbil} - \tau_{abil}*I_{rodoil} + \\ & 1/(X_b-X_{ai}-X_{ao})*I_{rodbila} - \tau_{ail}*I_{rodoil} + (1/X_{ao})*I_{rodaila} - \tau_l*I_{rodoil} \end{aligned}$$

;radoil

$$\begin{aligned} d/dt(L_{radoil}) = & \beta*S_{doil}*T_{Ira}/N_0 - (\mu+\gamma)*L_{radoil} + (1/(X_b-X_{ai}))*L_{radbil} + 1/(X_b-X_{ai}-X_{ao})*L_{radbila} \\ & + (1/X_{ao})*L_{radaila} \end{aligned}$$

$$\begin{aligned} d/dt(B_{radoil}) = & \gamma*L_{radoil} - (\mu+\sigma)*B_{radoil} - c_{Brado}*B_{radoil} + (1/(X_b-X_{ai}))*B_{radbil} - \tau_{abil}*B_{radoil} + \\ & 1/(X_b-X_{ai}-X_{ao})*B_{radbila} - \tau_{ail}*B_{radoil} + (1/X_{ao})*B_{radaila} - \tau_l*B_{radoil} \end{aligned}$$

$$d/dt(I_{radoil}) = \sigma * B_{radoil} - (\mu + \delta) * I_{radoil} - c_{Irado} * I_{radoil} + (1/(X_b - X_{ai})) * I_{radbil} - \tau_{abil} * I_{radoil} + 1/(X_b - X_{ai} - X_{ao}) * I_{radbila} - \tau_{ail} * I_{radoil} + (1/X_{ao}) * I_{radaila} - \tau_1 * I_{radoil}$$

;rbdoi1

$$d/dt(L_{rbdoi1}) = \beta * S_{doi1} * T_{Irb}/N_0 - (\mu + \gamma) * L_{rbdoi1} + (1/(X_b - X_{ai})) * L_{rbdbil} + 1/(X_b - X_{ai} - X_{ao}) * L_{rbdbila} + (1/X_{ao}) * L_{rbdaila}$$

$$d/dt(B_{rbdoi1}) = \gamma * L_{rbdoi1} - (\mu + \sigma) * B_{rbdoi1} - c_{Brbdo} * B_{rbdoi1} + (1/(X_b - X_{ai})) * B_{rbdbil} - \tau_{abil} * B_{rbdoi1} + 1/(X_b - X_{ai} - X_{ao}) * B_{rbdbila} - \tau_{ail} * B_{rbdoi1} + (1/X_{ao}) * B_{rbdaila} - \tau_1 * B_{rbdoi1}$$

$$d/dt(I_{rbdoi1}) = \sigma * B_{rbdoi1} - (\mu + \delta) * I_{rbdoi1} - c_{Irbdo} * I_{rbdoi1} + (1/(X_b - X_{ai})) * I_{rbdbil} - \tau_{abil} * I_{rbdoi1} + 1/(X_b - X_{ai} - X_{ao}) * I_{rbdbila} - \tau_{ail} * I_{rbdoi1} + (1/X_{ao}) * I_{rbdaila} - \tau_1 * I_{rbdoi1}$$

$$N_{doi1} = S_{doi1} + L_{rodoi1} + B_{rodoi1} + I_{rodoi1} + L_{radoil} + B_{radoil} + I_{radoil} + L_{rbdoi1} + B_{rbdoi1} + I_{rbdoi1}$$

;Treatment with artesunate in Intervention 1

; N_{daila}

;Box N_{daila}

init S_{daila} = 0

;init B_{daila} = B_{daila}0

;init L_{daila} = L_{daila}0

;init I_{daila} = I_{daila}0

$$L_{daila} = L_{rodaila} + L_{radaila} + L_{rbdaila}$$

$$B_{daila} = B_{rodaila} + B_{radaila} + B_{rbdaila}$$

$$I_{daila} = I_{rodaila} + I_{radaila} + I_{rbdaila}$$

init L_{rodaila} = 0

init L_{radaila} = 0

init L_{rbdaila} = 0

init B_{rodaila} = 0

init B_{radaila} = 0

init B_{rbdaila} = 0

init I_{rodaila} = 0

init I_{radaila} = 0

init I_{rbdaila} = 0

$$d/dt(S_{daila}) = -\mu * S_{daila} - \beta * S_{daila} * T_{Iro}/N_0 + \delta * I_{rodaila} + c_{Broda} * B_{rodaila} + c_{Iroda} * I_{rodaila} - \beta * S_{daila} * T_{Ira}/N_0 + \delta * I_{radaila} + c_{Brada} * B_{radaila} + c_{Irada} * I_{radaila} - \beta * S_{daila} * T_{Irb}/N_0 + \delta * I_{rbdaila} + c_{Brbda} * B_{rbdaila} + c_{Irbda} * I_{rbdaila} - (1/X_{ao}) * S_{daila}$$

;rodaila

$$d/dt(L_{rodaila}) = \beta * S_{daila} * T_{Iro}/N_0 - (\mu + \gamma) * L_{rodaila} - (1/X_{ao}) * L_{rodaila}$$

$$d/dt(B_{rodaila}) = \gamma * L_{rodaila} - (\mu + \sigma) * B_{rodaila} - c_{Broda} * B_{rodaila} + \tau_{ail} * B_{rodoi1} - (1/X_{ao}) * B_{rodaila}$$

$$d/dt(I_{rodaila}) = \sigma * B_{rodaila} - (\mu + \delta) * I_{rodaila} - c_{Iroda} * I_{rodaila} + \tau_{ail} * I_{rodoi1} - (1/X_{ao}) * I_{rodaila}$$

;radaila

$$d/dt(L_{radaila}) = \beta * S_{daila} * T_{Ira}/N_0 - (\mu + \gamma) * L_{radaila} - (1/X_{ao}) * L_{radaila}$$

$$d/dt(B_{radaila}) = \gamma * L_{radaila} - (\mu + \sigma) * B_{radaila} - c_{Brada} * B_{radaila} + \tau_{ail} * B_{radoil} - (1/X_{ao}) * B_{radaila}$$

$$d/dt(I_{radaila}) = \sigma * B_{radaila} - (\mu + \delta) * I_{radaila} - c_{Irada} * I_{radaila} + \tau_{ail} * I_{radoil} - (1/X_{ao}) * I_{radaila}$$

;rbdaila

$$d/dt(L_{rbdaila}) = \beta * S_{daila} * T_{Irb}/N_0 - (\mu + \gamma) * L_{rbdaila} - (1/X_{ao}) * L_{rbdaila}$$

$$d/dt(B_{rbdaila}) = \gamma * L_{rbdaila} - (\mu + \sigma) * B_{rbdaila} - c_{Brbda} * B_{rbdaila} + \tau_{ail} * B_{rbdail} - (1/X_{ao}) * B_{rbdaila}$$

$$d/dt(I_{rbdaila}) = \sigma * B_{rbdaila} - (\mu + \delta) * I_{rbdaila} - c_{Irbda} * I_{rbdaila} + \tau_{ail} * I_{rbdail} - (1/X_{ao}) * I_{rbdaila}$$

$$N_{daila} = S_{daila} + L_{rodaila} + B_{rodaila} + I_{rodaila} + L_{radaila} + B_{radaila} + I_{radaila} + L_{rbdaila} + B_{rbdaila} + I_{rbdaila}$$

;Treatment of infected patients with artesunate/piperaquine in Intervention 1

; N_{dabila}, N_{dbila}

;Box N_{dabila}

init S_{dabila} = 0

;init B_{dabila} = B_{dabila}0

;init L_{dabila} = L_{dabila}0

;init I_{dabila} = I_{dabila}0

$$L_{dabila} = L_{rodabila} + L_{radabila} + L_{rbdabila}$$

$$B_{dabila} = B_{rodabila} + B_{radabila} + B_{rbdabila}$$

$$I_{dabila} = I_{rodabila} + I_{radabila} + I_{rbdabila}$$

init L_{rodabila} = 0

init L_{radabila} = 0

init L_{rbdabila} = 0

init B_{rodabila} = 0

init B_{radabila} = 0

init B_{rbdabila} = 0

init I_{rodabila} = 0

init I_{radabila} = 0

init I_{rbdabila} = 0

$$d/dt(S_{dabila}) = -\mu * S_{dabila} - \beta * S_{dabila} * T_{Iro}/N_0 + \delta * I_{rodabila} + c_{Brodb} * B_{rodabila} + c_{Irodab} * I_{rodabila} - \beta * S_{dabila} * T_{Ira}/N_0 + \delta * I_{radabila} + c_{Bradab} * B_{radabila} + c_{Iradab} * I_{radabila} - \beta * S_{dabila} * T_{Irb}/N_0 + \delta * I_{rbdabila} + c_{Brbdab} * B_{rbdabila} + c_{Irbdab} * I_{rbdabila} - (1/X_{ao}) * S_{dabila}$$

;rodabila

$$d/dt(L_{rodabila}) = \beta * S_{dabila} * T_{Iro}/N_0 - (\mu + \gamma) * L_{rodabila} - (1/X_{ao}) * L_{rodabila}$$

$$\begin{aligned} d/dt(B_{rodabila}) &= \gamma * L_{rodabila} - (\mu + \sigma) * B_{rodabila} - c_{Brodab} * B_{rodabila} - (1/X_{ao}) * B_{rodabila} \\ d/dt(I_{rodabila}) &= \sigma * B_{rodabila} - (\mu + \delta) * I_{rodabila} - c_{Irodab} * I_{rodabila} - (1/X_{ao}) * I_{rodabila} \end{aligned}$$

;radabila

$$\begin{aligned} d/dt(L_{radabila}) &= \beta * S_{dabila} * T_{Ira}/N_0 - (\mu + \gamma) * L_{radabila} - (1/X_{ao}) * L_{radabila} \\ d/dt(B_{radabila}) &= \gamma * L_{radabila} - (\mu + \sigma) * B_{radabila} - c_{Bradab} * B_{radabila} - (1/X_{ao}) * B_{radabila} \\ d/dt(I_{radabila}) &= \sigma * B_{radabila} - (\mu + \delta) * I_{radabila} - c_{Iradab} * I_{radabila} - (1/X_{ao}) * I_{radabila} \end{aligned}$$

;rbdabila

$$\begin{aligned} d/dt(L_{rbdabila}) &= \beta * S_{dabila} * T_{Irb}/N_0 - (\mu + \gamma) * L_{rbdabila} - (1/X_{ao}) * L_{rbdabila} \\ d/dt(B_{rbdabila}) &= \gamma * L_{rbdabila} - (\mu + \sigma) * B_{rbdabila} - c_{Brbdab} * B_{rbdabila} + \tau_{ail} * B_{rdbil} - \\ & (1/X_{ao}) * B_{rbdabila} + \tau_{ail} * B_{rdbil} \\ d/dt(I_{rbdabila}) &= \sigma * B_{rbdabila} - (\mu + \delta) * I_{rbdabila} - c_{Irbdb} * I_{rbdabila} + \tau_{ail} * I_{rdbil} - (1/X_{ao}) * I_{rbdabila} + \\ & \tau_{ail} * I_{rdbil} \end{aligned}$$

$$N_{dabila} = S_{dabila} + L_{rodabila} + B_{rodabila} + I_{rodabila} + L_{radabila} + B_{radabila} + I_{radabila} + L_{rbdabila} + B_{rbdabila} + I_{rbdabila}$$

;Box N_{dbila}

$$\begin{aligned} \text{init } S_{dbila} &= 0 \\ \text{;init } B_{dbila} &= B_{dbila}0 \\ \text{;init } L_{dbila} &= L_{dbila}0 \\ \text{;init } I_{dbila} &= I_{dbila}0 \end{aligned}$$

$$\begin{aligned} L_{dbila} &= L_{rodabila} + L_{radabila} + L_{rbdabila} \\ B_{dbila} &= B_{rodabila} + B_{radabila} + B_{rbdabila} \\ I_{dbila} &= I_{rodabila} + I_{radabila} + I_{rbdabila} \end{aligned}$$

$$\begin{aligned} \text{init } L_{rodabila} &= 0 \\ \text{init } L_{radabila} &= 0 \\ \text{init } L_{rbdabila} &= 0 \\ \text{init } B_{rodabila} &= 0 \\ \text{init } B_{radabila} &= 0 \\ \text{init } B_{rbdabila} &= 0 \\ \text{init } I_{rodabila} &= 0 \\ \text{init } I_{radabila} &= 0 \\ \text{init } I_{rbdabila} &= 0 \end{aligned}$$

$$\begin{aligned} d/dt(S_{dbila}) &= -\mu * S_{dbila} - \beta * S_{dbila} * T_{Iro}/N_0 + \delta * I_{rodabila} + c_{Brodab} * B_{rodabila} + c_{Irodab} * I_{rodabila} - \\ & \beta * S_{dbila} * T_{Ira}/N_0 + \delta * I_{radabila} + c_{Bradab} * B_{radabila} + c_{Iradab} * I_{radabila} - \beta * S_{dbila} * T_{Irb}/N_0 + \delta * I_{rbdabila} + \\ & c_{Brbdab} * B_{rbdabila} + c_{Irbdb} * I_{rbdabila} + (1/X_{ao} * S_{dabila}) - 1/(X_b - X_{ai} - X_{ao}) * S_{dbila} \end{aligned}$$

;rodbila

$$d/dt(L_{rodbila}) = \beta * S_{dbila} * T_{lro}/N_0 - (\mu + \gamma) * L_{rodbila} + (1/X_{ao}) * L_{rodabila} - 1/(X_b - X_{ai} - X_{ao}) * L_{rodbila}$$

$$d/dt(B_{rodbila}) = \gamma * L_{rodbila} - (\mu + \sigma) * B_{rodbila} - c_{Brodb} * B_{rodbila} + (1/X_{ao}) * B_{rodabila} - 1/(X_b - X_{ai} - X_{ao}) * B_{rodbila}$$

$$d/dt(I_{rodbila}) = \sigma * B_{rodbila} - (\mu + \delta) * I_{rodbila} - c_{Irodb} * I_{rodbila} + (1/X_{ao}) * I_{rodabila} - 1/(X_b - X_{ai} - X_{ao}) * I_{rodbila}$$

;radbila

$$d/dt(L_{radbila}) = \beta * S_{dbila} * T_{lra}/N_0 - (\mu + \gamma) * L_{radbila} + (1/X_{ao}) * L_{radabila} - 1/(X_b - X_{ai} - X_{ao}) * L_{radbila}$$

$$d/dt(B_{radbila}) = \gamma * L_{radbila} - (\mu + \sigma) * B_{radbila} - c_{Brad} * B_{radbila} + (1/X_{ao}) * B_{radabila} - 1/(X_b - X_{ai} - X_{ao}) * B_{radbila}$$

$$d/dt(I_{radbila}) = \sigma * B_{radbila} - (\mu + \delta) * I_{radbila} - c_{Irad} * I_{radbila} + (1/X_{ao}) * I_{radabila} - 1/(X_b - X_{ai} - X_{ao}) * I_{radbila}$$

;rdbbila

$$d/dt(L_{rdbbila}) = \beta * S_{dbila} * T_{lrb}/N_0 - (\mu + \gamma) * L_{rdbbila} + (1/X_{ao}) * L_{rdbabila} - 1/(X_b - X_{ai} - X_{ao}) * L_{rdbbila}$$

$$d/dt(B_{rdbbila}) = \gamma * L_{rdbbila} - (\mu + \sigma) * B_{rdbbila} - c_{Brdb} * B_{rdbbila} - \tau_{ail} * B_{rdbbila} + (1/X_{ao}) * B_{rdbabila} - 1/(X_b - X_{ai} - X_{ao}) * B_{rdbbila}$$

$$d/dt(I_{rdbbila}) = \sigma * B_{rdbbila} - (\mu + \delta) * I_{rdbbila} - c_{Irdb} * I_{rdbbila} - \tau_{ail} * I_{rdbbila} + (1/X_{ao}) * I_{rdbabila} - 1/(X_b - X_{ai} - X_{ao}) * I_{rdbbila}$$

$$N_{dbila} = S_{dbila} + L_{rodbila} + B_{rodbila} + I_{rodbila} + L_{radbila} + B_{radbila} + I_{radbila} + L_{rdbbila} + B_{rdbbila} + I_{rdbbila}$$

;Switch to ACT (artesunate + piperazine) for treatment in place of artesunate monotherapy

;also Intervention 3: MSAT with ACT

; N_{dabio}, N_{dbio}

;Box N_{dabio}

init S_{dabio} = 0

;init B_{dabio} = B_{dabio}0

;init L_{dabio} = L_{dabio}0

;init I_{dabio} = I_{dabio}0

$$L_{dabio} = L_{rodabio} + L_{radabio} + L_{rdbabio}$$

$$B_{dabio} = B_{rodabio} + B_{radabio} + B_{rdbabio}$$

$$I_{dabio} = I_{rodabio} + I_{radabio} + I_{rdbabio}$$

init L_{rodabio} = 0

init L_{radabio} = 0

init L_{rdbabio} = 0

init $B_{\text{rodabio}} = 0$
 init $B_{\text{radabio}} = 0$
 init $B_{\text{rbdabio}} = 0$
 init $I_{\text{rodabio}} = 0$
 init $I_{\text{radabio}} = 0$
 init $I_{\text{rbdabio}} = 0$

$$\begin{aligned}
 d/dt(S_{\text{dabio}}) = & -\mu * S_{\text{dabio}} - \beta * S_{\text{dabio}} * T_{\text{Iro}}/N_0 + \delta * I_{\text{rodabio}} + c_{\text{Brodab}} * B_{\text{rodabio}} + c_{\text{Irodab}} * I_{\text{rodabio}} - \\
 & \beta * S_{\text{dabio}} * T_{\text{Ira}}/N_0 + \delta * I_{\text{radabio}} + c_{\text{Bradab}} * B_{\text{radabio}} + c_{\text{Iradab}} * I_{\text{radabio}} - \beta * S_{\text{dabio}} * T_{\text{Irb}}/N_0 + \delta * I_{\text{rbdabio}} + \\
 & c_{\text{Brbdab}} * B_{\text{rbdabio}} + c_{\text{Irbdbab}} * I_{\text{rbdabio}} - (1/X_{\text{ai}}) * S_{\text{dabio}} + c_{\text{Lp}} * (L_{\text{rodabio}} + L_{\text{radabio}} + L_{\text{rbdabio}}) + \\
 & c_{\text{Ip}} * (I_{\text{rodabio}} + I_{\text{radabio}} + I_{\text{rbdabio}})
 \end{aligned}$$

;rodabio

$$\begin{aligned}
 d/dt(L_{\text{rodabio}}) = & \beta * S_{\text{dabio}} * T_{\text{Iro}}/N_0 - (\mu + \gamma) * L_{\text{rodabio}} - (1/X_{\text{ai}}) * L_{\text{rodabio}} - c_{\text{Lp}} * L_{\text{rodabio}} \\
 d/dt(B_{\text{rodabio}}) = & \gamma * L_{\text{rodabio}} - (\mu + \sigma) * B_{\text{rodabio}} - c_{\text{Brodab}} * B_{\text{rodabio}} + \tau_{\text{ab}} * B_{\text{rodoio}} - (1/X_{\text{ai}}) * B_{\text{rodabio}} \\
 d/dt(I_{\text{rodabio}}) = & \sigma * B_{\text{rodabio}} - (\mu + \delta) * I_{\text{rodabio}} - c_{\text{Irodab}} * I_{\text{rodabio}} + \tau_{\text{ab}} * I_{\text{rodoio}} - (1/X_{\text{ai}}) * I_{\text{rodabio}} - \\
 & c_{\text{Ip}} * I_{\text{rodabio}}
 \end{aligned}$$

;radabio

$$\begin{aligned}
 d/dt(L_{\text{radabio}}) = & \beta * S_{\text{dabio}} * T_{\text{Ira}}/N_0 - (\mu + \gamma) * L_{\text{radabio}} - (1/X_{\text{ai}}) * L_{\text{radabio}} - c_{\text{Lp}} * L_{\text{radabio}} \\
 d/dt(B_{\text{radabio}}) = & \gamma * L_{\text{radabio}} - (\mu + \sigma) * B_{\text{radabio}} - c_{\text{Bradab}} * B_{\text{radabio}} + \tau_{\text{ab}} * B_{\text{radoio}} - (1/X_{\text{ai}}) * B_{\text{radabio}} \\
 d/dt(I_{\text{radabio}}) = & \sigma * B_{\text{radabio}} - (\mu + \delta) * I_{\text{radabio}} - c_{\text{Iradab}} * I_{\text{radabio}} + \tau_{\text{ab}} * I_{\text{radoio}} - (1/X_{\text{ai}}) * I_{\text{radabio}} - \\
 & c_{\text{Ip}} * I_{\text{radabio}}
 \end{aligned}$$

;rbdabio

$$\begin{aligned}
 d/dt(L_{\text{rbdabio}}) = & \beta * S_{\text{dabio}} * T_{\text{Irb}}/N_0 - (\mu + \gamma) * L_{\text{rbdabio}} - (1/X_{\text{ai}}) * L_{\text{rbdabio}} - c_{\text{Lp}} * L_{\text{rbdabio}} \\
 d/dt(B_{\text{rbdabio}}) = & \gamma * L_{\text{rbdabio}} - (\mu + \sigma) * B_{\text{rbdabio}} - c_{\text{Brbdab}} * B_{\text{rbdabio}} + \tau_{\text{ab}} * B_{\text{rbdio}} - (1/X_{\text{ai}}) * B_{\text{rbdabio}} + \\
 & \tau_{\text{ab}} * B_{\text{rdbio}} \\
 d/dt(I_{\text{rbdabio}}) = & \sigma * B_{\text{rbdabio}} - (\mu + \delta) * I_{\text{rbdabio}} - c_{\text{Irbdbab}} * I_{\text{rbdabio}} + \tau_{\text{ab}} * I_{\text{rbdio}} - (1/X_{\text{ai}}) * I_{\text{rbdabio}} + \\
 & \tau_{\text{ab}} * I_{\text{rdbio}} - c_{\text{Ip}} * I_{\text{rbdabio}}
 \end{aligned}$$

$$N_{\text{dabio}} = S_{\text{dabio}} + L_{\text{rodabio}} + B_{\text{rodabio}} + I_{\text{rodabio}} + L_{\text{radabio}} + B_{\text{radabio}} + I_{\text{radabio}} + L_{\text{rbdabio}} + B_{\text{rbdabio}} + I_{\text{rbdabio}}$$

;Box N_{dbio}

init $S_{\text{dbio}} = 0$
 ;init $B_{\text{dbio}} = B_{\text{dbio}}0$
 ;init $L_{\text{dbio}} = L_{\text{dbio}}0$
 ;init $I_{\text{dbio}} = I_{\text{dbio}}0$

$$\begin{aligned}
 L_{\text{dbio}} &= L_{\text{rodabio}} + L_{\text{radabio}} + L_{\text{rbdabio}} \\
 B_{\text{dbio}} &= B_{\text{rodabio}} + B_{\text{radabio}} + B_{\text{rbdabio}}
 \end{aligned}$$

$$I_{dbio} = I_{rodbio} + I_{radbio} + I_{rdbio}$$

$$\text{init } L_{rodbio} = 0$$

$$\text{init } L_{radbio} = 0$$

$$\text{init } L_{rdbio} = 0$$

$$\text{init } B_{rodbio} = 0$$

$$\text{init } B_{radbio} = 0$$

$$\text{init } B_{rdbio} = 0$$

$$\text{init } I_{rodbio} = 0$$

$$\text{init } I_{radbio} = 0$$

$$\text{init } I_{rdbio} = 0$$

$$\begin{aligned} d/dt(S_{dbio}) = & -\mu * S_{dbio} - \beta * S_{dbio} * T_{Iro}/N_0 + \delta * I_{rodbio} + c_{Brodb} * B_{rodbio} + c_{Irodb} * I_{rodbio} - \\ & \beta * S_{dbio} * T_{Ira}/N_0 + \delta * I_{radbio} + c_{Bradb} * B_{radbio} + c_{Iradb} * I_{radbio} - \beta * S_{dbio} * T_{Irb}/N_0 + \delta * I_{rdbio} + \\ & c_{Brdb} * B_{rdbio} + c_{Irbdb} * I_{rdbio} + (1/X_{ai}) * S_{dabio} - (1/(X_b - X_{ai})) * S_{dbio} \end{aligned}$$

;rodbio

$$d/dt(L_{rodbio}) = \beta * S_{dbio} * T_{Iro}/N_0 - (\mu + \gamma) * L_{rodbio} + (1/X_{ai}) * L_{rodabio} - (1/(X_b - X_{ai})) * L_{rodbio}$$

$$d/dt(B_{rodbio}) = \gamma * L_{rodbio} - (\mu + \sigma) * B_{rodbio} - c_{Brodb} * B_{rodbio} + (1/X_{ai}) * B_{rodabio} - (1/(X_b - X_{ai})) * B_{rodbio}$$

$$d/dt(I_{rodbio}) = \sigma * B_{rodbio} - (\mu + \delta) * I_{rodbio} - c_{Irodb} * I_{rodbio} + (1/X_{ai}) * I_{rodabio} - (1/(X_b - X_{ai})) * I_{rodbio}$$

;radbio

$$d/dt(L_{radbio}) = \beta * S_{dbio} * T_{Ira}/N_0 - (\mu + \gamma) * L_{radbio} + (1/X_{ai}) * L_{radabio} - (1/(X_b - X_{ai})) * L_{radbio}$$

$$d/dt(B_{radbio}) = \gamma * L_{radbio} - (\mu + \sigma) * B_{radbio} - c_{Bradb} * B_{radbio} + (1/X_{ai}) * B_{radabio} - (1/(X_b - X_{ai})) * B_{radbio}$$

$$d/dt(I_{radbio}) = \sigma * B_{radbio} - (\mu + \delta) * I_{radbio} - c_{Iradb} * I_{radbio} + (1/X_{ai}) * I_{radabio} - (1/(X_b - X_{ai})) * I_{radbio}$$

;rdbio

$$d/dt(L_{rdbio}) = \beta * S_{dbio} * T_{Irb}/N_0 - (\mu + \gamma) * L_{rdbio} + (1/X_{ai}) * L_{rdbabio} - (1/(X_b - X_{ai})) * L_{rdbio}$$

$$d/dt(B_{rdbio}) = \gamma * L_{rdbio} - (\mu + \sigma) * B_{rdbio} - c_{Brdb} * B_{rdbio} + (1/X_{ai}) * B_{rdbabio} - (1/(X_b - X_{ai})) * B_{rdbio} - \tau_{ab} * B_{rdbio}$$

$$d/dt(I_{rdbio}) = \sigma * B_{rdbio} - (\mu + \delta) * I_{rdbio} - c_{Irbdb} * I_{rdbio} + (1/X_{ai}) * I_{rdbabio} - (1/(X_b - X_{ai})) * I_{rdbio} - \tau_{ab} * I_{rdbio}$$

$$N_{dbio} = S_{dbio} + L_{rodbio} + B_{rodbio} + I_{rodbio} + L_{radbio} + B_{radbio} + I_{radbio} + L_{rdbio} + B_{rdbio} + I_{rdbio}$$

;Intervention 2

;MSAT using atovaquone/proguanil

; N_{dvghi2}, N_{dvgi2}, N_{dvi2}, N_{doi2}

;Box N_{dvghi2}

$$\text{init } S_{dvghi2} = 0$$

```
;init Bdvghi2 = Bdvghi20
;init Ldvghi2 = Ldvghi20
;init Idvghi2 = Idvghi20
```

```
Ldvghi2 = Lrodvghi2+Lradvghi2+Lrbdvghi2
Bdvghi2 = Brodvghi2+Bradvghi2+Brbdvghi2
Idvghi2 = Irodvghi2+Iradvghi2+Irbdvghi2
```

```
init Lrodvghi2 = 0
init Lradvghi2 = 0
init Lrbdvghi2 = 0
init Brodvghi2 = 0
init Bradvghi2 = 0
init Brbdvghi2 = 0
init Irodvghi2 = 0
init Iradvghi2 = 0
init Irbdvghi2 = 0
```

```
d/dt(Sdvghi2) = - μ*Sdvghi2 - β*Sdvghi2*TIro/N0 + δ*Irodvghi2 + cLdv*Lrodvghi2 + cBdv*Brodvghi2 +
cLdv*Irodvghi2 - β*Sdvghi2*TIra/N0 + δ*Iradvghi2 + cLdv*Lradvghi2 + cBdv*Bradvghi2 +
cLdv*Iradvghi2 - β*Sdvghi2*TIrb/N0 + δ*Irbdvghi2 + cLdv*Lrbdvghi2 + cBdv*Brbdvghi2 + cLdv*Irbdvghi2
- (1/Xp)*Sdvghi2 + cLp*(Lrodvghi2 + Lradvghi2 + Lrbdvghi2) + clp*(Irodvghi2 + Iradvghi2 + Irbdvghi2)
```

```
;rodvghi2
```

```
d/dt(Lrodvghi2) = β*Sdvghi2*TIro/N0 - (μ+γ)*Lrodvghi2 - (1/Xp)*Lrodvghi2 - cLp*Lrodvghi2 -
cLdv*Lrodvghi2
d/dt(Brodvghi2) = γ*Lrodvghi2 - (μ+σ)*Brodvghi2 + τ2*Brodoio + τ2*Brodaio - (1/Xp)*Brodvghi2 -
cBdv*Brodvghi2 + τ2*Brodoi2 + τ2*Bradabi2 + τ2*Bradbi2
d/dt(Irodvghi2) = σ*Brodvghi2 - (μ+δ)*Irodvghi2 + τ2*Irodoio + τ2*Irodaio - (1/Xp)*Irodvghi2 -
clp*Irodvghi2 - cLdv*Irodvghi2 + τ2*Irodoi2 + τ2*Iradabi2 + τ2*Iradbi2
```

```
;radvghi2
```

```
d/dt(Lradvghi2) = β*Sdvghi2*TIra/N0 - (μ+γ)*Lradvghi2 - (1/Xp)*Lradvghi2 - cLp*Lradvghi2 -
cLdv*Lradvghi2
d/dt(Bradvghi2) = γ*Lradvghi2 - (μ+σ)*Bradvghi2 + τ2*Brodoio + τ2*Brodaio - (1/Xp)*Bradvghi2 -
cBdv*Bradvghi2 + τ2*Brodoi2 + τ2*Bradabi2 + τ2*Bradbi2
d/dt(Iradvghi2) = σ*Bradvghi2 - (μ+δ)*Iradvghi2 + τ2*Irodoio + τ2*Irodaio - (1/Xp)*Iradvghi2 -
clp*Iradvghi2 - cLdv*Iradvghi2 + τ2*Irodoi2 + τ2*Iradabi2 + τ2*Iradbi2
```

```
;rbdvghi2
```

```
d/dt(Lrbdvghi2) = β*Sdvghi2*TIrb/N0 - (μ+γ)*Lrbdvghi2 - (1/Xp)*Lrbdvghi2 - cLp*Lrbdvghi2 -
cLdv*Lrbdvghi2
d/dt(Brbdvghi2) = γ*Lrbdvghi2 - (μ+σ)*Brbdvghi2 + τ2*Brodoio + τ2*Brodaio - (1/Xp)*Brbdvghi2 -
cBdv*Brbdvghi2 + τ2*Brodoi2 + τ2*Bradabi2 + τ2*Bradbi2
```

$$d/dt(I_{rbdvgpi2}) = \sigma * B_{rbdvgpi2} - (\mu + \delta) * I_{rbdvgpi2} + \tau_2 * I_{rbdio} + \tau_2 * I_{rbdai} - (1/X_p) * I_{rbdvgpi2} - c_{lp} * I_{rbdvgpi2} - c_{ldvg} * I_{rbdvgpi2} + \tau_2 * I_{rbdoi2} + \tau_2 * I_{rbdabi2} + \tau_2 * I_{rdbi2}$$

$$N_{dvvgpi2} = S_{dvvgpi2} + L_{rodvgpi2} + B_{rodvgpi2} + I_{rodvgpi2} + L_{radvgpi2} + B_{radvgpi2} + I_{radvgpi2} + L_{rbdvgpi2} + B_{rbdvgpi2} + I_{rbdvgpi2}$$

;Box N_{dvvgi2}

init S_{dvvgi2} = 0

;init B_{dvvgi2} = B_{dvvgi2}0

;init L_{dvvgi2} = L_{dvvgi2}0

;init I_{dvvgi2} = I_{dvvgi2}0

$$L_{dvvgi2} = L_{rodvgi2} + L_{radvgi2} + L_{rbdvgi2}$$

$$B_{dvvgi2} = B_{rodvgi2} + B_{radvgi2} + B_{rbdvgi2}$$

$$I_{dvvgi2} = I_{rodvgi2} + I_{radvgi2} + I_{rbdvgi2}$$

init L_{rodvgi2} = 0

init L_{radvgi2} = 0

init L_{rbdvgi2} = 0

init B_{rodvgi2} = 0

init B_{radvgi2} = 0

init B_{rbdvgi2} = 0

init I_{rodvgi2} = 0

init I_{radvgi2} = 0

init I_{rbdvgi2} = 0

$$d/dt(S_{dvvgi2}) = -\mu * S_{dvvgi2} - \beta * S_{dvvgi2} * T_{Iro}/N_0 + \delta * I_{rodvgi2} - \beta * S_{dvvgi2} * T_{Ira}/N_0 + \delta * I_{radvgi2} - \beta * S_{dvvgi2} * T_{Irb}/N_0 + \delta * I_{rbdvgi2} + (1/X_p) * S_{dvvgpi2} - (1/(X_g - X_p)) * S_{dvvgi2} + c_{ldvg} * (L_{rodvgi2} + L_{radvgi2} + L_{rbdvgi2}) + c_{bdvg} * (B_{rodvgi2} + B_{radvgi2} + B_{rbdvgi2}) + c_{ldvg} * (I_{rodvgi2} + I_{radvgi2} + I_{rbdvgi2})$$

;rodvgi2

$$d/dt(L_{rodvgi2}) = \beta * S_{dvvgi2} * T_{Iro}/N_0 - (\mu + \gamma) * L_{rodvgi2} + (1/X_p) * L_{rodvgpi2} - (1/(X_g - X_p)) * L_{rodvgi2} - c_{ldvg} * L_{rodvgi2}$$

$$d/dt(B_{rodvgi2}) = \gamma * L_{rodvgi2} - (\mu + \sigma) * B_{rodvgi2} + (1/X_p) * B_{rodvgpi2} - (1/(X_g - X_p)) * B_{rodvgi2} - c_{bdvg} * B_{rodvgi2}$$

$$d/dt(I_{rodvgi2}) = \sigma * B_{rodvgi2} - (\mu + \delta) * I_{rodvgi2} + (1/X_p) * I_{rodvgpi2} - (1/(X_g - X_p)) * I_{rodvgi2} - c_{ldvg} * I_{rodvgi2}$$

;radvgi2

$$d/dt(L_{radvgi2}) = \beta * S_{dvvgi2} * T_{Ira}/N_0 - (\mu + \gamma) * L_{radvgi2} + (1/X_p) * L_{radvgpi2} - (1/(X_g - X_p)) * L_{radvgi2} - c_{ldvg} * L_{radvgi2}$$

$$\begin{aligned} d/dt(B_{\text{radvgi2}}) &= \gamma * L_{\text{radvgi2}} - (\mu + \sigma) * B_{\text{radvgi2}} + (1/X_p) * B_{\text{radvgpi2}} - (1/(X_g - X_p)) * B_{\text{radvgi2}} - \\ & c_{\text{Bdvg}} * B_{\text{radvgi2}} \\ d/dt(I_{\text{radvgi2}}) &= \sigma * B_{\text{radvgi2}} - (\mu + \delta) * I_{\text{radvgi2}} + (1/X_p) * I_{\text{radvgpi2}} - (1/(X_g - X_p)) * I_{\text{radvgi2}} - c_{\text{Idvg}} * I_{\text{radvgi2}} \end{aligned}$$

$$\begin{aligned} ;\text{rbdvgi2} \\ d/dt(L_{\text{rbdvgi2}}) &= \beta * S_{\text{dvgi2}} * T_{\text{Irb}}/N_0 - (\mu + \gamma) * L_{\text{rbdvgi2}} + (1/X_p) * L_{\text{rbdvgpi2}} - (1/(X_g - X_p)) * L_{\text{rbdvgi2}} - \\ & c_{\text{Ldvg}} * L_{\text{rbdvgi2}} \\ d/dt(B_{\text{rbdvgi2}}) &= \gamma * L_{\text{rbdvgi2}} - (\mu + \sigma) * B_{\text{rbdvgi2}} + (1/X_p) * B_{\text{rbdvgpi2}} - (1/(X_g - X_p)) * B_{\text{rbdvgi2}} - \\ & c_{\text{Bdvg}} * B_{\text{rbdvgi2}} \\ d/dt(I_{\text{rbdvgi2}}) &= \sigma * B_{\text{rbdvgi2}} - (\mu + \delta) * I_{\text{rbdvgi2}} + (1/X_p) * I_{\text{rbdvgpi2}} - (1/(X_g - X_p)) * I_{\text{rbdvgi2}} - c_{\text{Idvg}} * I_{\text{rbdvgi2}} \end{aligned}$$

$$N_{\text{dvgi2}} = S_{\text{dvgi2}} + L_{\text{rodvgi2}} + B_{\text{rodvgi2}} + I_{\text{rodvgi2}} + L_{\text{radvgi2}} + B_{\text{radvgi2}} + I_{\text{radvgi2}} + L_{\text{rbdvgi2}} + B_{\text{rbdvgi2}} + I_{\text{rbdvgi2}}$$

;Box N_{dvi2}

init S_{dvi2} = 0
;init B_{dvi2} = B_{dvi2}0
;init L_{dvi2} = L_{dvi2}0
;init I_{dvi2} = I_{dvi2}0

$$\begin{aligned} L_{\text{dvi2}} &= L_{\text{rodvi2}} + L_{\text{radvi2}} + L_{\text{rbdvi2}} \\ B_{\text{dvi2}} &= B_{\text{rodvi2}} + B_{\text{radvi2}} + B_{\text{rbdvi2}} \\ I_{\text{dvi2}} &= I_{\text{rodvi2}} + I_{\text{radvi2}} + I_{\text{rbdvi2}} \end{aligned}$$

init L_{rodvi2} = 0
init L_{radvi2} = 0
init L_{rbdvi2} = 0
init B_{rodvi2} = 0
init B_{radvi2} = 0
init B_{rbdvi2} = 0
init I_{rodvi2} = 0
init I_{radvi2} = 0
init I_{rbdvi2} = 0

$$\begin{aligned} d/dt(S_{\text{dvi2}}) &= -\mu * S_{\text{dvi2}} - \beta * S_{\text{dvi2}} * T_{\text{Iro}}/N_0 + \delta * I_{\text{rodvi2}} - \beta * S_{\text{dvi2}} * T_{\text{Ira}}/N_0 + \delta * I_{\text{radvi2}} - \\ & \beta * S_{\text{dvi2}} * T_{\text{Irb}}/N_0 + \delta * I_{\text{rbdvi2}} + (1/(X_g - X_p)) * S_{\text{dvgi2}} - (1/(X_v - X_g - X_p)) * S_{\text{dvi2}} + c_{\text{Ldv}} * (L_{\text{rodvi2}} + \\ & L_{\text{radvi2}} + L_{\text{rbdvi2}}) + c_{\text{Bdv}} * (B_{\text{rodvi2}} + B_{\text{radvi2}} + B_{\text{rbdvi2}}) + c_{\text{Idv}} * (I_{\text{rodvi2}} + I_{\text{radvi2}} + I_{\text{rbdvi2}}) \end{aligned}$$

;rodvi2

$$d/dt(L_{rodvi2}) = \beta * S_{dvi2} * T_{Iro}/N_0 - (\mu + \gamma) * L_{rodvi2} - c_{Ldv} * L_{rodvi2} + (1/(X_g - X_p)) * L_{rodvgi2} - (1/(X_v - X_g - X_p)) * L_{rodvi2}$$

$$d/dt(B_{rodvi2}) = \gamma * L_{rodvi2} - (\mu + \sigma) * B_{rodvi2} - c_{Bdv} * B_{rodvi2} + (1/(X_g - X_p)) * B_{rodvgi2} - (1/(X_v - X_g - X_p)) * B_{rodvi2}$$

$$d/dt(I_{rodvi2}) = \sigma * B_{rodvi2} - (\mu + \delta) * I_{rodvi2} - c_{Idv} * I_{rodvi2} + (1/(X_g - X_p)) * I_{rodvgi2} - (1/(X_v - X_g - X_p)) * I_{rodvi2}$$

;radvi2

$$d/dt(L_{radvi2}) = \beta * S_{dvi2} * T_{Ira}/N_0 - (\mu + \gamma) * L_{radvi2} - c_{Ldv} * L_{radvi2} + (1/(X_g - X_p)) * L_{radvgi2} - (1/(X_v - X_g - X_p)) * L_{radvi2}$$

$$d/dt(B_{radvi2}) = \gamma * L_{radvi2} - (\mu + \sigma) * B_{radvi2} - c_{Bdv} * B_{radvi2} + (1/(X_g - X_p)) * B_{radvgi2} - (1/(X_v - X_g - X_p)) * B_{radvi2}$$

$$d/dt(I_{radvi2}) = \sigma * B_{radvi2} - (\mu + \delta) * I_{radvi2} - c_{Idv} * I_{radvi2} + (1/(X_g - X_p)) * I_{radvgi2} - (1/(X_v - X_g - X_p)) * I_{radvi2}$$

;rbdvi2

$$d/dt(L_{rbdvi2}) = \beta * S_{dvi2} * T_{Irb}/N_0 - (\mu + \gamma) * L_{rbdvi2} - c_{Ldv} * L_{rbdvi2} + (1/(X_g - X_p)) * L_{rbdvgi2} - (1/(X_v - X_g - X_p)) * L_{rbdvi2}$$

$$d/dt(B_{rbdvi2}) = \gamma * L_{rbdvi2} - (\mu + \sigma) * B_{rbdvi2} - c_{Bdv} * B_{rbdvi2} + (1/(X_g - X_p)) * B_{rbdvgi2} - (1/(X_v - X_g - X_p)) * B_{rbdvi2}$$

$$d/dt(I_{rbdvi2}) = \sigma * B_{rbdvi2} - (\mu + \delta) * I_{rbdvi2} - c_{Idv} * I_{rbdvi2} + (1/(X_g - X_p)) * I_{rbdvgi2} - (1/(X_v - X_g - X_p)) * I_{rbdvi2}$$

$$N_{dvi2} = S_{dvi2} + L_{rodvi2} + B_{rodvi2} + I_{rodvi2} + L_{radvi2} + B_{radvi2} + I_{radvi2} + L_{rbdvi2} + B_{rbdvi2} + I_{rbdvi2}$$

;Box Ndoi2

$$\text{init } S_{doi2} = 0$$

$$I_{doi2} = 0$$

$$L_{doi2} = L_{rodoi2} + L_{radoi2} + L_{rbdoi2}$$

$$B_{doi2} = B_{rodoi2} + B_{radoi2} + B_{rbdoi2}$$

$$I_{doi2} = I_{rodoi2} + I_{radoi2} + I_{rbdoi2}$$

$$\text{init } L_{rodoi2} = 0$$

$$\text{init } L_{radoi2} = 0$$

$$\text{init } L_{rbdoi2} = 0$$

$$\text{init } B_{rodoi2} = 0$$

$$\text{init } B_{radoi2} = 0$$

$$\text{init } B_{rbdoi2} = 0$$

$$\text{init } I_{rodoi2} = 0$$

$$\text{init } I_{radoi2} = 0$$

$$\text{init } I_{rbdoi2} = 0$$

$$\begin{aligned} d/dt(S_{doi2}) = & -\mu * S_{doi2} - \beta * S_{doi2} * T_{Iro}/N_0 + \delta * I_{rodoi2} + c_{Brodo} * B_{rodoi2} + c_{Irodo} * I_{rodoi2} - \\ & \beta * S_{doi2} * T_{Ira}/N_0 + \delta * I_{radoi2} + c_{Brado} * B_{radoi2} + c_{Irado} * I_{radoi2} - \beta * S_{doi2} * T_{Irb}/N_0 + \delta * I_{rbdoi2} + \\ & c_{Brbdo} * B_{rbdoi2} + c_{Irbdo} * I_{rbdoi2} + (1/(X_v - X_g - X_p)) * S_{dvi2} + 1/(X_b - X_{ai}) * S_{dbi2} + (1/X_{ao}) * S_{dai2} \end{aligned}$$

;rodoi2

$$\begin{aligned} d/dt(L_{rodoi2}) = & \beta * S_{doi2} * T_{Iro}/N_0 - (\mu + \gamma) * L_{rodoi2} + (1/(X_v - X_g - X_p)) * L_{rodvi2} + 1/(X_b - X_{ai}) * L_{rodbi2} \\ & + (1/X_{ao}) * L_{rodai2} \end{aligned}$$

$$\begin{aligned} d/dt(B_{rodoi2}) = & \gamma * L_{rodoi2} - (\mu + \sigma) * B_{rodoi2} - c_{Brodo} * B_{rodoi2} + (1/(X_v - X_g - X_p)) * B_{rodvi2} - \\ & \tau_{abi2} * B_{rodoi2} + 1/(X_b - X_{ai}) * B_{rodbi2} - \tau_{ai2} * B_{rodoi2} + (1/X_{ao}) * B_{rodai2} - \tau_2 * B_{rodoi2} \end{aligned}$$

$$\begin{aligned} d/dt(I_{rodoi2}) = & \sigma * B_{rodoi2} - (\mu + \delta) * I_{rodoi2} - c_{Irodo} * I_{rodoi2} + (1/(X_v - X_g - X_p)) * I_{rodvi2} - \tau_{abi2} * I_{rodoi2} + \\ & 1/(X_b - X_{ai}) * I_{rodbi2} - \tau_{ai2} * I_{rodoi2} + (1/X_{ao}) * I_{rodai2} - \tau_2 * I_{rodoi2} \end{aligned}$$

;radoi2

$$\begin{aligned} d/dt(L_{radoi2}) = & \beta * S_{doi2} * T_{Ira}/N_0 - (\mu + \gamma) * L_{radoi2} + (1/(X_v - X_g - X_p)) * L_{radvi2} + 1/(X_b - X_{ai}) * L_{radbi2} + \\ & (1/X_{ao}) * L_{radai2} \end{aligned}$$

$$\begin{aligned} d/dt(B_{radoi2}) = & \gamma * L_{radoi2} - (\mu + \sigma) * B_{radoi2} - c_{Brado} * B_{radoi2} + (1/(X_v - X_g - X_p)) * B_{radvi2} - \tau_{abi2} * B_{radoi2} \\ & + 1/(X_b - X_{ai}) * B_{radbi2} - \tau_{ai2} * B_{radoi2} + (1/X_{ao}) * B_{radai2} - \tau_2 * B_{radoi2} \end{aligned}$$

$$\begin{aligned} d/dt(I_{radoi2}) = & \sigma * B_{radoi2} - (\mu + \delta) * I_{radoi2} - c_{Irado} * I_{radoi2} + (1/(X_v - X_g - X_p)) * I_{radvi2} - \tau_{abi2} * I_{radoi2} + \\ & 1/(X_b - X_{ai}) * I_{radbi2} - \tau_{ai2} * I_{radoi2} + (1/X_{ao}) * I_{radai2} - \tau_2 * I_{radoi2} \end{aligned}$$

;rbdoi2

$$\begin{aligned} d/dt(L_{rbdoi2}) = & \beta * S_{doi2} * T_{Irb}/N_0 - (\mu + \gamma) * L_{rbdoi2} + (1/(X_v - X_g - X_p)) * L_{rbdvi2} + 1/(X_b - X_{ai}) * L_{rdbbi2} \\ & + (1/X_{ao}) * L_{rbdai2} \end{aligned}$$

$$\begin{aligned} d/dt(B_{rbdoi2}) = & \gamma * L_{rbdoi2} - (\mu + \sigma) * B_{rbdoi2} - c_{Brbdo} * B_{rbdoi2} + (1/(X_v - X_g - X_p)) * B_{rbdvi2} - \\ & \tau_{abi2} * B_{rbdoi2} + 1/(X_b - X_{ai}) * B_{rdbbi2} - \tau_{ai2} * B_{rbdoi2} + (1/X_{ao}) * B_{rbdai2} - \tau_2 * B_{rbdoi2} \end{aligned}$$

$$\begin{aligned} d/dt(I_{rbdoi2}) = & \sigma * B_{rbdoi2} - (\mu + \delta) * I_{rbdoi2} - c_{Irbdo} * I_{rbdoi2} + (1/(X_v - X_g - X_p)) * I_{rbdvi2} - \tau_{abi2} * I_{rbdoi2} + \\ & 1/(X_b - X_{ai}) * I_{rdbbi2} - \tau_{ai2} * I_{rbdoi2} + (1/X_{ao}) * I_{rbdai2} - \tau_2 * I_{rbdoi2} \end{aligned}$$

$$N_{doi2} = S_{doi2} + L_{rodoi2} + B_{rodoi2} + I_{rodoi2} + L_{radoi2} + B_{radoi2} + I_{radoi2} + L_{rbdoi2} + B_{rbdoi2} + I_{rbdoi2}$$

;Treatment with artesunate in intervention 2

; N_{dai2}

;Box N_{dai2}

init S_{dai2} = 0

;init B_{dai2} = B_{dai2}0

;init L_{dai2} = L_{dai2}0

;init I_{dai2} = I_{dai2}0

L_{dai2} = L_{rodai2} + L_{radai2} + L_{rbdai2}

B_{dai2} = B_{rodai2} + B_{radai2} + B_{rbdai2}

$$I_{dai2} = I_{rodai2} + I_{radai2} + I_{rbdai2}$$

$$\text{init } L_{rodai2} = 0$$

$$\text{init } L_{radai2} = 0$$

$$\text{init } L_{rbdai2} = 0$$

$$\text{init } B_{rodai2} = 0$$

$$\text{init } B_{radai2} = 0$$

$$\text{init } B_{rbdai2} = 0$$

$$\text{init } I_{rodai2} = 0$$

$$\text{init } I_{radai2} = 0$$

$$\text{init } I_{rbdai2} = 0$$

$$\begin{aligned} d/dt(S_{dai2}) = & -\mu * S_{dai2} - \beta * S_{dai2} * T_{Iro}/N_0 + \delta * I_{rodai2} + c_{Broda} * B_{rodai2} + c_{Iroda} * I_{rodai2} - \\ & \beta * S_{dai2} * T_{Ira}/N_0 + \delta * I_{radai2} + c_{Brada} * B_{radai2} + c_{Irada} * I_{radai2} - \beta * S_{dai2} * T_{Irb}/N_0 + \delta * I_{rbdai2} + \\ & c_{Brbda} * B_{rbdai2} + c_{Irbda} * I_{rbdai2} - (1/X_{ao}) * S_{dai2} \end{aligned}$$

;rodai2

$$d/dt(L_{rodai2}) = \beta * S_{dai2} * T_{Iro}/N_0 - (\mu + \gamma) * L_{rodai2} - (1/X_{ao}) * L_{rodai2}$$

$$d/dt(B_{rodai2}) = \gamma * L_{rodai2} - (\mu + \sigma) * B_{rodai2} - c_{Broda} * B_{rodai2} + \tau_{ai2} * B_{rodoi2} - (1/X_{ao}) * B_{rodai2}$$

$$d/dt(I_{rodai2}) = \sigma * B_{rodai2} - (\mu + \delta) * I_{rodai2} - c_{Iroda} * I_{rodai2} + \tau_{ai2} * I_{rodoi2} - (1/X_{ao}) * I_{rodai2}$$

;radai2

$$d/dt(L_{radai2}) = \beta * S_{dai2} * T_{Ira}/N_0 - (\mu + \gamma) * L_{radai2} - (1/X_{ao}) * L_{radai2}$$

$$d/dt(B_{radai2}) = \gamma * L_{radai2} - (\mu + \sigma) * B_{radai2} - c_{Brada} * B_{radai2} + \tau_{ai2} * B_{radoi2} - (1/X_{ao}) * B_{radai2}$$

$$d/dt(I_{radai2}) = \sigma * B_{radai2} - (\mu + \delta) * I_{radai2} - c_{Irada} * I_{radai2} + \tau_{ai2} * I_{radoi2} - (1/X_{ao}) * I_{radai2}$$

;rbdai2

$$d/dt(L_{rbdai2}) = \beta * S_{dai2} * T_{Irb}/N_0 - (\mu + \gamma) * L_{rbdai2} - (1/X_{ao}) * L_{rbdai2}$$

$$d/dt(B_{rbdai2}) = \gamma * L_{rbdai2} - (\mu + \sigma) * B_{rbdai2} - c_{Brbda} * B_{rbdai2} + \tau_{ai2} * B_{rbdoi2} - (1/X_{ao}) * B_{rbdai2}$$

$$d/dt(I_{rbdai2}) = \sigma * B_{rbdai2} - (\mu + \delta) * I_{rbdai2} - c_{Irbda} * I_{rbdai2} + \tau_{ai2} * I_{rbdoi2} - (1/X_{ao}) * I_{rbdai2}$$

$$N_{dai2} = S_{dai2} + L_{rodai2} + B_{rodai2} + I_{rodai2} + L_{radai2} + B_{radai2} + I_{radai2} + L_{rbdai2} + B_{rbdai2} + I_{rbdai2}$$

;Treatment with ACT (artemesunate + piperazine) during intervention 2

$$; N_{dabi2}, N_{dbi2}$$

;Box N_{dabi2}

$$\text{init } S_{dabi2} = 0$$

$$; \text{init } B_{dabi2} = B_{dabi2}0$$

$$; \text{init } L_{dabi2} = L_{dabi2}0$$

$$; \text{init } I_{dabi2} = I_{dabi2}0$$

$$L_{dabi2} = L_{rodabi2} + L_{radabi2} + L_{rbdabi2}$$

$$B_{dabi2} = B_{rodabi2} + B_{radabi2} + B_{rbdabi2}$$

$$I_{dabi2} = I_{rodabi2} + I_{radabi2} + I_{rbdabi2}$$

```

init Lrodabi2 = 0
init Lradabi2 = 0
init Lrdbabi2 = 0
init Brodabi2 = 0
init Bradabi2 = 0
init Brdbabi2 = 0
init Irodabi2 = 0
init Iradabi2 = 0
init Irdbabi2 = 0

```

$$\begin{aligned}
 d/dt(S_{dabi2}) = & -\mu * S_{dabi2} - \beta * S_{dabi2} * T_{Iro}/N_0 + \delta * I_{rodabi2} + c_{Brodab} * B_{rodabi2} + c_{Irodab} * I_{rodabi2} - \\
 & \beta * S_{dabi2} * T_{Ira}/N_0 + \delta * I_{radabi2} + c_{Bradab} * B_{radabi2} + c_{Iradab} * I_{radabi2} - \beta * S_{dabi2} * T_{Irb}/N_0 + \delta * I_{rdbabi2} + \\
 & c_{Brbdab} * B_{rdbabi2} + c_{Irbdb} * I_{rdbabi2} - (1/X_{ai}) * S_{dabi2}
 \end{aligned}$$

```

;rodabi2

```

$$\begin{aligned}
 d/dt(L_{rodabi2}) &= \beta * S_{dabi2} * T_{Iro}/N_0 - (\mu + \gamma) * L_{rodabi2} - (1/X_{ai}) * L_{rodabi2} \\
 d/dt(B_{rodabi2}) &= \gamma * L_{rodabi2} - (\mu + \sigma) * B_{rodabi2} - c_{Brodab} * B_{rodabi2} - (1/X_{ai}) * B_{rodabi2} + \tau_{abi2} * B_{rodoi2} - \\
 & \tau_2 * B_{rodabi2} \\
 d/dt(I_{rodabi2}) &= \sigma * B_{rodabi2} - (\mu + \delta) * I_{rodabi2} - c_{Irodab} * I_{rodabi2} - (1/X_{ai}) * I_{rodabi2} + \tau_{abi2} * I_{rodoi2} - \\
 & \tau_2 * I_{rodabi2}
 \end{aligned}$$

```

;radabi2

```

$$\begin{aligned}
 d/dt(L_{radabi2}) &= \beta * S_{dabi2} * T_{Ira}/N_0 - (\mu + \gamma) * L_{radabi2} - (1/X_{ai}) * L_{radabi2} \\
 d/dt(B_{radabi2}) &= \gamma * L_{radabi2} - (\mu + \sigma) * B_{radabi2} - c_{Bradab} * B_{radabi2} - (1/X_{ai}) * B_{radabi2} + \tau_{abi2} * B_{radoi2} - \\
 & \tau_2 * B_{radabi2} \\
 d/dt(I_{radabi2}) &= \sigma * B_{radabi2} - (\mu + \delta) * I_{radabi2} - c_{Iradab} * I_{radabi2} - (1/X_{ai}) * I_{radabi2} + \tau_{abi2} * I_{radoi2} - \\
 & \tau_2 * I_{radabi2}
 \end{aligned}$$

```

;rdbabi2

```

$$\begin{aligned}
 d/dt(L_{rdbabi2}) &= \beta * S_{dabi2} * T_{Irb}/N_0 - (\mu + \gamma) * L_{rdbabi2} - (1/X_{ai}) * L_{rdbabi2} \\
 d/dt(B_{rdbabi2}) &= \gamma * L_{rdbabi2} - (\mu + \sigma) * B_{rdbabi2} - c_{Brbdab} * B_{rdbabi2} + \tau_{ab} * B_{rdbi2} - (1/X_{ai}) * B_{rdbabi2} + \\
 & \tau_{abi2} * B_{rdbdoi2} - \tau_2 * B_{rdbabi2} \\
 d/dt(I_{rdbabi2}) &= \sigma * B_{rdbabi2} - (\mu + \delta) * I_{rdbabi2} - c_{Irbdb} * I_{rdbabi2} + \tau_{ab} * I_{rdbi2} - (1/X_{ai}) * I_{rdbabi2} + \\
 & \tau_{abi2} * I_{rdbdoi2} - \tau_2 * I_{rdbabi2}
 \end{aligned}$$

$$N_{dabi2} = S_{dabi2} + L_{rodabi2} + B_{rodabi2} + I_{rodabi2} + L_{radabi2} + B_{radabi2} + I_{radabi2} + L_{rdbabi2} + B_{rdbabi2} + I_{rdbabi2}$$

```

;Box Ndabi2

```

```

init Sdabi2 = 0

```

```
;init Bdbi2 = Bdbi20
;init Ldbi2 = Ldbi20
;init Idbi2 = Idbi20
```

```
Ldbi2 = Lrodbi2+Lradbi2+Lrdbi2
Bdbi2 = Brodbi2+Bradbi2+Brdbi2
Idbi2 = Irodbi2+Iradbi2+Irdbi2
```

```
init Lrodbi2 = 0
init Lradbi2 = 0
init Lrdbi2 = 0
init Brodbi2 = 0
init Bradbi2 = 0
init Brdbi2 = 0
init Irodbi2 = 0
init Iradbi2 = 0
init Irdbi2 = 0
```

```
d/dt(Sdbi2) = - μ*Sdbi2 - β*Sdbi2*TIro/N0 + δ*Irodbi2 + cBrodb*Brodbi2 + cIrodb*Irodbi2 -
β*Sdbi2*TIra/N0 + δ*Iradbi2 + cBrad*Bradbi2 + cIrad*Iradbi2 - β*Sdbi2*TIrb/N0 + δ*Irdbi2 +
cBrdb*Brdbi2 + cIrdb*Irdbi2 + (1/Xai*Sdabi2) - 1/(Xb-Xai)*Sdbi2
```

```
;rodbi2
d/dt(Lrodbi2) = β*Sdbi2*TIro/N0 - (μ+γ)*Lrodbi2 + (1/Xai)*Lrodabi2 - 1/(Xb-Xai)*Lrodbi2
d/dt(Brodbi2) = γ*Lrodbi2 - (μ+σ)*Brodbi2 - cBrodb*Brodbi2 + (1/Xai)*Brodabi2 - 1/(Xb-Xai)*Brodbi2 -
τ2*Brodbi2
d/dt(Irodbi2) = σ*Brodbi2 - (μ+δ)*Irodbi2 - cIrodb*Irodbi2 + (1/Xai)*Irodabi2 - 1/(Xb-Xai)*Irodbi2 -
τ2*Irodbi2
```

```
;radbi2
d/dt(Lradbi2) = β*Sdbi2*TIra/N0 - (μ+γ)*Lradbi2 + (1/Xai)*Lradabi2 - 1/(Xb-Xai)*Lradbi2
d/dt(Bradbi2) = γ*Lradbi2 - (μ+σ)*Bradbi2 - cBrad*Bradbi2 + (1/Xai)*Bradabi2 - 1/(Xb-Xai)*Bradbi2 -
τ2*Bradbi2
d/dt(Iradbi2) = σ*Bradbi2 - (μ+δ)*Iradbi2 - cIrad*Iradbi2 + (1/Xai)*Iradabi2 - 1/(Xb-Xai)*Iradbi2 -
τ2*Iradbi2
```

```
;rdbi2
d/dt(Lrdbi2) = β*Sdbi2*TIrb/N0 - (μ+γ)*Lrdbi2 + (1/Xai)*Lrdbabi2 - 1/(Xb-Xai)*Lrdbi2
d/dt(Brdbi2) = γ*Lrdbi2 - (μ+σ)*Brdbi2 - cBrdb*Brdbi2 - τab*Brdbi2 + (1/Xai)*Brdbabi2 -
1/(Xb-Xai)*Brdbi2 - τ2*Brdbi2
d/dt(Irdbi2) = σ*Brdbi2 - (μ+δ)*Irdbi2 - cIrdb*Irdbi2 - τab*Irdbi2 + (1/Xai)*Irdbabi2 - 1/(Xb-
Xai)*Irdbi2 - τ2*Irdbi2
```

$$N_{dbi2} = S_{dbi2} + L_{rodbi2} + B_{rodbi2} + I_{rodbi2} + L_{radbi2} + B_{radbi2} + I_{radbi2} + L_{rdbbi2} + B_{rdbbi2} + I_{rdbbi2}$$

;CHECKS AND TOTALS

$$\text{check} = N_0 - (N_{doio} + N_{daio} + N_{dabi1} + N_{dbi1} + N_{doi1} + N_{dabio} + N_{dbio} + N_{dai1a} + N_{dabi1a} + N_{dbi1a} + N_{dvghi2} + N_{dvgi2} + N_{dvi2} + N_{doi2} + N_{dai2} + N_{dabi2} + N_{dbi2})$$

$$T_N = N_{doio} + N_{daio} + N_{dabi1} + N_{dbi1} + N_{doi1} + N_{dabio} + N_{dbio} + N_{dai1a} + N_{dabi1a} + N_{dbi1a} + N_{dvghi2} + N_{dvgi2} + N_{dvi2} + N_{doi2} + N_{dai2} + N_{dabi2} + N_{dbi2}$$

$$N_{ra} = (I_{radoio} + I_{radaio} + I_{radabi1} + I_{radbi1} + I_{radoi1} + I_{radabio} + I_{radbio} + I_{radai1a} + I_{radabi1a} + I_{radbi1a}) + (B_{radoio} + B_{radaio} + B_{radabi1} + B_{radbi1} + B_{radoi1} + B_{radabio} + B_{radbio} + B_{radai1a} + B_{radabi1a} + B_{radbi1a}) + (I_{radvgpi2} + I_{radvgi2} + I_{radvi2} + I_{radoi2} + I_{radai2} + I_{radabi2} + I_{radbi2}) + (B_{radvgpi2} + B_{radvgi2} + B_{radvi2} + B_{radoi2} + B_{radai2} + B_{radabi2} + B_{radbi2})$$

$$N_{rb} = (I_{rbdoio} + I_{rbdaio} + I_{rbdabi1} + I_{rdbi1} + I_{rbdoi1} + I_{rbdabio} + I_{rdbbio} + I_{rbdai1a} + I_{rbdabi1a} + I_{rdbbi1a}) + (B_{rbdai1a} + B_{rbdoio} + B_{rbdaio} + B_{rbdabi1} + B_{rdbi1} + B_{rbdoi1} + B_{rbdabio} + B_{rdbbio} + B_{rbdai1a} + B_{rbdabi1a} + B_{rdbbi1a}) + (I_{rbdvgpi2} + I_{rbdvgi2} + I_{rbdvi2} + I_{rbdoi2} + I_{rbdai2} + I_{rbdabi2} + I_{rdbi2}) + (B_{rbdai2} + B_{rbdvgpi2} + B_{rbdvgi2} + B_{rbdvi2} + B_{rbdoi2} + B_{rbdai2} + B_{rbdabi2} + B_{rdbi2})$$

$$T_{IB} = (I_{doio} + I_{daio} + I_{dabi1} + I_{dbi1} + I_{doi1} + I_{dabio} + I_{dbio} + I_{dai1a} + I_{dabi1a} + I_{dbi1a}) + (B_{doio} + B_{daio} + B_{dabi1} + B_{dbi1} + B_{doi1} + B_{dabio} + B_{dbio} + B_{dai1a} + B_{dabi1a} + B_{dbi1a}) + (I_{dvghi2} + I_{dvgi2} + I_{dvi2} + I_{doi2} + I_{dai2} + I_{dabi2} + I_{dbi2}) + (B_{dvghi2} + B_{dvgi2} + B_{dvgi2} + B_{doi2} + B_{dai2} + B_{dabi2} + B_{dbi2})$$

$$\beta = \text{amp} * ((1 - \beta_n) * \beta_n) * \cos(2 * 3.14159 * (\text{time} - \pi)) + ((1 - \beta_n) * \beta_n)$$

$\pi = 0.5$; peak time for malaria transmission with 0=January

$\text{amp} = 0.67$; amplitude of seasonal variation with value 0 to 1

$$q = (\mu + \gamma) * (\mu + \sigma) * (\mu + \delta) / (\sigma * \gamma)$$

$\text{cost}_a = 0$; cost of resistance to artesunate

$\text{cost}_b = 0$; cost of resistance to piperaquine

$$T_{Iro} = I_{rodoio} + I_{rodaio} + I_{rodabi1} + I_{rodbi1} + I_{rodoi1} + I_{rodabio} + I_{rodbio} + I_{rodai1a} + I_{rodabi1a} + I_{rodbi1a} + I_{rodvgpi2} + I_{rodvgi2} + I_{rodvi2} + I_{rodoi2} + I_{rodai2} + I_{rodabi2} + I_{rodbi2}$$

$$T_{Ira} = (1 - \text{cost}_a) * (I_{radoio} + I_{radaio} + I_{radabi1} + I_{radbi1} + I_{radoi1} + I_{radabio} + I_{radbio} + I_{radai1a} + I_{radabi1a} + I_{radbi1a} + I_{radvgpi2} + I_{radvgi2} + I_{radvi2} + I_{radoi2} + I_{radai2} + I_{radabi2} + I_{radbi2})$$

$$T_{Irb} = (1 - \text{cost}_b) * (I_{rbdoio} + I_{rbdaio} + I_{rbdabi1} + I_{rdbi1} + I_{rbdoi1} + I_{rbdabio} + I_{rdbbio} + I_{rbdai1a} + I_{rbdabi1a} + I_{rdbbi1a} + I_{rbdvgpi2} + I_{rbdvgi2} + I_{rbdvi2} + I_{rbdoi2} + I_{rbdai2} + I_{rbdabi2} + I_{rdbi2})$$

$$T_I = T_{Iro} + T_{Ira} + T_{Irb}$$

$$T_{ilcum}' = \tau_1 * (N_{doio} + N_{daio})$$

$$T_{ilcumcov} = (100 * T_{ilcum}) / T_N$$

$$\text{init } T_{ilcum} = 0$$

$\text{perc}_{ra} = 100 \cdot N_{ra} / T_{IB}$; percent of blood stage infections resistant to artesunate
 $\text{perc}_{rb} = 100 \cdot N_{rb} / T_{IB}$; percent of blood stage infections resistant to piperaquine
 $\text{perc}_{IB} = 100 \cdot T_{IB} / T_N$; percent of infections that are blood stage

$\text{init}(IT_{roda})=0$

$IT_{roda}' = B_{rodaio} + B_{rodaila} + L_{rodaio} + L_{rodaila} + I_{rodaio} + I_{rodaila} + B_{rodabio} + B_{rodabil} + B_{rodabila} +$
 $L_{rodabio} + L_{rodabil} + L_{rodabila} + I_{rodabio} + I_{rodabil} + I_{rodabila} + B_{rodabi2} + L_{rodabi2} + I_{rodabi2} + B_{rodai2} +$
 $L_{rodai2} + I_{rodai2}$

$\text{init}(IT_{rodb})=0$

$IT_{rodb}' = B_{rodbio} + B_{rodbil} + B_{rodbila} + L_{rodbio} + L_{rodbil} + L_{rodbila} + I_{rodbio} + I_{rodbil} + I_{rodbila} + B_{rodabio} +$
 $B_{rodabil} + B_{rodabila} + L_{rodabio} + L_{rodabil} + L_{rodabila} + I_{rodabio} + I_{rodabil} + I_{rodabila} + B_{rodabi2} +$
 $L_{rodabi2} + I_{rodabi2} + B_{rodbi2} + L_{rodbi2} + I_{rodbi2}$

$\text{init}(IT_{rada})=0$

$IT_{rada}' = B_{radaio} + B_{radaila} + L_{radaio} + L_{radaila} + I_{radaio} + I_{radaila} + B_{radabio} + B_{radabil} + B_{radabila} + L_{radabio} +$
 $L_{radabil} + L_{radabila} + I_{radabio} + I_{radabil} + I_{radabila} + B_{radabi2} + L_{radabi2} + I_{radabi2} + B_{radai2} + L_{radai2} +$
 I_{radai2}

$\text{init}(IT_{radb})=0$

$IT_{radb}' = B_{radbio} + B_{radbil} + B_{radbila} + L_{radbio} + L_{radbil} + L_{radbila} + I_{radbio} + I_{radbil} + I_{radbila} + B_{radabio} +$
 $B_{radabil} + B_{radabila} + L_{radabio} + L_{radabil} + L_{radabila} + I_{radabio} + I_{radabil} + I_{radabila} + B_{radabi2} + L_{radabi2} +$
 $I_{radabi2} + B_{radbi2} + L_{radbi2} + I_{radbi2}$

$\text{init}(IT_{rbda})=0$

$IT_{rbda}' = B_{rbdaio} + B_{rbdaila} + L_{rbdaio} + L_{rbdaila} + I_{rbdaio} + I_{rbdaila} + B_{rbdabio} + B_{rbdabil} + B_{rbdabila} +$
 $L_{rbdabio} + L_{rbdabil} + L_{rbdabila} + I_{rbdabio} + I_{rbdabil} + I_{rbdabila} + B_{rbdabi2} + L_{rbdabi2} + I_{rbdabi2} + B_{rbdai2} +$
 $L_{rbdai2} + I_{rbdai2}$

$\text{init}(IT_{rddb})=0$

$IT_{rddb}' = B_{rddbio} + B_{rddbil} + B_{rddbila} + L_{rddbio} + L_{rddbil} + L_{rddbila} + I_{rddbio} + I_{rddbil} + I_{rddbila} + B_{rddbabio} +$
 $B_{rddbabil} + B_{rddbabila} + L_{rddbabio} + L_{rddbabil} + L_{rddbabila} + I_{rddbabio} + I_{rddbabil} + I_{rddbabila} + B_{rddbabi2} + L_{rddbabi2} +$
 $I_{rddbabi2} + B_{rddbbi2} + L_{rddbbi2} + I_{rddbbi2}$

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