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Investigating the use of routine malaria surveillance data to evaluate the effectiveness of pyrethroid vector control interventions

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Declaration of Own Work

All work presented in this thesis was undertaken by the candidate under the supervision of Dr Thomas S Churcher and Prof Azra C Ghani with the few minor exceptions listed below. All sources used in the thesis have been cited appropriately.

- In Chapter 5, to estimate the proportions of mosquito bites taken outside during the evening, I used the results of a previous meta-analysis carried out by Janetta Skarp (BSc project, Imperial College London, submitted June 2006). “Investigating the impact of *Anopheles* mosquitoes’ biting time on the efficacy of bednets against malaria”.
- I wrote the code for a deterministic version of Imperial College malaria transmission model (including my additions to the model) in a specific programming language for a package for the R statistical software called “Odin”. I undertook all work bar the package development, this was done by Rich Fitzjohn at Imperial College London. (The package can be accessed at: https://github.com/mrc-ide/odin as of 25/09/2018).

The work in Chapter 3 has been published as the following paper, the candidate conducted the analysis and wrote the first draft:

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Abstract

Malaria control is increasingly being tailored to local needs, this is especially necessary in humanitarian settings where resources are poor. Pyrethroids are the most widely used class of insecticides for mosquito control. Using them effectively requires measuring their epidemiological impact and understanding how this is reduced by the emergence of pyrethroid resistance in mosquitoes. In the first two chapters of this thesis, I consider how we could measure the impact of pyrethroid vector control tools using the prevalence of infection in pregnant women, a potentially cheaper and more reliable alternative to clinical incidence or prevalence in children. In Chapter 2, I fit a Bayesian regression model to show that the malaria burden measured at hospitals near internally displaced populations is higher than the regional average. In Chapter 3, I demonstrate that the prevalence of infection in pregnant women and the clinical incidence in children change together over time. Collecting routine data from pregnant women seems promising as a measure for assessing malaria burden trends. In the second half of the thesis I explore the impact of different pyrethroid-based interventions in a variety of contexts. In Chapter 4, I expand an existing mathematical model of malaria transmission to predict the impact of distributing emanators (a type of spatial repellent) where insecticidal nets are not commonplace. In Chapter 5, I establish how outdoor evening biting could sustain transmission in places where insecticidal nets are used but residual transmission remains. In Chapter 6, I investigate a sub-lethal effect of pyrethroid bed nets that I call temporary feeding inhibition, mosquitoes that are exposed to pyrethroids do not die but are unable to bite humans for a short while afterwards. Together the work shows how statistical and transmission dynamics models can be used to understand the efficacy of vector control interventions and measure their effectiveness in the field.

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Acronyms and Abbreviations

EIR – Entomological Inoculation Rate

WHO – World Health Organisation

VSA – Variant surface antigens

PfEMP-1 – *Plasmodium falciparum* erythrocyte membrane protein 1

RDT – Rapid diagnostic test

PCR – Polymerase chain reaction

LLINs – Long-lasting insecticidal nets

ITNs – Insecticide treated nets

PBO – Piperonyl Butoxide

IRS – Indoor residual spraying

TFI – Temporary feeding interruption

RCT – Randomised controlled trial

MDA – Mass drug administration

SMC – Seasonal malaria chemoprevention

SP – Sulphadoxine Pyrimethamine

AQ – Amodiaquine

IPT – Intermittent preventative treatment

MSP – Merozoite surface protein

CSA – Chondroitin sulphate A

LBW – Low birth weight

IUGR – Intrauterine growth restriction

UN – United Nations

DRC – Democratic Republic of the Congo

MSF – Médicines Sans Frontières

IDPs – Internally displaced peoples

DHS – Demographic and Health Surveys

ANC – Ante-natal clinic/care

DIC – Deviance information criterion

MCMC – Markov Chain Monte Carlo

ACT – Artemisinin combination therapy

IST – Intermittent screening and treatment

VAR – Vector autoregression model

AR – Autoregressive model

AIC – Akaike information criterion

DLNM – Distributed lag non-linear model

1 Introduction

Malaria is a huge burden on global health, featuring in the "big 3" infectious diseases alongside HIV and tuberculosis that cause severe morbidity, mortality and continue cycles of poverty (Bhutta *et al.*, 2014). Understanding the epidemiology and drivers of malaria transmission is key to reducing the disease burden. This chapter is an introduction to malaria epidemiology and the mathematical modelling of malaria, building a foundation for the rest of the work in this thesis to expand upon. It will also cover the specific topics of malaria during pregnancy and malaria in the Democratic Republic of Congo (DRC) to contextualise some of the data used in this thesis.

1.1 Malaria Epidemiology

1.1.1 The Burden of Malaria

Malaria is the disease caused by the *Plasmodium* parasite, common symptoms include intermittent fevers followed by chills and shaking, headaches, nausea, and vomiting (Bruce CLJ, 1980). The six species that infect humans and inflict harm are *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale wallikeri, Plasmodium ovale curtisi, Plasmodium knowlesi* and *Plasmodium malariae* (Calderaro *et al.*, 2013). The two main parasite species have a rough geographic divide, with *P. falciparum* dominating in Africa and *P. vivax* being the most prevalent in South East Asia. These two species have different life cycles, and cause different morbidity and mortality rates, which may be due to their adaptation to different climates (Bruce CLJ, 1980). In 2016 there were an estimated 216 million clinical cases and 445,000 deaths due to *Plasmodium* parasites across the world, with 90% of cases and 91% of deaths in Africa (World Health Organization, 2017b). These values nevertheless represent a significant improvement since the turn of the century, with the widespread scale-up of malaria control programmes beginning in 2000 reducing the incidence of clinical malaria episodes by 40% over the next 15 years (Bhatt *et al.*, 2015).

1.1.2 Malaria transmission

The intensity of malaria transmission is measured in terms of the entomological inoculation rate (EIR), which is the number of infectious mosquito bites that a person receives each year (WHO 2013). The EIR at a particular location is estimated by multiplying the daily mosquito biting rate on humans by the proportion of mosquitoes that are infectious to humans and then multiplying the result by 365 (Kilama *et al.*, 2014). The daily mosquito biting rate is measured using human landing catches where a volunteer sits outside during mosquito feeding times and counts the number of mosquitoes that attempt to feed upon them (Gimnig *et al.*, 2013). The EIR can change throughout the year depending on the seasonal influence in the size of the mosquito population, typically rising in the rainy season and falling during the dry season (White, Griffin, *et al.*, 2011). The degree of seasonality in a location is determined by the proportion of infectious bites or cases each year that fall within a short seasonal window (Reiner *et al.*, 2015). In the most seasonal settings the mosquito biting rate becomes negligible during the dry season so there is very little malaria transmission (Jawara *et al.*, 2008). Countries close to the equator can sustain mosquito populations that cause malaria transmission all year round, this is known as stable or holo-endemic transmission. Further away from the equator, malaria transmission might only be possible when certain drivers of malaria transmission change. For example, if there is an unexpectedly large rainfall then there could be a resulting malaria epidemic due to the mosquito population size increasing (Teklehaimanot *et al.*, 2004). These malaria epidemics happen in areas of unstable transmission, in contrast to stable transmission happening constantly. They have particularly high mortality because the population have no naturally acquired immunity to malaria, meaning that they are more likely to develop serious clinical complications (Checchi *et al.*, 2006).

Malaria transmission is heterogeneous at all spatial scales: within a country (Okello *et al.*, 2006), within a province (Bejon *et al.*, 2010; Ahmed *et al.*, 2013), and within a town (Ferrari *et al.*, 2016) or village (Creasey *et al.*, 2004). Mosquitoes are predominantly only thought to travel a few kilometres in search of a blood meal, meaning that malaria transmission is highly clustered in “hot spots” (Kaufmann and Briegel, 2004; Kreuels *et al.*, 2008). People travelling in and out of these hot spots transport malaria into other areas when they are bitten by mosquitoes in new places (Martens and Hall, 2000; Wesolowski *et al.*, 2012). Human migration has moved drug-resistant strains of *P. falciparum* around the globe and imported

cases of malaria into places where the disease had previously been eliminated (Roper *et al.*, 2004; Cotter *et al.*, 2013).

The environmental factors that have a strong influence on the epidemiology of malaria can also interact, for example rainfall and temperature: heavy rain in a hot environment causes a much quicker rise in malaria transmission than heavy rain in a cold environment (Teklehaimanot *et al.*, 2004). This is because Anopheline mosquito larvae which are capable of transmitting malaria develop faster, and adult mosquitoes live longer, in warmer temperatures up until around 33 degrees Celsius (Beck-Johnson *et al.*, 2013). There is also a positive, but complex, relationship between temperature and the time that the parasite takes to develop in the mosquito (its extrinsic incubation period). In general, the parasite develops faster at warmer temperatures, but recent studies have found that the magnitude of the daily variance in temperature is also important (Gething *et al.*, 2011; Blanford *et al.*, 2013) with daily fluctuations in temperature having a greater impact on the extrinsic incubation period when the mean temperature is higher (Paaijmans, Read and Thomas, 2009). Mathematical models that predict malaria incidence based on climatic factors have become more feasible in recent years due to large, fine-scale remote-sensing satellite data covering Sub-Saharan Africa (Gething *et al.*, 2011; Christiansen-Jucht *et al.*, 2015; Zinszer *et al.*, 2015). This has made it possible to produce risk maps for malaria at a fine spatial resolution of 1km based on the local climate and rainfall (Garske, Ferguson and Ghani, 2013).

Efforts to reduce malaria transmission are categorised roughly into three aims: control, elimination, or eradication. Malaria control generally refers to ways of managing malaria transmission to reduce the disease burden. A country or region can achieve malaria elimination when there is no ongoing within-country malaria transmission, allowing that imported cases may occur due to people travelling to and from other countries that have not eliminated the disease (J. M. Cohen *et al.*, 2010). Malaria eradication refers to the much more complex task of achieving zero incidence of malaria worldwide. It is hotly contested as to whether such a goal is possible (Roberts and Enserink, 2007; Feachem *et al.*, 2010; Liu *et al.*, 2013; Tanner *et al.*, 2015). Malaria elimination has been shown to be theoretically possible in many parts of the world, but there remain areas where malaria is so endemic that no combination of current tools is predicted to achieve elimination (Griffin *et al.*, 2010).

1.1.3 Parasite lifecycle

The malaria parasite has a complex lifecycle, as passing between human hosts and mosquito vector requires several specific life-stages. Transmission from an infected mosquito to a human occurs when the mosquito takes a blood meal. First, sporozoites in the salivary glands are injected into the bloodstream (Figure 1A). The sporozoites travel through the bloodstream until they reach the liver where they invade liver cells called hepatocytes (Figure 1.1B). Within the hepatocytes the sporozoite generates thousands of merozoites (the asexual stage of the parasite) before the hepatocyte bursts and releases the merozoites into the bloodstream. This is called the blood stage of the infection (Figure 1.1C). In the blood the merozoites bind to and invade red blood cells (erythrocytes), producing around 20 copies of themselves over the course of 48 hours in the case of *P. falciparum*. The parasite causes changes in the invaded erythrocyte that make it attach to the endothelial walls of blood vessels and organs while it is reproducing, a process called sequestration. The invaded cells generally sequester at the same time, causing a periodic pattern in the parasite density in the blood stream, with the period varying by *Plasmodium* species (Hawking, Worms and Gammage, 1968). Merozoites being released into the blood causes the same periodic symptoms in the human (Bruce CLJ, 1980). Sequestration is advantageous to the parasite because it prevents infected red blood cells from circulating to the liver or spleen, where they may be destroyed (White, 2017).

At some point during the infection a subset of merozoites will switch to producing gametocytes, the sexual stage of the parasite (Figure 1.1D). It is unclear exactly what causes this behaviour, but various theories have been proposed, including: the density of asexual parasites in the blood (Sowunmi *et al.*, 2004), the intensity of the host immune response, and the level of anaemia in the human host (Sowunmi *et al.*, 2004; Bousema and Drakeley, 2011). High densities of gametocytes in the blood make it more likely that a biting mosquito will ingest at least one male and one female gametocyte while taking a blood meal (Churcher *et al.*, 2013; Da *et al.*, 2015) (Figure 1.1E). After ingestion, conditions in the mosquito midgut prompts gametocytes to prepare for reproduction. The male gametocyte produces up to 8 motile gametes that move around in the blood meal until they meet a female gametocyte to fertilise. After reproduction occurs the newly formed parasite stage pierces the midgut wall of the mosquito and forms a sack called an oocyst (Figure 1.1F). The oocyst produces thousands of sporozoites until it bursts after a minimum of 10 days. Sporozoites migrate to the salivary

gland of the mosquito where they are then ready to infect another human and complete the life-cycle (Figure 1.1G).

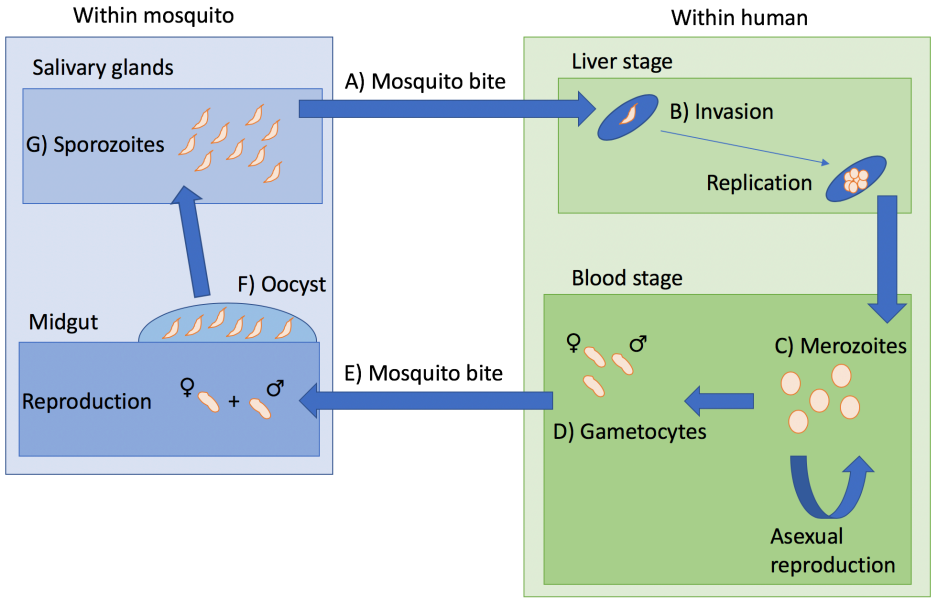

Figure 1.1: The lifecycle of Plasmodium falciparum. (A) Sporozoites enter the human blood stream during a mosquito bite. (B) The sporozoites hide in the liver and replicate before re-entering the blood stream as (C) merozoites. The merozoites reproduce asexually in the blood stream causing clinical symptoms. (D) some merozoites diverge into gametocytes, the sexual stage of the parasite. (E) biting mosquitoes ingest gametocytes that reproduce in their midgut. (F) Fertilisation occurs and the gamete forms an oocyst on the wall of the mosquito midgut. (G) The oocyst fills with sporozoites until it bursts, the sporozoites migrate to the salivary glands to repeat the transmission process.

1.1.4 Malaria morbidity and mortality

In naïve human hosts the number of merozoites in the blood rises quickly, causing symptoms around 9-14 days after the infectious bite (Bruce CLJ, 1980). Infected erythrocytes that enter the spleen trigger the immune system to start making pro-inflammatory cytokines, which prevent infected erythrocytes from binding to endothelial walls but also cause fever, shaking and nausea. Infected erythrocytes sequestering in organs and blood vessels can cause blood clots and damage to endothelial walls (Bruce CLJ, 1980). In the lungs this damage can lead to pulmonary oedema (Taylor, Cañon and White, 2006). As the parasites destroy erythrocytes the human host can develop anaemia, as well as acidosis and hypoglycaemia due to renal system dysfunction (Trampuz *et al.*, 2003). Cerebral malaria occurs when invaded erythrocytes sequestering in the small capillaries of the brain cause clots, leading to seizures, coma and a high likelihood of death (Idro *et al.*, 2010). Those who survive cerebral malaria

may be left with lasting brain injuries and cognitive impairment (Fernando, Rodrigo and Rajapakse, 2010).

Aside from clinical cases of malaria, a significant proportion of individuals in endemic areas unknowingly harbour an asymptomatic parasite infection (Bousema *et al.*, 2014; Chen *et al.*, 2016). These individuals are protected from clinical episodes of malaria by their immune response, but long-term chronic infection with *P. falciparum* is associated with a variety of poor health outcomes. Malaria interventions often reduce all-cause mortality even if malaria-specific mortality is not reduced, suggesting that asymptomatic parasite infections aggravate other diseases (Chen *et al.*, 2016). Long-term asymptomatic infections have also been specifically linked to anaemia (Looareesuwan *et al.*, 1987), systemic bacterial infections (Biggs *et al.*, 2014), and poor school performance (Fernando *et al.*, 2006; Clarke *et al.*, 2008).

1.1.5 Immunity to malaria

Specific anti-sporozoite antibodies are capable of blocking sporozoites from infecting the liver, preventing an infection from establishing (Dups, Pepper and Cockburn, 2014). If this fails, then the infection will progress to the blood stage with circulating merozoites and the corresponding immune response will change. The immune response to the emergence of blood stage parasites from the liver is split into the immediate non-specific innate immune response and the slower adaptive immune response that is a malaria-specific response. The innate immune response aims to quickly stunt parasite development. While this occurs the adaptive immune response becomes primed to combat the infection (Stevenson and Riley, 2004). The adaptive immune response to malaria is much more comprehensive, blocking merozoites from invading erythrocytes, preventing infected erythrocytes from sequestering, inciting macrophages to consume (phagocytose) infected erythrocytes and merozoites circulating in the bloodstream, and inhibiting the inflammatory cytokine cascade that causes the clinical symptoms of malaria (Good and Doolan, 1999; Marsh and Kinyanjui, 2006). Effective immunity against clinical episodes of malaria is acquired over many parasite infections. Where malaria transmission is high this happens quickly; and adults will therefore have a well-developed immune response against the asexual stage of the parasite so they infrequently have clinical episodes of malaria despite parasitaemia (Doolan, Dobaño and Baird, 2009). Where malaria transmission is highly seasonal or unstable individuals may not be exposed to parasite infections frequently enough to develop a good clinical immunity, hence the majority of infections will lead to a clinical episode of malaria (Rono *et al.*, 2015).

Plasmodium falciparum has many genetically distinct strains, which have different polymorphisms in parasite surface proteins that the immune system uses to recognise the infection (Su *et al.*, 1995). This means that exposure to many different parasite strains is needed for adequate protection against future clinical episodes. On top of different parasite strains, *P. falciparum* will also vary the proteins that it expresses on the surface of an infected erythrocyte over the course of a single infection (Frech and Chen, 2013). These changeable proteins are known as variant surface antigens (VSA). A common VSA is *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP-1). At each cycle of parasite replication, a proportion of subsequent parasites express a genetically distinct version of PfEMP-1. This change delays the hosts immune response since it now has to begin targeting different proteins. The combination of different parasite strains and different VSAs within an infection mean that an individual's immune system must learn to recognise a large variety of antigens before it can mount a thorough defence against the asexual stage parasites. The accumulation of immunity against certain strains and VSAs explains the age patterns of clinical malaria episodes, especially where there is high transmission intensity. Building up immunity against a wide selection of VSAs takes time because you need to have experienced many previous infections, children are therefore the most susceptible to clinical episodes of malaria (Drakeley *et al.*, 2006). In high transmission areas of Sub-Saharan Africa, nearly all malaria morbidity and mortality associated with malaria is in children under five years old (Murray *et al.*, 2012), who have not been exposed to enough infections to have acquired immunity.

1.1.6 Malaria diagnostics

Malaria is difficult to diagnose clinically because many of its symptoms are shared with other common but life-threatening diseases. It is therefore important to be able to determine whether such symptoms are due to malaria quickly and accurately. The two most common malaria diagnostics used in Sub-Saharan Africa are microscopy and rapid diagnostic tests (RDTs). Microscopy involves examining stained blood smears under a microscope to look for merozoites, gametocytes, or erythrocytes that have been invaded. By counting the number of parasites in a given area, a quick assessment can be made of the parasite density. Microscopy is inexpensive but requires trained health workers, and is time consuming because of the time taken to stain and process each blood sample (Payne, 1988; Ochola *et al.*, 2006). Microscopy also requires a relatively high parasite density threshold for detection compared to other malaria diagnostics (Okell *et al.*, 2012). The precise level depends on the

skill of the person performing the microscopy, but it is estimated to be around 50-100 parasites per microlitre of blood for the average microscopist (Payne, 1988). This is around the same level as some brands of RDT but a lot higher than <5 parasites per microlitre for molecular diagnostic methods (Tangpukdee *et al.*, 2009).

RDTs detect parasite antigens in the blood, offering an inexpensive, fast method of detecting *P. falciparum* without requiring any laboratory equipment. This allows health workers to diagnose malaria in rural settings with minimal training (Carrara *et al.*, 2006). A huge benefit of the speed and simplicity of RDTs has been that clinicians can now rule out malaria in patients that have malaria-like symptoms, allowing faster diagnosis of other life-threatening conditions, saving money on antimalarial drug use, and preventing over-use of drugs (Odaga *et al.*, 2014). Since RDTs detect specific antigens on the surface of parasites, only some brands are able to also detect other *Plasmodium* species (Murray *et al.*, 2008). Most RDTs use antibodies to detect the antigen Histidine-rich protein 2 (HRP2), although this antigen remains in the bloodstream after parasite clearance leading to a false positive test result for a median of 35-42 days afterwards (Grandesso *et al.*, 2016).

Polymerase chain reaction (PCR) methods amplify genetic material present only on the surface of *Plasmodium* parasites. Primers determine which genetic material is amplified and can be selected to discriminate between species. PCR testing has an extremely high sensitivity (Johnston *et al.*, 2006) and low detection threshold (Tangpukdee *et al.*, 2009). Unfortunately, the costly equipment, training and stringent laboratory conditions required for PCR analysis mean that it can only be undertaken at the wealthiest research institutions. As such, PCR is mainly used to estimate the sensitivity of other less expensive diagnostics or to perform genetic analysis on parasites. An analysis of data used to compare diagnostic methods found that of all infections detected by PCR, RDTs detected 41% of these, and microscopy detects 87% of those detected by RDT (Wu *et al.*, 2015).

1.2 Methods for controlling malaria

Malaria interventions broadly belong in two categories, those that target the mosquito vectors and those that target the parasite infection in the human host. Vector control methods aim to reduce the mosquito biting rate on humans, thereby reducing transmission. The parasite can be also be controlled in the human population to prevent infection from occurring, to prevent morbidity from infections that occur, and to reduce transmission from human to mosquito.

The next section will describe the most widely used and promising malaria control interventions. Special consideration is given to the methods used to evaluate vector control interventions.

1.2.1 Vector control

1.2.1.1 Contact-based approaches

Insecticide treated nets (ITNs) are a physical and chemical barrier between sleeping humans and *Anopheles* mosquito species that transmit malaria, which predominantly prefer to take bloodmeals from humans indoors between dusk and dawn (Bruce CLJ, 1980). ITNs where the insecticide is incorporated into the net fabric are referred to as long-lasting insecticidal nets (LLINs). The serviceable life of an LLIN is thought to be three years, although some evidence suggests that it is closer to two years (Gnanguenon *et al.*, 2014). A huge number of LLINs have been distributed since the year 2000 as part of the Roll Back Malaria initiative and other public health campaigns (Bhatt *et al.*, 2015). They have been highly effective at reducing malaria mortality and clinical episodes of the disease in a range of transmission settings across Africa (Lengeler, 2004). Bhatt et al. (2015), estimate that 663 million clinical cases of malaria were prevented between 2000 and 2015, and that LLINs were responsible for 68% of these. Since LLINs kill mosquitoes that attempt to take a bloodmeal, they are responsible for both a personal protection effect for the person sleeping under the net, as well as a community-level protection effect, because mosquitoes that are killed cannot then go on to infect others (Howard *et al.*, 2000; Maxwell *et al.*, 2002). The major problems associated with LLINs for malaria control involve achieving wide-scale and equal coverage (Barat *et al.*, 2004; Sexton, 2011), incorrect or inconsistent LLIN use (Atkinson *et al.*, 2009; MacIntyre *et al.*, 2012; Xu *et al.*, 2014), and mosquitoes developing resistance to pyrethroids, the only class of insecticides currently widely used on LLINs (N'Guessan *et al.*, 2007; Strode *et al.*, 2014; Lindblade *et al.*, 2015).

The public health impact of pyrethroid resistance remains unclear, in part because resistance cannot be randomised between sites, preventing its evaluation in randomised control-trials (Kleinschmidt *et al.*, 2015). A recent large-scale study across 5 countries found no association between the level of resistance (as measured by a discriminating dose bioassay) and the incidence of malaria (Kleinschmidt *et al.*, 2018). It also showed that LLINs still provide substantial personal protection to those using them. The interpretation of this study

on the public health impact of pyrethroid resistance is difficult, as the method of assessing the level of resistance has high measurement error and resistance might be associated with the endemicity of the disease. The strongest evidence for pyrethroid resistance diminishing the effectiveness of LLINs comes from a recent randomised control trial from Tanzania. Here the prevalence of malaria was shown to be substantially lower in villages given LLINs which contained pyrethroid and the synergist piperonyl butoxide (PBO), compared to similar LLINs with pyrethroid only (Protopopoff *et al.*, 2018).

Another widely used method of vector control is Indoor Residual Spraying (IRS), where insecticide is sprayed directly onto the walls of houses to kill mosquitoes that rest there and/or deter mosquitoes from entering the house. There is a lower level of personal protection attributable to IRS than LLINs because there is no actual physical barrier between human and mosquito, a vector may still bite before it rests on the walls of the house and dies. If high IRS coverage is achieved then the average population density and life-expectancy of the mosquito population will decline, reducing transmission. IRS is responsible for an estimated 13% of the predicted reduction in cases between 2000 and 2015 (Bhatt *et al.*, 2015). Pyrethroid insecticides have historically been used as indoor residual sprays throughout Africa, though the fear of pyrethroid resistant mosquitoes has caused the transition to other classes of insecticide (Organització Mundial de la Salut Global Malaria Programme, 2012). This move towards insecticide resistance management has been easier for IRS than LLINs, as although the number of alternative insecticide options available are limited for LLINs, there are a few non-pyrethroid IRS products on the market. This is in part because, unlike LLINs, the insecticide used in IRS does not have as much direct contact with humans so a wider variety of insecticides can be used (Ossè *et al.*, 2012). IRS is applied directly to the walls of permanent structures so there is a lower chance of improper use. However, the effectiveness of IRS depends heavily on the willingness of populations to tolerate repeated rounds of spraying and on the building materials used locally, which can significantly impact how long the insecticide remains effective on the wall (Mutagahywa *et al.*, 2015). IRS is also not very amenable to mosquito control in areas with refugee or displaced populations as people do not live in permanent structures and the logistics of spraying are difficult (Rowland and Nosten, 2001; Graham, 2004). Alternatives to IRS such as insecticidal wall hangings and blankets have been investigated for use by displaced populations (Rowland *et al.*, 1999; Graham *et al.*, 2002, 2004; Mittal *et al.*, 2011) and as longer lasting alternatives to repeated spraying rounds in urban and rural settings (Messenger, Matias, *et al.*, 2012; Messenger, Miller, *et al.*, 2012; Ngufor, Tungu, *et al.*, 2014).

1.2.1.2 Airborne pyrethroids

While the pyrethroids used in LLINs and IRS act when a mosquito contacts them, an alternative route of insecticide delivery is through the air or in the "vapour phase". This may involve burning coils or suffusing a material with a pyrethroid such as transfluthrin, which has a low melting point and will be passively released into the air (Ogoma, Moore and Maia, 2012). At high enough concentrations airborne pyrethroids have been found to induce mortality in mosquitoes and deter them from entering the protected area (hereby referred to as mosquito deterrence) (Ogoma, Ngonyani, *et al.*, 2014). Volatile pyrethroids have also been shown to induce other entomological effects that may reduce mosquito vectoral capacity. Mosquitoes exposed to transfluthrin coils showed increased mortality within the next 24 hours and the ones that survived and managed to feed produced 97% fewer eggs than control mosquitoes (Ogoma, Lorenz, *et al.*, 2014). One method of releasing airborne pyrethroids is to have them passively evaporate from a material such as hessian cloth soaked in transfluthrin (Ogoma *et al.*, 2012). This wider category of interventions are referred to as emanators (Ogoma *et al.*, 2017). Emanators require relatively little user interaction to work and can be fashioned into decorative wall hangings for use outside homes, bars, or in other places where people gather (Masalu *et al.*, 2017). Small-scale trials of emanators have shown them to be particularly effective at preventing bites on users sat nearby, even with low concentrations of pyrethroids (Ogoma *et al.*, 2017).

Airborne transfluthrin also causes blood feeding inhibition, which is a term routinely used to describe the reduction in the percentage of mosquitoes that successfully acquire a blood meal. Blood feeding inhibition could be induced by multiple, non-exclusive, distinct processes. For example, LLINs might reduce blood feeding by a mosquito preferentially avoiding the intervention (i.e. being deterred from entering a house with a net), being killed by the insecticide when landing on the net, or by exiting the house without a blood meal due to the insecticide or physical barrier of the LLIN. There is evidence that the blood feeding inhibition induced by airborne pyrethroids is due to a more pronounced effect than merely the mosquito deterrence mentioned above. Around half of mosquitoes exposed to smoke from transfluthrin coils remained unable to take up an opportunity for a blood meal 12 hours after exposure (Ogoma, Ngonyani, *et al.*, 2014). Airborne pyrethroids interfere with mosquito sensory organs, preventing them from responding to host cues that they use to find a blood meal (Bohbot and Dickens, 2010). This insecticide-induced sub-lethal morbidity that prevents

mosquitoes from blood-feeding for a period of time following exposure will be referred to as temporary feeding interruption (TFI) to differentiate it from the broader blood-feeding inhibition that it causes. Several other studies have looked at the impact of airborne pyrethroids and have had similar findings. For example, in Thailand, vectors of the species *Anopheles dirus* were less likely to land on volunteers inside huts treated with the pyrethroid deltamethrin than in control huts (Malaithong *et al.*, 2010). Other studies have found that contact with permethrin corresponded with reduced attempts to land on a host in *An. gambiae s.s* (Siegert, Walker and Miller, 2009), and use of metafluthrin reduced mosquito landing attempts in *Aedes albopictus* and *Aedes taeniorhynchus* (Xue *et al.*, 2012).

This insecticide-induced TFI effect joins an increasing list of other morbidity effects of insecticides such as reduced fecundity or delayed mortality (>24 hours after exposure). Pyrethroid-resistant mosquitoes that were regularly exposed to pyrethroids on LLINs to mimic blood feeding behaviour did not die upon contact with the LLIN, but instead had their overall lifespans reduced by over half (Viana *et al.*, 2016). Delayed mortality is posited as a reason why LLINs remain effective against pyrethroid resistant mosquitoes that they no longer kill (at least immediately upon contact). Combinations of sub-lethal effects could exacerbate the impact of interventions and diminish the public health impact of pyrethroid resistance.

1.2.1.3 Gene drive

A novel vector control method is mosquito gene drives, which use genetic techniques to introduce new genes into wild mosquito populations that eliminate the mosquitoes or make them less effective as malaria vectors (Burt, 2003). Mosquito population genetics models suggest that it is possible to release mosquitoes with a "selfish" gene that has a greater than 50% chance of selection during reproduction. These selfish genes can eventually establish themselves in the population and completely replace any previous variants (Deredec, Godfray and Burt, 2011). Since these selfish genes are so persistent once they are introduced it does not matter if they have a corresponding fitness cost for subsequent generations, although such a fitness cost would usually cause the eventual extinction of a non-selfish gene. If the selfish gene introduced into the population affects the fertility of mosquito offspring, then increasingly more mosquitoes with low fertility will be preferentially produced generation after generation until the population is eliminated (Hammond *et al.*, 2016). Alternatively, modified male mosquitoes may be released that are far more likely to produce male offspring,

which again are predicted to eventually cause population collapse (Burt, 2003; Deredec, Godfray and Burt, 2011). Another method is to introduce a gene that makes mosquitoes resistant to malaria infection, with the aim of making all wild mosquitoes resistant to malaria infection (Gantz *et al.*, 2015). Again, even if this resistance gene came with a fitness cost, it being preferentially passed down during reproduction would ensure that it eventually proliferated throughout the entire mosquito population. Recent mathematical modelling work that assessed the potential effectiveness of gene drives as a malaria eradication tool found that their effectiveness depends on the lowest level of mosquito density experienced each year (Eckhoff *et al.*, 2017). In highly seasonal settings the mosquito population may become highly segmented in the dry season, hence pockets of mosquitoes without any gene-driven mosquitoes in them will likely remain. These pockets of mosquitoes can then prolong the survival of the population for another transmission season.

1.2.1.4 Evaluating vector control interventions

The proscribed development pipeline for vector control tools involves three phases of trials, after which the WHO makes recommendations for using the tool in the field (Vontas *et al.*, 2014). Some institutions that buy and distribute vector control tools adhere strictly to WHO recommendations, whereas others are free to do what they perceive is best. Phase I trials involve testing the intervention using laboratory-based assays to determine how it affects mosquitoes. Phase II trials assess the entomological efficacy against free-flying mosquitoes in small semi-field, or field trials. Phase III trials measure the epidemiological impact of a tool, ideally using a randomised controlled trial (RCT) to measure the difference in disease between control and treatment groups. Phase III RCTs need to be designed and undertaken very carefully to make sure that they have sufficient statistical power to detect changes in their chosen epidemiological outcome, as well as carefully monitoring that the vector control tool is thoroughly distributed and actually used by the population (Wilson *et al.*, 2015).

Experimental hut trials are the most widely used method of evaluating the entomological efficacy of interventions that target mosquitoes in the home, such as LLINs or IRS. They are a basic representation of the housing style in a broad geographical region of Africa that can be built to the same specification across the region, allowing different products to be tested in identical huts in different locations. There are currently three main types of experimental hut used in Africa: East African (Smith, 1965), West African (Darriet *et al.*, 2002) and Ifakara (Okumu *et al.*, 2012) huts. The types of hut differ in their layout and building materials to

best represent general differences in the styles of housing across Africa (World Health Organization, 2013a). Volunteers sleep in the hut to attract mosquitoes into the structure where they become trapped, either through flying through one-way valves in the windows or eaves of the house, or through being caught in exit traps. Comparisons between a control hut with an untreated bed net and a hut with a LLIN in can show how the intervention deters mosquitoes from entering the hut (reduced hut entry), kills mosquitoes (increased mortality), and prevents mosquitoes from feeding (reducing blood feeding rates) (Massue *et al.*, 2016). Most newly developed insecticides in Phase I evaluation cause high mosquito mortality within the first 24 hours following exposure, though some chemistries of insecticide are thought to take longer to induce their effect (Agossa *et al.*, 2018). Experimental hut trials try to estimate the efficacy of the compound against mosquitoes looking for a blood meal under more natural conditions, though here too endpoint measurement may need to be delayed according to the mode of action of the insecticide.

1.2.2 Drug treatment

Effective drug treatment clears parasite infections from humans and prevents immediate onwards transmission. In many endemic countries most infections are asymptomatic, with people carrying low-density infections for long periods of time, so focusing only on treating the visible, clinical episodes of malaria will be insufficient to halt malaria transmission (Bousema *et al.*, 2014). Mass drug administration (MDA) campaigns circumvent this problem by providing everyone in a population with a dose of anti-malarial drugs at the same time, regardless of whether they have symptoms of parasite infection. Clearing the entire asymptomatic parasite reservoir in humans can potentially end or significantly reduce malaria transmission because no humans will be infectious to biting mosquitoes. However, observations from previous MDA campaigns (Von Seidlein and Greenwood, 2003; Poirot *et al.*, 2013; Newby *et al.*, 2015) and contemporary mathematical modelling work (Gu *et al.*, 2003; Okell *et al.*, 2011) show that while MDA can have a large impact on parasite prevalence in the short term, the prevalence of infection returns to normal levels soon after the campaign has finished. This is due to several reasons: no MDA will have 100% coverage of the population, so some people will be left untreated; the anti-malarial will not have 100% efficacy at clearing infections, so some people will remain infectious to mosquitoes; current MDA does not kill mosquitoes that are currently infectious, which will re-infect the population after the campaign has finished. These factors mean that an MDA campaign is unlikely to permanently interrupt malaria transmission, apart from in very isolated settings

with very low transmission (Kaneko *et al.*, 2000), or if MDA is combined with other interventions such as LLINs or IRS (Okell *et al.*, 2011).

In areas of seasonal malaria transmission, mass distributing anti-malarial drugs with a long prophylactic effect several times at the start of the transmission season may substantially delay the build-up in the number of cases and truncate the transmission season (Meremikwu *et al.*, 2012). The general administration of anti-malarial drugs for prophylaxis is known as Intermittent Preventative Treatment (IPT). When this is performed at the beginning of the transmission season it is known as Seasonal Malaria Chemoprevention (SMC). In places where malaria transmission is highly seasonal, where the majority of clinical episodes occur within a period of a few weeks, SMC has been found to significantly lower disease burden (Cissé *et al.*, 2006; Dicko *et al.*, 2008, 2011; Konaté *et al.*, 2011; Wilson, 2011; Meremikwu *et al.*, 2012) for a relatively low cost (Ross *et al.*, 2011; Nonvignon *et al.*, 2016). A meta-analysis of 12 IPT trials that distributed a monthly dose of Sulphadoxine-Pyrimethamine plus Amodiaquine (SP+AQ) to children during the transmission season estimated an 83% reduction in the incidence of clinical episodes of malaria (Wilson, 2011). The main considerations for using SMC are which age ranges to distribute anti-malarial drugs to, how to distribute them to ensure good and timely coverage, and when in the year the distributions should take place. IPT is usually targeted at infants (IPTi), pregnant women (IPTp), or school age children (IPTc).

1.2.3 Vaccine

A more permanent alternative to repeated drug distribution would be a vaccine to induce long-lasting immunity in the population. Developing a malaria vaccine has proven difficult due to the complexity of malaria immunology and the need for the vaccine to be safe to administer at scale (Crompton, Pierce, and Miller 2010). The most advanced candidate, RTS,S, initiated an immune response against a circumsporozoite protein, which enabled the immune system to target infections at the sporozoite stage before the parasite can enter the liver and go on to develop into a blood-stage infection (J. Cohen *et al.*, 2010). Results of a recent RCT found that 20 months after administration the vaccine efficacy (the percentage reduction in disease in the vaccinated group compared to a control) was 27% in 6-12 week old children and 45.1% in 5-17 month old children (Mahmoudi and Keshavarz 2017; The RTS,S Clinical Trials Partnership 2015). By 2 years the vaccine efficacy had dropped even further, with the drop being slightly reduced if the child received a booster dose of the

vaccine at 18 months (The RTS,S Clinical Trials Partnership 2015). The poor longevity and efficacy of protection of the RTS,S vaccine means that its mass distribution is unlikely to be cost-effective in most transmission settings in Africa (Winskill *et al.*, 2017).

1.2.4 Summary

It is widely regarded that effective malaria control will require a combination of interventions (Walker *et al.*, 2016). The most cost-effective intervention package will depend on the seasonality of transmission, vector species composition, housing, mosquito and human behaviour, and the intensity of malaria transmission. Variation in these factors at a fine spatial scale means that province-level intervention strategies are often far more cost effective than country-level strategies. Recent mathematical modelling studies have suggested that the most cost-effective combination across all settings would be the distribution of LLINs combined with IRS or SMC depending on the degree of seasonality at the location (Walker *et al.*, 2016; Winskill *et al.*, 2017). This study however assumes that LLINs are working optimally and are not influenced by the rise of pyrethroid resistant mosquitoes. In highly seasonal settings a short burst of three to four months of SMC across the wet season was suggested to be more cost effective than IRS, because SMC only needs to be mobilised for a short window of the year. In endemic settings where SMC would be required for a longer duration, IRS at high population coverage became more cost-effective.

If malaria elimination is to be achieved, then additional control interventions will be needed. Evaluation of potential tools is time consuming and costly as novel interventions need to show additional benefit over the current standard-of-care. Mathematical models of malaria transmission can be used as a low-cost method to estimate the impact of different interventions in different settings and support the evaluation process. These mathematical models can vary greatly, so the next section introduces the most widely used models and illustrates their potential utility.

1.3 Mathematical models of malaria transmission

Mathematical models have been used for many years to predict the behaviour of malaria and to estimate the impact of control interventions. Most mathematical models of malaria transmission are both mechanistic, meaning that they try to mathematically represent the physical systems that they are approximating, and dynamical, meaning that the states in the

model are dependent on time. The canonical model of malaria transmission is the Ross-MacDonald model, which consists of linked ordinary differential equations explaining the proportions of human and mosquito populations that are currently susceptible or infected (D. L. Smith *et al.*, 2012). The Ross-MacDonald model was developed in the 1950s, but the main form and assumptions of the model can still be seen in many models used today (D. L. Smith *et al.*, 2012). Over time mathematical models of malaria have become more complex, allowing them to capture different facets of malaria transmission. For example, they may try to incorporate seasonal variation of mosquito abundance (Reiner *et al.*, 2013), or how humans acquire clinical immunity to the parasite (Filipe *et al.*, 2007). The Bill and Melinda Gates Foundation convenes the Malaria Modelling Consortium, with the aim of directing research and encouraging collaboration between different modelling groups. The consortium currently includes groups from Swiss TPH (Smith, Killeen, *et al.*, 2006; Smith *et al.*, 2008), Imperial College London (Griffin *et al.*, 2010) and Intellectual Ventures (Eckhoff, 2011), amongst others. These three groups have each developed their own model of *P. falciparum* malaria transmission, each with different mathematical properties, modelling assumptions, and focus on particular aspects of malaria transmission and control.

1.3.1 The Intellectual Ventures model

The Intellectual Ventures model began as a mosquito population dynamics model that aimed to capture the effects of weather and intervention combinations on vector populations (Eckhoff, 2011). The mosquito population varies depending on available habitat, rainfall, and temperature, with each of these drivers having their own impact on larval development rates, larval mortality, and the maximum mosquito population size that an area can support. Adult female mosquitoes then attempt to take blood meals, which can succeed or fail and result in the mosquito dying, feeding on an animal, or being repelled. The complex representation of mosquito feeding enables the model to estimate how changes in mosquito behaviour alters the success of vector control interventions, such as mosquitoes resting outdoors after a bloodmeal or the propensity that a mosquito will bite a human rather than an animal (Eckhoff, 2011). Later, the vector dynamics model was coupled with an individual-based model of a human population that allows for multiple genetically distinct parasite infections and a corresponding immune response that depends on previous exposure to three different types of antigenic component of the merozoite (P. A. Eckhoff, 2012; P. Eckhoff, 2012). Each infection has a merozoite surface protein (MSP) variant, a current *P. falciparum* erythrocyte membrane protein (PfEMP-1), and variants of minor surface epitopes. Each modelled

infection has a repertoire of 50 unique PfEMP-1 variants, which it switches between during an infection to evade the host immune response. Over time the individuals in a model simulation build up a repository of previously-seen antigenic components, mimicking the acquired immunity to clinical disease that is observed in the field (Wenger and Eckhoff, 2013). The model output has been compared to observed data by showing matching patterns of how prevalence of infection varies with age across transmission settings (P. A. Eckhoff, 2012). The model allows for different quantities of unique antigenic components, which produces prevalence-age relationships that are closer to observed data when more components are used (P. A. Eckhoff, 2012). The coupled vector and human model has since been used to estimate the impact of current and potential malaria vaccines (Wenger and Eckhoff, 2013), MDA campaigns with a variety of anti-malarial drug combinations (Gerardin, Eckhoff and Wenger, 2015), and the size of the infectious reservoir of malaria (Gerardin *et al.*, 2015), amongst other work.

1.3.2 The Swiss TPH model

The model developed by researchers as the Swiss Tropical and Public Health Institute incorporates many aspects of the epidemiology of malaria highlighted in the previous section and is built out of separate modules that focus on different parts of malaria epidemiology, such as: the relationship between the mosquito biting rate on humans and the force of infection (Smith, Maire, *et al.*, 2006), human immunity to the asexual stage of the parasite (Maire *et al.*, 2006), how infectious humans are to mosquitoes (Killeen, Ross and Smith, 2006; Ross, Killeen and Smith, 2006), immunity to the pre-erythrocytic stage of the parasite (Smith, Maire, *et al.*, 2006), the occurrence of clinical episodes (Smith, Ross, *et al.*, 2006), severe disease (Ross *et al.*, 2006) and mortality (Ross and Smith, 2006). The model has been used to investigate many aspects of malaria epidemiology. For example, different methods of vaccine distribution were also explored, investigating whether it was delivered in a mass vaccination campaign or incorporated into the Extended Programme on Immunisation (T. Smith *et al.*, 2012). The team use an ensemble modelling technique, whereby the results of many versions of the same model that have different underlying assumptions are run and compared to increase the robustness of predictions (T. Smith *et al.*, 2012; Penny *et al.*, 2016).

1.3.3 The Imperial College Model

The malaria transmission model developed by researchers at Imperial College London is extended and used as part of this thesis, so a greater level of background and detail is provided. It is an individual-based, compartmental model that tracks the infection status of individuals through time, as they age and acquire exposure-driven immunity. Each section is highlighted in turn.

1.3.3.1 Human population model

At any point in time humans belong to one of six compartments that represent their different infection statuses (Griffin *et al.*, 2010) (Figure 1.2). The proportion of the population in each compartment gives a measure of the extent of parasite infection in the community. Individuals begin in the susceptible compartment (*S*) and experience a force of infection that depends on the current number of infectious mosquito bites per unit of time. Once a person is infected, they develop clinical disease or an asymptomatic infection depending on their level of blood-stage immunity. Each clinical disease case has a probability of being treated. Treatment clears parasite infection and they enter the treated disease compartment (*T*) before entering the temporary prophylaxis compartment (*P*) and eventually returning to being susceptible. If they are not treated they enter the untreated clinical disease compartment (*D*). As the individuals slowly naturally clear the infection they will eventually end up in the asymptomatic parasite infection compartment (*A*), which is thought to form a large part of the infectious reservoir in humans (Bousema *et al.*, 2014). An individual with an asymptomatic infection can be re-infected and face a new clinical episode, or may lower their parasite burden further until it becomes a sub-patent infection, (*U)*, where parasite densities are so low that they are difficult to detect using most malaria diagnostics (Okell *et al.*, 2012). An individual with a sub-patent infection can be re-infected again or will eventually clear their infection, returning to the susceptible compartment.

Individuals in the model have four distinct types of malaria-related immunity (Griffin, Ferguson and Ghani, 2014). Newly born babies have maternal immunity from antibodies that are passed down to them from their mother, and this immunity wanes quickly after birth. As children in the model are exposed to the parasite they develop immunity against clinical disease upon being infected (blood-stage immunity), as well as immunity against an infection taking hold upon receiving an infectious bite (transmission-blocking immunity). Individuals

also develop immunity from parasite exposure, this is modelled as an individual's ability to suppress parasite densities (Griffin, Ferguson and Ghani, 2014). Lower parasite densities are harder to detect with microscopy, and the model output can reflect this shortcoming in diagnostic sensitivity. It is important to capture this dynamic since low density infections may still contribute significantly to onward transmission (Okell *et al.*, 2009).

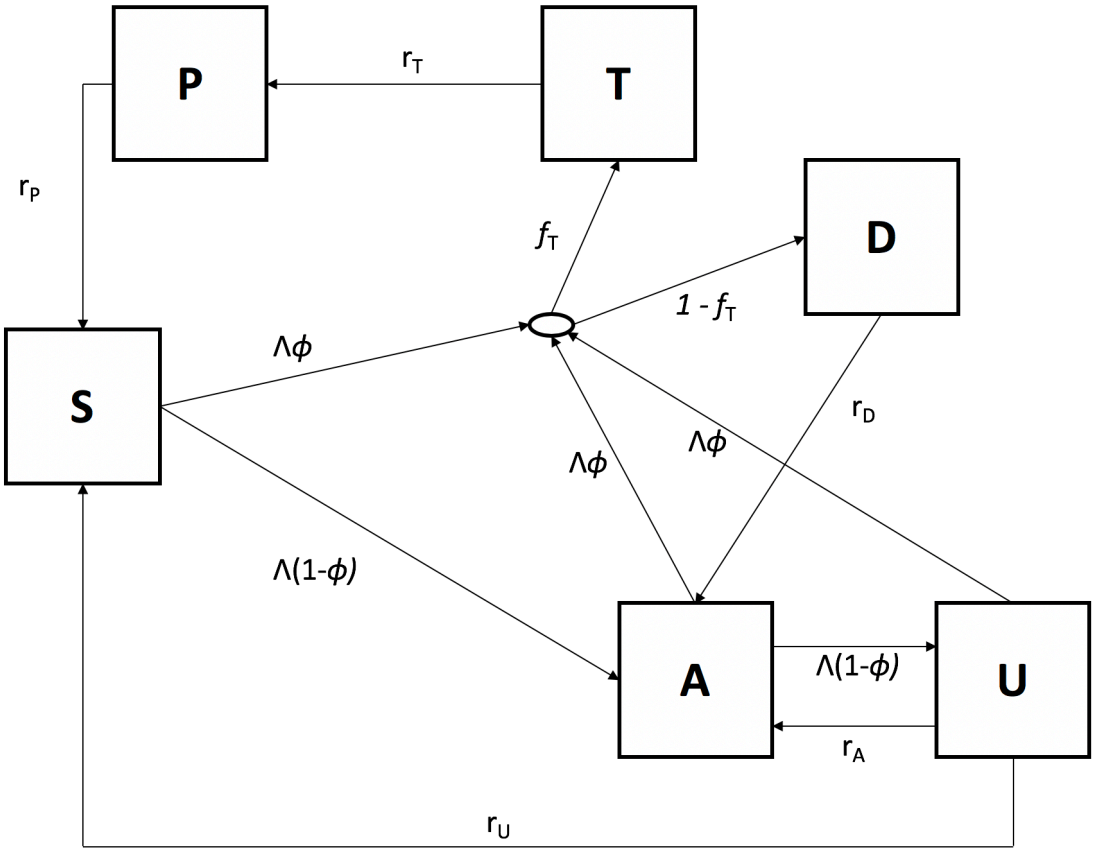

Figure 1.2: Flowchart of the structure of the Imperial College malaria transmission model. Susceptible (S) humans are infected at a rate based on the force of infection (Λ). Infected people develop clinical disease or an asymptomatic infection (A) depending on their probability of developing clinical disease (ϕ). If they develop a clinical disease, they either move to the treated (T) or untreated disease (D) categories, depending on the proportion of treated cases (f_T). Treated cases have a prophylactic stage (P) before returning to being susceptible. Asymptomatic cases can either change into being clinical episodes or become sub-patent infections (U). Sub-patent infections can either return to being asymptomatic infections, become clinical episodes or be cleared by the body and return to being susceptible.

1.3.3.2 Mosquito population model

Mosquitoes have their own population model and there is another separate model for larval development. The production of larvae is driven by a location-specific seasonality profile that represents rainfall over the course of a year (White, Griffin, *et al.*, 2011). Rain provides

breeding grounds for mosquitoes and the larval population will grow until the competition for resources curbs population growth. The larvae go through several developmental stages until they reach maturity, upon which the female mosquitoes will hatch and begin to take blood-meals. Like humans, mosquitoes begin life free of infection (susceptible) until they bite an infectious human host. The probability that a human infects a mosquito depends on which compartment the human is in. Humans in compartments that represent high density parasite infections are more likely to transmit the infection (Griffin *et al.*, 2010). After becoming infected, mosquitoes remain in a latently infected compartment for 10 days while the parasite develops in their midgut. After parasite development, mosquitoes move to an infectious compartment where they remain throughout their life-time. Adult mosquitoes are assumed to die at a constant background rate.

On average the rate at which humans are bitten is relative to their age as it is assumed that the larger their body size the more bites they will receive (irrespective of their use of control interventions). This assumption has recently been supported by studies which used genetic fingerprinting to link blood-meals to different people within a community (Gonçalves, Kapulu, *et al.*, 2017). In addition the model also incorporates an underlying relative biting rate that reflects observed heterogeneity in mosquito biting rates between individuals of the same age (Smith *et al.*, 1995).

1.3.3.3 Model fitting

During the initial creation of the Imperial College model by Griffin *et al.* (2010), the model was partly parameterised using independently estimated values collated from relevant literature. These parameters are listed in Griffin *et al.*, (2010) and Griffin, Ferguson and Ghani (2014).The parameter values that could not be obtained from existing literature were estimated by fitting the equilibrium solution of the model to parasite prevalence and clinical disease incidence by age through a range of transmission settings (Griffin *et al.*, 2010; Griffin, Ferguson and Ghani, 2014). Since the Imperial College model does not explicitly model within-host parasite densities, the fitted parameters related primarily to human immunity and human infectiousness to mosquitoes and how these change with parasite exposure. The equilibrium solution of the compartmental model is the solution where the derivative of each of the differential equations is zero, so the population in each compartment stays constant. For each transmission setting, EIR estimates were used to construct a prior distribution of EIR values. The EIR value changes the mosquito carrying capacity that varies

the level of endemicity in the model. After the EIR is set, the values of the fitted parameters were chosen to reproduce the corresponding age-prevalence profiles. The fitting process used Bayesian Markov chain Monte Carlo methods, producing a posterior distribution for each parameter that can then be explored during sensitivity analysis.

1.3.3.4 Vector control interventions

The most widely used control interventions, LLINs and IRS, are incorporated into the model by reducing the probability that a mosquito will be able to feed on humans, reducing the mosquito population size (as measured by the number of mosquitoes per human), increasing the mosquito death rate and increasing the time that mosquitoes spend finding a blood meal (Griffin *et al.*, 2010). The proportion of bites taken on humans relative to animals and the proportion of bites taken on unprotected humans relative to protected humans both change in response to interventions. The effectiveness of LLINs and IRS are calibrated using data from experimental hut trials, which is used to predict the probability (per feeding attempt) that a mosquito will be deterred from entering, exit the hut without feeding, successfully blood-feed, or be killed. Mosquitoes that are repelled will go on to try and find another blood meal. The overall effectiveness of interventions depends on the coverage in the population, higher coverage increases the time that a mosquito spends looking for a meal on an unprotected human and increases the probability that a mosquito will encounter a LLIN or IRS and die (Le Menach *et al.*, 2007). The correlation between intervention coverage is controlled using a multivariate normal distribution. The mean parameters control the probability that each individual receives an intervention and the covariance matrix alters the probability that a user receives another intervention if they already have one or more. Changing the values in the covariance matrix explores how random or highly correlated distributions of multiple interventions can alter the overall impact on transmission.

The species composition of the local vector population is specified using local data where available, with each species having their own entomological parameters such as susceptibility to insecticides, propensity to feed on humans rather than animals and when they prefer to bite (i.e. when humans are indoors or in bed). Different mosquito species are assumed to act independently of one another. Mosquito pyrethroid resistance is incorporated into the model by linking the outcome of a discriminating dose bioassay to the performance of LLINs and IRS in experimental hut trials (Churcher *et al.*, 2016). As resistance increases, the maximum probability of a newly distributed LLIN killing or deterring a mosquito decreases. The

probabilities of an LLIN or IRS killing or deterring mosquitoes decay faster when mosquitoes are pyrethroid resistant, the insecticide concentration on the net diminishes and quickly reaches a level where it is wholly ineffective on local mosquitoes. As a result, the magnitude and length of LLIN impact are greatly reduced by pyrethroid resistance. Malaria prevalence and clinical incidence are predicted to rise as the severity of pyrethroid resistance increases, unless alternative methods of malaria control can be found.

1.3.3.5 Imperial College malaria model development

The deterministic version of the Imperial College malaria model was previously run in a software called Berkeley Madonna, there were two main issues with this: the model could not easily be run many times with varying sets of parameters and the model could not be run on the high-performance computing cluster. During my thesis I wrote a version of the Imperial College malaria model that runs in the statistical software R, this makes working with the model far easier since you can define your parameter values, run the model and then plot the output all within R. Although I did not develop the actual mathematical structure of the model, writing this updated version of the model involved a significant amount of work since it involved translating nearly 1000 lines of code between two programming languages. The code is available as an R package for other members of my research group, it is now annotated to make it easier to understand and has documentation to help new group members get started on working with the model. In the future, I hope to make the R package available online so that modelling results published by the Imperial College Malaria group are reproducible for anyone.

1.3.4 Model differences

All three models vary in their modelling assumptions and the complexity with which they choose to model certain aspects of malaria transmission. The different modelling assumptions create slightly divergent incidence-prevalence profiles for each age group (Cameron *et al.*, 2015). The different way that the models are fitted and run requires different amounts of computational power and time. One benefit of the Imperial College model is that it can be run on a personal computer and produce predictions several years into the future within a reasonable amount of time. This is because the Imperial College model is less complex since it does not model blood-stage parasite densities within each individual. Instead, a proportion of infected individuals develop clinical disease depending on acquired immunity to blood-stage infections that develops with age and level of exposure. The probability of a mosquito becoming infected after biting a human depends on the proportion of the population in each infectious state, with weightings given to each state depending on the likely level of

circulating gametocytes (i.e. asymptomatic and sub-microscopic cases have less gametocytes and are therefore less likely to infect (Bousema and Drakeley, 2011; Churcher *et al.*, 2013)). On the other hand, the Swiss TPH and Intellectual Ventures models explicitly model the parasite densities within each individual, the densities respond to host immunity and becoming super infected. In these two models humans become sick based on their parasite density passing a pyrogenic threshold that is based on age and previous exposure (Rogier, Commences and Trape, 1996; Smith, Ross, *et al.*, 2006). Both of these models also explicitly model gametocyte density in each individual and this is used to determine the likelihood that a biting mosquito becomes infected (Maire *et al.*, 2006; P. Eckhoff, 2012). Modelling within-host parasite dynamics makes the Swiss TPH and Intellectual Ventures models far more complex but does not seem to produce results that are wildly different to the Imperial College model when used in real-world modelling scenarios. For example, all three of the models summarised here were used in an ensemble modelling project that estimated the health impact and cost-effectiveness of the transmission blocking RTS,S malaria vaccine (Penny *et al.*, 2016). The authors conclude that "The estimated underlying protection against infection was similar across models during the first 18 months, but diverged as the projections extended beyond the trial period". This model divergence is likely to be due to precisely how transmission blocking immunity wanes in each model.

The three models also vary in the level of stochasticity that is involved in the modelling process. Models that have higher stochasticity will require more model runs to produce a prediction since they must account for variation that is introduced during the model run. As mentioned in Section 1.3.3.3 the Imperial College model produces uncertainty estimates by taking 1000 independent draws from the posterior distributions of each parameter (produced during the original model fit to incidence data from 23 sites across Africa) (Griffin *et al.*, 2010). The model is then run using each of these 1000 parameter sets to produce a range of uncertainty around the model prediction, this means that uncertainty in the parameter value is incorporated into an uncertainty around the model predictions. The Intellectual Ventures model used incremental mixture importance sampling to explore the parameter space and selected parameter sets with sufficiently high likelihood when producing model simulations for four sites in Tanzania and Nigeria (McCarthy *et al.*, 2015). The Swiss TPH model produces uncertainty by combining the results of an ensemble of up to 14 sub-models that employ different model assumptions for aspects of transmission such as immune decay, transmission heterogeneity, and access to treatment (Smith, Killeen, *et al.*, 2006).

1.4 Malaria in pregnancy

This section introduces the epidemiology of malaria in pregnant women, since it differs slightly from the epidemiology of the general population. These type of data (specifically the prevalence of infection in pregnant women) are used in Chapter 2 and Chapter 3 and are referred to throughout the thesis. Ante-natal clinics (ANC) can collect this type of data by recording the number of pregnant women that test positive for malaria parasites when they attend ante-natal clinic appointments.

Falciparum malaria can utilise particular variant surface antigens on the surface of infected erythrocytes to bind to chondroitin sulphate A (CSA), which is only present on tissue in the placenta (Feng *et al.*, 2009). These variant surface antigens are unrecognisable to an individual's immune response that has only previously experienced non-placental infections, which usually bind infected erythrocytes to the CD36 ligand (Fried and Duffy, 1998; Desai *et al.*, 2007). This means that a woman who has not experienced a *P. falciparum* infection during pregnancy before will be unable to prevent the parasite invading the placenta. This feature of placental infections mean that placental parasite infections can quickly grow to high parasite densities within the placenta and remain this way for a long period of time over the pregnancy (Desai *et al.*, 2007; Walker *et al.*, 2014). The prevalence of parasite infections during pregnancy is highest in primigravidae (women pregnant with their first child) and decreases with subsequent pregnancies because women have had more exposure to the CSA-binding variant surface antigen (Rogerson *et al.*, 2007). The effect of gravidity upon prevalence of infection is most pronounced in endemic settings where women are highly likely to have had a placental infection during a previous pregnancy. In low transmission settings women of any gravidity will have poorer immunity, predisposing them to high density parasite infections and more severe outcomes such as clinical episodes and maternal death (Ndam *et al.*, 2017).

Placental malaria infection has been found to be a significant cause of adverse birth outcomes. This is concerning given that an estimated 41.2% of women across Africa had a placental malaria infection at some point during their pregnancy in the year 2010 (Walker *et al.*, 2014). Placental malaria infections have been shown to be responsible for babies with low birth weight (LBW), intrauterine growth restriction (IUGR), and in rarer cases pre-term delivery or foetal death (Desai *et al.*, 2007). Malaria in pregnancy is responsible for between 75,000 and 250,000 infant deaths per year (Rogerson *et al.*, 2007), mostly in endemic

settings. In areas of high transmission placental parasite infections are the cause of nearly 20% of all LBW deliveries, 70% of IUGR, and 36% of pre-term deliveries (Desai *et al.*, 2007). Placental malaria infections are also strongly correlated with maternal anaemia, causing an estimated 26% of severe anaemia cases amongst pregnant women in high transmission settings (Desai *et al.*, 2007).

Mathematical modelling indicates that pregnancy-specific malaria immunity acts to clear placental infections quickly, rather than preventing placental infection from occurring in the first place (Walker *et al.*, 2013). Infections that existed before pregnancy that then become placental infections around 7-12 weeks into a new pregnancy when the placenta develops, have been estimated to account for 70% of cases of malaria in pregnancy (Walker *et al.*, 2013). In multigravidae women, the pre-existing infections that go on to invade the placenta will be cleared more quickly, which may be why there are fewer adverse birth outcomes in these women (Kalilani-Phiri *et al.*, 2013).

The timing of placental parasite infections during pregnancy has implications for the deployment of the pregnancy-specific malaria intervention intermittent preventative treatment during pregnancy (IPTp). Doses of anti-malarial drugs, usually Sulfadoxine-Pyrimethamine (SP), are given at regular intervals to clear existing infections and induce a prophylactic effect against parasite infection for up to 4-6 weeks (White, 2005). The WHO currently recommends a monthly dose of SP from the second trimester onwards within Africa (World Health Organization, 2013b). Presumptive IPTp to clear infections and the distribution of LLINs to prevent new infections are particularly appropriate methods for controlling malaria in pregnancy because they don't require a diagnosis of placental infection, which requires histology and so cannot be done easily before delivery (Kattenberg *et al.*, 2011). There is some evidence that RDTs (that detect the surface antigen HRP2) have a higher sensitivity than blood smear microscopy on both placental and peripheral blood samples but this has not been rigorously tested using placental histology as a standard reference (Kattenberg *et al.*, 2011; Fried, Muehlenbachs and Duffy, 2012). Pregnant women, especially those pregnant for the first time, often have infections with high parasite densities that are likely to be detected by RDT or microscopy (Gonçalves, Walker, *et al.*, 2017). The prevalence of infection in pregnant women is strongly associated with the prevalence of infection in children under five years old, many countries are exploring the routine surveillance of pregnant women as a way of monitoring malaria trends (van Eijk *et al.*, 2015; Willilo *et al.*, 2016).

1.5 Malaria in Eastern Democratic Republic of Congo

The data used in Chapter 2 on this thesis is collected in the Democratic Republic of Congo (DRC), one of the most malaria-endemic countries in the world (Hay *et al.*, 2010). In 2013 Nigeria and DRC accounted for 40% of global malaria cases (World Health Organization, 2014). The natural landscape of the DRC is highly variable, prevalence estimates from the 2007 DHS survey ranged from 0% to 82%, with cases being clustered together spatially (Messina *et al.*, 2011). Data available on malaria transmission from the DRC is poor due to the scale of the country and data collection being prevented by long-term violent conflict (Messina *et al.*, 2011). A UN peacekeeping mission has been present in the country since 1999, but violence continues to prevent the implementation of a robust health service in the Eastern part of the country (Ahoua *et al.*, 2006; Kalisya *et al.*, 2015).

The North and South Kivu regions of the DRC lie on the eastern border with Rwanda and Burundi. These two regions have been the focal point for decades of ethnic tensions and conflict, with an estimated 517,000 refugees and 1.5 million internally displaced peoples (IDPs) in 2015 (IDMC, 2016). Due to the widespread destruction of healthcare infrastructure (Kalisya *et al.*, 2015; Stasse *et al.*, 2015), the majority of healthcare provision for many people in the Kivus is provided by non-governmental organisations such as Médicines Sans Frontières (MSF). Where ministry of health structures do exist they are often supported by NGOs such as MSF. Refugees and IDPs often face barriers to healthcare, meaning that they face a higher disease burden. A recent study of an IDP camp in Eastern DRC found that the prevalence of infection for *P. falciparum* was higher there than in a nearby town with an indigenous population (Charchuk *et al.*, 2016). National malaria control and elimination programmes often neglect refugees and IDPs (Williams, Hering and Spiegel, 2013).

MSF operates in both “open” and “closed” camp settings, this refers mostly to whether the people living in them are in their own homes or are living somewhere else temporarily (Schmidt, 2003). Open settings are areas where indigenous communities that are living in their own homes (but may have IDPs living among them) are affected by conflict and the problems associated with this: poor access to healthcare, high insecurity and frequent flight. Closed settings are areas where people are living in temporary shelters having fled their homes. Closed settings can be formal, where things like settlement layout or food distributions are centrally planned, or informal, where groups of people fleeing violence settle in an area that may or may not have services nearby. The MSF sites in the DRC referred to in this work are all open settings and informal closed settings unless otherwise specified.

Malaria control in refugee and IDP settings has its own unique set of challenges due to poor security and the transient nature of the population. Historically MSF and other humanitarian organisations have found it difficult to use systematic control methods of vector control such as IRS, hence they have concentrated more on emergency distributions of LLINs, case management and

chemoprevention. An MSF survey in 2013 found that in North Kivu 77.62% of households owned an LLIN, which corresponded to 28.57% of the population each having their own LLIN (Isidro, Martín and Arnold, 2013). The survey found that beyond not owning an LLIN, the most common reasons people gave for not using an LLIN were that their LLIN was stolen or missing, or that they were unable to hang them (Table 1.1). Notably, 28 people (6.38% of the total) in the 'Other' category (Table 1.1) gave the reason that they did not have an LLIN because they were fleeing. At the same time, IRS is logistically hard to do given security concerns and its residual-efficacy is likely to be short given the precarious nature of housing. Humanitarian organisations are therefore seeking to invest in novel-low cost vector control interventions that may be more appropriate in some settings in which they operate. However, many of the products being considered have not been formally evaluated through WHO or other evaluation agencies so there is a need for robust pilot studies to build up the evidence base to justify future deployment.

Table 1.1: Reasons given for no LLIN use. Reproduced with permission from "Survey on Knowledge, Attitudes and Practice (KAP) survey of Long-Lasting Insecticide-treated bedNets (LLINs), in the Democratic Republic of Congo (DRC)" Martín, A., Arnold, M., Ariti, C., Siddiqui, R. MSF August 2013.

Reason for no LLIN use	**Number of people (% of total)**
Not enough LLINs	248 (56.49%)
LLIN is missing/stolen/sold	88 (20.05%)
LLIN is too difficult to hang	49 (11.16%)
LLIN is too hot to sleep under	4 (0.91%)
LLIN used for other things	6 (1.37%)
Other	44 (9.79%)
(missing)	1 (0.23%)
Total	439 (100%)

1.6 Conclusion

Malaria transmission is complex and there are many factors that need to be observed and understood before deciding the optimum method of control. Many malaria endemic areas also happen to be some of the poorest and most politically unstable areas in the world, which makes collecting the data needed to inform malaria control difficult.

This thesis will investigate how the epidemiological impact of novel vector control tools can be assessed in pilot studies in very low resource environments. The thesis firstly examines how the routine testing of pregnant women in IPTp programmes in refugee camp settings in the DRC can be used to monitor malaria transmission. It does this by assessing the relationship between the prevalence

of malaria in pregnant women and the clinical incidence of disease, a metric central to malaria control programmes and cost-effectiveness analysis but rarely reliably recorded in areas of humanitarian need. It will then go on to expand an existing mathematical model of *P. falciparum* malaria to investigate the public health impact of volatile pyrethroid emanators. Their utility shall initially be assessed in populations that do not own an LLIN (similar to many refugees or IDPs) before examining how they could be used to prevent biting during the evening (a time when there are no widely used effective vector control tool). This work highlights the potential epidemiological importance of sub-lethal exposure of mosquitoes to pyrethroids. The importance of this feeding inhibition effect shall be further investigated using a meta-analysis of experimental hut trial data to determine how it may influence the efficacy of LLINs in areas with pyrethroid resistant mosquitoes. This will have important implications for the use of LLINs in humanitarian settings and more broadly across Africa as local mosquito populations become increasingly resistant to pyrethroid insecticides.

2 Comparing the malaria burden faced by MSF to other data sources for eastern Democratic Republic of Congo

2.1 Introduction

Comparison of prevalence of malaria infection estimates allows governments and malaria control programmes to assess the malaria burden difference between locations, or at the same location at different times. Accurately measuring the prevalence of infection is difficult due to the need to find an accessible, unbiased sample of the population who can then be tested using microscopy or RDTs. When starting malaria control programmes, measuring the change in the prevalence of infection before and afterwards will evaluate how effective the control measures have been. The prevalence of infection is a good measure of disease endemicity because it is robust to changes in the population size. Estimating the prevalence of infection using rapid diagnostic tests (RDTs) will also detect asymptomatic malaria infections in the population as well as clinical episodes. In endemic settings, the number of asymptomatic infections is a good indicator of the level of malaria transmission in the immediate past.

In the hospitals and health centres in the Democratic Republic of Congo (DRC) where MSF work, the only consistent malaria prevalence data available is for pregnant women attending ante-natal clinics. Free ante-natal clinic (ANC) appointments are provided to all expectant mothers, anecdotal evidence from MSF suggests that most pregnant women around the clinics attend at least one ANC appointment. During each ANC appointment an RDT is used to screen for infection whether the woman is symptomatic or not; women that test positive are treated according to the protocols of MSF and the country within which they are operating. Those that test negative are given Sulphadoxine Pyremethamine (SP).

The usual practice for routine malaria surveillance is to measure the prevalence of infection in children between 0 and 5 years old. The prevalence estimates for infants and pregnant women cannot be compared directly, since differences in pregnancy and age alter malaria immunology (Sections 1.1.5 and 1.4). Age correlates strongly with the level of immunity to blood-stage infection when transmission is reasonably high, such as in the DRC (Carneiro *et al.*, 2010; Griffin, Ferguson and Ghani, 2014). Women who have reached child-bearing age will likely have had many previous clinical episodes of malaria that allow them to suppress the parasite density of new infections when they are not pregnant, leading to ongoing asymptomatic infections (Doolan, Dobaño and Baird, 2009). Pregnant women are particularly susceptible to placental parasite infections, which their immune system will not be able to prevent unless they have previously experienced malaria during pregnancy (Desai *et al.*, 2007). On the other hand, young children have low acquired immunity due to fewer

previous infections and are therefore more likely to experience clinical episodes of malaria instead of ongoing asymptomatic infections.

The goal of this chapter is to ascertain whether the malaria burden, in terms of the prevalence of infection in pregnant women, observed by MSF at their sites in North and South Kivu in the DRC, is higher than would be expected for the region. This information would support MSF logistical planning and inform global distribution maps of malaria burden. There is anecdotal evidence from MSF staff that the burden of malaria experienced at their sites is far higher than would be expected for North and South Kivu using Demographic Health Survey data (USAID, 2013), the results of this chapter will allow MSF to answer this question empirically. This goal is achieved by fitting nested statistical models to a dataset comparing the prevalence of infection in pregnant women and prevalence of infection in children under 5 years old across Africa. The statistical model allows for conversion between the prevalence of infection in pregnant women and children under 5 years old. This allows for comparison between the estimates of the prevalence of infection in children produced by the model for MSF sites and observations from larger field studies in the North and South Kivu region.

A meta-analysis of 18 studies containing 57 sub-studies (where one study has multiple sites or sampling times) from across Sub-Saharan Africa that measured prevalence of infection in pregnant women and children under 5 simultaneously found that the prevalence of infection in children is generally higher than that of pregnant women, with a strong positive correlation between the two prevalence measures (van Eijk *et al.*, 2015). This is understandable, since a change in the number of infectious bites received by all human hosts will simultaneously increase or decrease the prevalence of infection in all sub-populations to differing degrees – dependent upon the immunological factors mentioned earlier. However, a measure of correlation does not allow for conversion between the prevalence of infection in each group. In this Chapter I build upon the analysis done by van Eijk *et al*. (2015) to fit a flexible Bayesian regression model that predicts the prevalence of infection in children under 5 years using the prevalence of infection in pregnant women as an explanatory variable. The prevalence of infection in pregnant women observed by MSF in the DRC is converted into an estimate of the prevalence of infection in children under 5, which is then compared to Demographic and Health Surveys (DHS) data for the corresponding North or South Kivu region of the DRC. This will determine whether the malaria burden observed at MSF hospitals and health centres is comparable to that found in the DHS data, which has a much wider geographical scope and is widely used to assess the impact of malaria control initiatives.

In addition, the estimated prevalence of infection in children under 5 at the MSF site Walikale is then compared to a recent estimate of the prevalence of infection in children under 5 years old living in an IDP camp in Walikale. The survey aimed to quantify the disparity in malaria burden between children

under 5 in an IDP camp and children under 5 living in a neighbouring village, both of which were in Walikale (Charchuk *et al.*, 2016). The survey tested 200 children in the IDP camp and 200 children in a nearby village, finding that the prevalence of infection was significantly higher in the IDP camp (17.5% compared to 7.5% in the village). The main reason for this discrepancy was thought to be lack of LLIN use and ownership in the IDP camp, lack of education regarding malaria prevention in the mothers in the IDP camp compared to the village, increased vectoral capacity in the IDP camp due to standing water providing breeding grounds for mosquitoes, and a lack of shelter leaving children more exposed to mosquito biting at night. Walikale is the site of a lot of population movement due to conflict, with 7% of the one million IDPs in North Kivu living in Walikale, as well as 10.8% of those IDPs living in displacement camps in North Kivu originating from Walikale (MSF, 2017). Due to the amount of population displacement, it is interesting to determine whether the malaria burden observed by MSF at Walikale is closer to that of the local village or IDP camp.

2.2 Methods

2.2.1 Data

The van Eijk *et al.* (2015) dataset was collated from a systematic review of studies that contemporaneously measured the prevalence of infection in children and in pregnant women. Data from the 57 sub-studies were collated between 1983 and 2015 in a range of countries in Sub-Saharan Africa. In this analysis the number of women and children tested, as well as the number of tests that returned a positive result, were extracted from the manuscript.

The DHS data used in this analysis refers to the Democratic Republic of Congo Standard DHS survey conducted between 2013 and 2014 (USAID, 2013). Households were randomly sampled in each province and children under 5 were tested for malaria by microscopy. DHS surveys are repeated approximately every 5 years and form the basis of much modelling work that predicts malaria burden reduction over time (Bhatt *et al.*, 2015).

The MSF data used in this analysis is collected routinely at ante-natal care clinics at 4 sites in North and South Kivu where MSF operate hospitals and health centres (Figure 2.1). At every ante-natal clinic visit every pregnant woman is tested by RDT, each month the number of women tested, and the number of positive tests is totalled up. The months of data used coincide with the time during which the DRC DHS survey 2013-14 was being carried out.

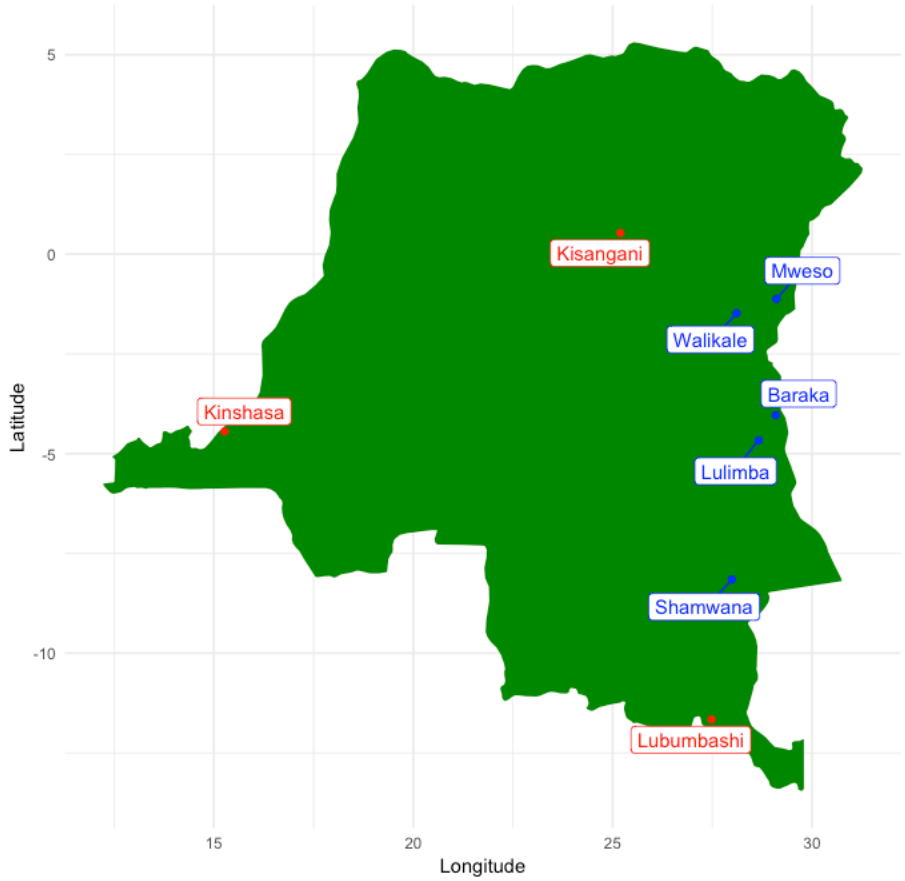

Figure 2.1: A map of the Democratic Republic of Congo. Points in blue are the five locations where MSF work that are examined in this work (only four are introduced in this chapter, Chapter 3 also uses data from Shamwana). Points in red are three large towns in DRC, including the capital Kinshasa, to contextualise the map.

2.2.2 Bayesian log-odds regression

Two flexible, nested, Bayesian models were fitted to the dataset compiled by van Eijk et al. (2015), containing the estimates of the prevalence of infection in pregnant women and in children. The first model (referred to as the simple model) fitted a linear relationship between the log-odds of the prevalence in each group (Sharp and Thompson, 2000; Wu *et al.*, 2015). The log-odds of the prevalence of infection in pregnant women in the i^{th} study is denoted Ω_i^{ANC} and is given by:

$$\Omega_i^{ANC} = log\left(\frac{r_i^{ANC}}{r_i^{ANC} - n_i^{ANC}}\right), \qquad 2.1$$

where r_i^{ANC} is the number of pregnant women that tested positive in the i^{th} study and n_i^{ANC} is the number of pregnant women tested. It is assumed that the number of pregnant women that tested positive was distributed binomially with an unknown probability of success π_i^{ANC},

$$r_i^{ANC} \sim Bin\left(n_i^{ANC}, \pi_i^{ANC}\right). \qquad 2.2$$

The log-odds prevalence of infection in children (denoted by superscript *INF*) can be calculated in the same fashion using a parallel assumption about the number of children that tested positive in each trial:

$$\Omega_i^{INF} = log\left(\frac{r_i^{INF}}{r_i^{INF} - n_i^{INF}}\right) \quad 2.3$$

$$\mathrm{r}_i^{\mathrm{INF}} \sim Bin\left(\mathrm{n}_i^{\mathrm{INF}}, \pi_i^{\mathrm{INF}}\right) \quad 2.4$$

The model fits a linear relationship between the log-odds of the prevalence of infection in pregnant women (*ANC*) and children (*INF*),

$$\Omega_i^{INF} = \Omega_i^{ANC} + \delta_i \,. \quad 2.5$$

Here, δ_i is the log-odds ratio that quantifies the difference between the prevalence of infection in each group for the *i*th study. This can be further broken down into:

$$\delta_i = \delta_i' + \beta\Omega_i^{ANC} \quad 2.6$$

The value δ_i' is the expected log-odds ratio for the *i*th study, which is assumed to be normally distributed across studies: $\delta_i' \sim Normal(\delta, \tau)$. This means that δ is the mean log-odds ratio between the two groups over all studies and τ is an estimate of much this varies between studies. The variable β is the regression coefficient, meaning that changes in Ω_i^{ANC} have a linear effect on the log-odds ratio between the two groups, which matches previous work comparing malaria diagnostics (Wu *et al.*, 2015). The second model (referred as the interaction model) modifies Equation 2.6 to include the malaria diagnostic used (denoted m_i) as a categorial variable and as an interaction effect with endemicity:

$$\delta_i = \delta_i' + \beta\Omega_i^{ANC} + \beta_1 m_i + \beta_2 m_i \Omega_i^{ANC} \quad 2.7$$

The interaction model uses the same assumptions as the simple model regarding the distributions of the number of women and infants testing positive by RDT and the log-odds ratio (Equations 2.1-2.5). The additional diagnostic terms show whether the relationship between the prevalence in each group changes depending on the diagnostic method used. The models were fit using the statistical software WINBUGS, which uses a Markov chain Monte Carlo method to fit Bayesian models (Lunn *et al.*, 2000). The unknown parameters $\delta, \tau, \beta, \beta_1$, and β_2 were given flat priors (described in Table 2.1) and

their final values were estimated by taking the mean of >500 effective posterior samples for each parameter thinned from 4 Markov chains run in parallel.

The fit of each model was assessed using a leave one out cross-validation method, whereby the model is fit to all data except for one point. This unseen data point is then predicted and the accuracy of all of the predictions is recorded. This process is repeated for each data point and the correlation coefficient between the predicted and observed values gives an indication of how well the model fits the data. Each model run in the leave-one-out analysis ran 4 MCMC chains for 20000 iterations in WINBUGs. The two models are also compared using their Deviance Information criterion (DIC), which gives a relative score based on the predictive ability of each model. The model with the smallest DIC value is the preferred model.

2.3 Results

2.3.1 Relationship between metrics for all data

The two log-odds regression models were fit to 57 studies from 13 different countries in Sub-Saharan Africa, where a total of 54,798 children between 0 and 5 years old, and 9,205 pregnant women were tested using either diagnostic. Of the tests performed on children, 22,845 of them (41.7%) were undertaken by RDT, where the estimated prevalence ranged from 0.5% to 70%. The other 31,953 tests on children (58.3%) were undertaken by microscopy, where the estimated prevalence ranged from 0.4% to 78%. In pregnant women 2,563 of the tests (27.8%) used an RDT, finding an estimated prevalence range between 0% and 45.8%. The other 6,642 tests (72.2%) were performed using microscopy and estimates of prevalence ranged between 0% and 47.1%.

The simple model (Equation 2.6, no diagnostic terms) found that the prevalence of infection in children was higher than that in pregnant women. This was the case across all endemicity levels, with the gap between the prevalence in each group growing as the prevalence of malaria increased (Figure 2.2A). The 95% credible interval calculated from the posterior distributions of the fitted parameters did not include the possibility that the prevalence of infection in the two groups could be equal at any endemicity level (other than zero). The relatively simple shape of the fitted model broadly captures the observed relationship though there is wide variability in the observed data points away from the best fit line.

2.3.2 Relationship between metrics for different diagnostic methods

Including the diagnostic used as a variable in the model slightly improved the model DIC from 777.6 for the simple model to 775.3 for the interaction model, indicating a modest improvement in predictive power when stratifying by malaria diagnostic used. The interaction model (Equation 2.7)

found that the difference between the prevalence of infection in pregnant women and children was larger when studies used RDTs as the diagnostic method (19 trials out of 57, Figure 2.2B). The disparity between the prevalence of infection in the two groups was both generally larger when measured by RDT (Table 2.1, β_1) and this disparity grew larger as endemicity increased (Table 2.1, β_2). Trials using microscopy as a diagnostic showed a lower difference between the prevalence in each group (Figure 2.2C). When using microscopy as a diagnostic, the 95% credible interval of the relationship between the prevalence in each group included the possibility that the they might be equal at very high endemicity (>50% prevalence in each group).

The estimated values for the model parameters for both models are shown in Table 2.1. In the interaction model, the value of β_1 is negative, showing that the expected log-odds ratio between the prevalence in each group (the general difference between the prevalence of infection in each group before considering a linear endemicity effect) is higher for studies using RDTs. In the simple model, the 95% credible interval for the parameter β contains negative and positive values for the model fit to all data or studies that used microscopy. This means that the simple model cannot definitively say that the gap between the prevalence of infection in each group grows as the prevalence of infection in pregnant women (a proxy for transmission intensity) increases. In the interaction model the value of $\beta_2 + \beta$ is negative, which explains why in Figure 2.2C the relationship bends downwards as the prevalence of pregnant women increases. The interaction model fit for studies that used RDTs does find that the difference between the prevalence of infection in each group widens as transmission increases.

Both models performed well at leave-one-out cross-validation (Figure 2.3). The Pearson correlation coefficient between the values predicted by each model using the unseen data and the actual observed data was 0.863 for the simple model and 0.867 for the interaction model. The cross-validation results are shown visually in Figure 2.3, comparing the observed prevalence of infection in children with their out-of-sample estimate. Both models show a good level of agreement between the model prediction and the excluded observations with no significant bias in these data, suggesting that the linear relationship (on the log scale) adequately captures the underlying differences.

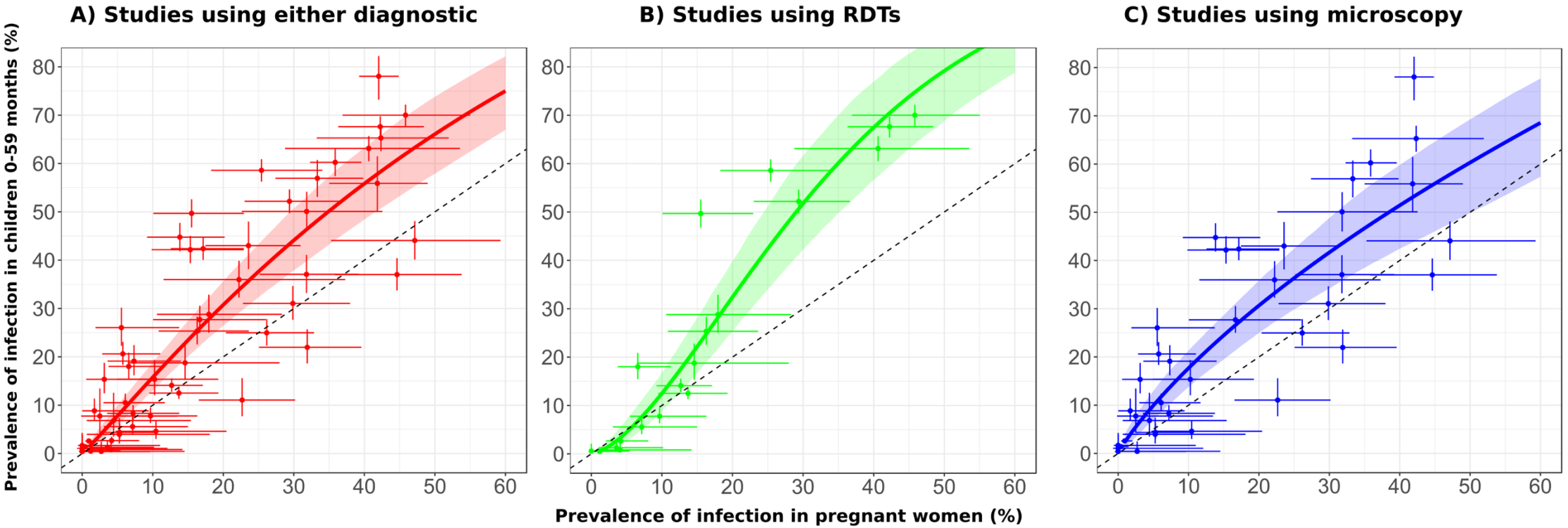

Figure 2.2: The cross-sectional relationship between malaria prevalence in children under 5 years and pregnant women fit by the simple model (A - red), fit by the interaction model to studies using RDT (B - green), and fit by the interaction model to studies using microscopy (C - blue). Points show observed values from the studies and error bars show corresponding 95% confidence intervals. The thick, coloured lines display the best fit log-odds regression model fit with the shaded interval around the line denotes the 95% credible interval constructed from posterior samples of the model parameters. The dotted black line shows where the prevalence of infection in each group would be equivalent.

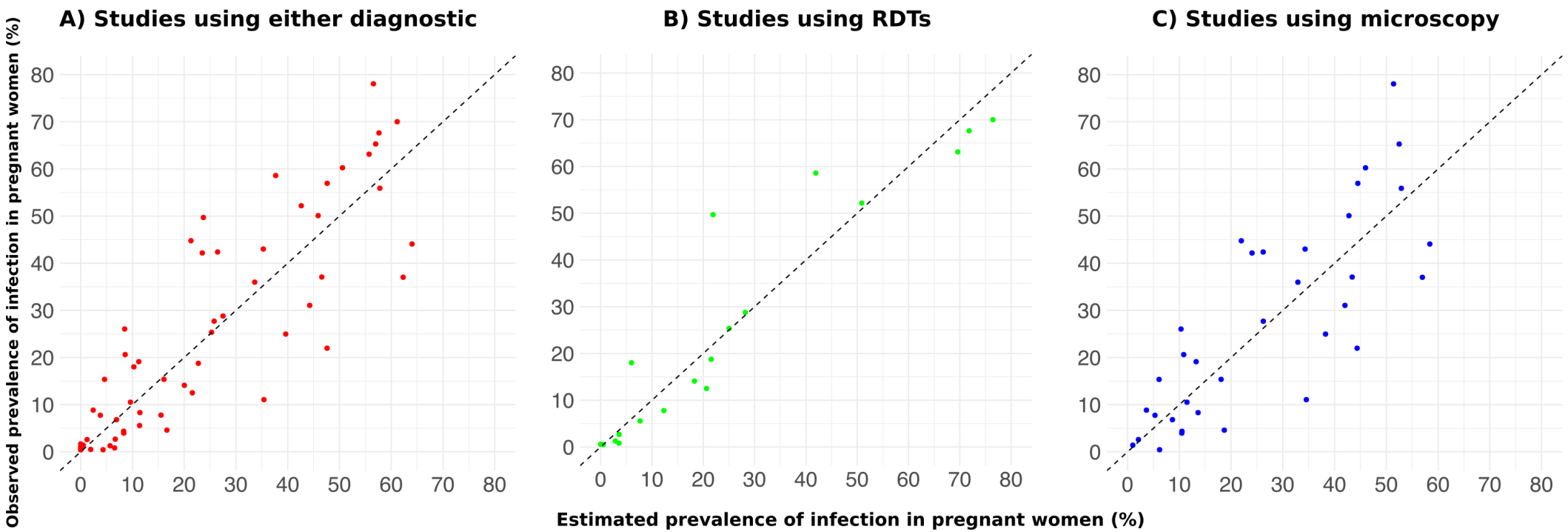

Figure 2.3: Estimates of the accuracy of model predictions using the leave-one-out cross validation procedure. The x-axis displays the estimated prevalence of infection in pregnant women from the simple model fit (A – red), interaction model for RDT (B – green), and interaction model for microscopy (C – blue). The y-axis shows the observed prevalence of infection in pregnant women for the corresponding study. The dotted black line denotes where the prediction is equal to the observation, points close to this line are accurate predictions. The fitted models predict the relationships relatively accurately, with points falling either side of the line for the whole range of endemicities.

*Table 2.1: A breakdown of the priors used for each parameter in the three log-odds regression models and the posterior mean and 95% credible interval. Credible intervals marked with a * contain the possibility that the parameter value is equal to zero.*

Parameter	**Prior**	**Simple model - Equation 2.6 Posterior mean (95% credible interval)**	**Interaction model – Equation 2.7 Posterior mean (95% credible interval)**
δ	N(0,1000)	0.672 (0.353,1.035)	1.241 (0.579,1.859)
β	N(0,10000)	0.072 (-0.095,0.246)*	0.450 (0.143,0.799)
τ	Gamma(0.1,0.001)	2.4996 (1.371,4.084)	2.829 (1.638,4.518)
β_1 (baseline = microscopy)	N(0,10000)	-	-0.820 (-1.588,-0.074)*
β_2	N(0,10000)	-	-0.550 (-0.936,-0.194)*

2.3.3 Comparison between MSF and DHS data

MSF measure their prevalence in pregnant women using RDTs and the DHS surveys measure their prevalence of infection in children using microscopy, so the simple model that doesn't differentiate between diagnostic was used to convert the prevalence of infection in pregnant women observed by MSF into estimates of the prevalence of infection in children. These predictions can then be directly compared with the corresponding regional estimates of the prevalence of infection in children collected by the DHS. The DHS data for North and South Kivu were collected between November 2013 and January 2014, therefore the prevalence of infection in pregnant women for each of these 3 months measured at each MSF location were used to generate predictions from the simple model. The results are shown in Figure 2.4. At all locations the estimated prevalence of infection in children from MSF data was significantly higher than that found by the DHS survey. In North Kivu, the prevalence of infection in children measured by the DHS was 5.3%, nearly four times less than the 22.3% estimated prevalence of infection in children predicted using the MSF data from Walikale (Figure 2.4A).

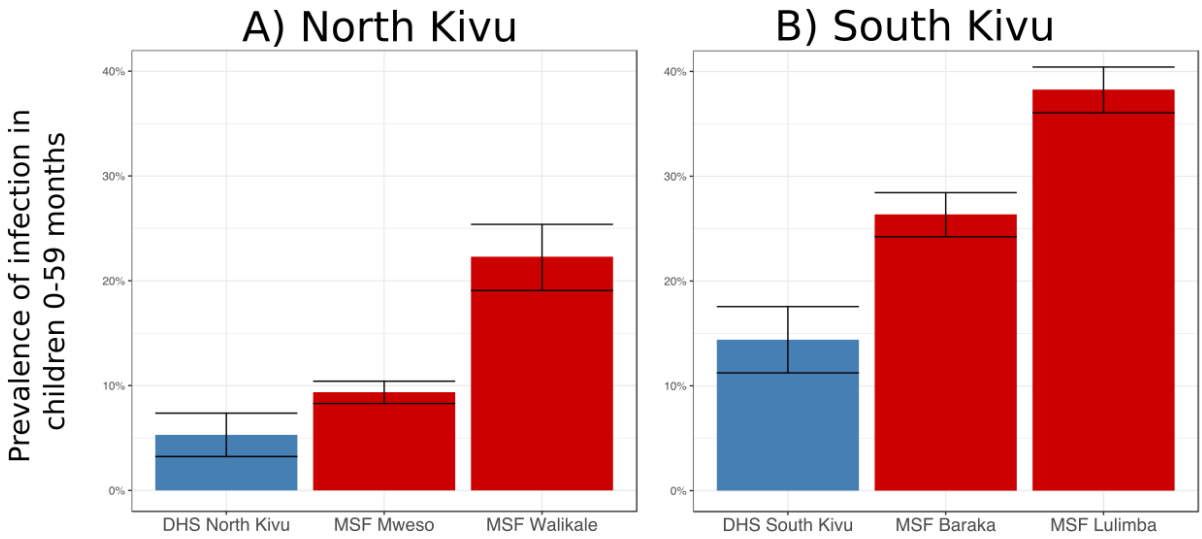

Figure 2.4: A comparison of the observed prevalence of infection in children from the DHS data (blue bars) and the predicted prevalence of infection in children for the refugee camp settings in which MSF operates in the area (using the log-odds regression model fitted to data from all studies) (red bars). The error bars for the DHS data show 95% confidence intervals using a normal approximation method. The error bars around the log-odds regression predictions show 95% credible intervals for the prediction (See Figure 2.2A).

2.3.4 Comparing MSF, DHS and IDP camp data

The prevalence of infection in children estimated from MSF aggregated data can also be compared to a separate study that tested children living in an IDP camp or local village in the "Walikale district" of North Kivu during August 2013. Figure 2.5 shows the estimated prevalence of infection in children using the MSF data for August 2013 (red), the DHS estimate for the North Kivu region in 2013 (blue, data collected Nov 2013-Jan 2014) and the estimates taken in the IDP camp and neighbouring village in August 2013 (purple). The prevalence estimate taken in the village in Walikale is close to the province-wide estimate for North Kivu performed by the DHS, whereas the prevalence estimate for the IDP camp is higher. Slightly higher than that still is the prevalence estimate calculated using the log-odds regression model on the aggregated data for MSF sites in Walikale.

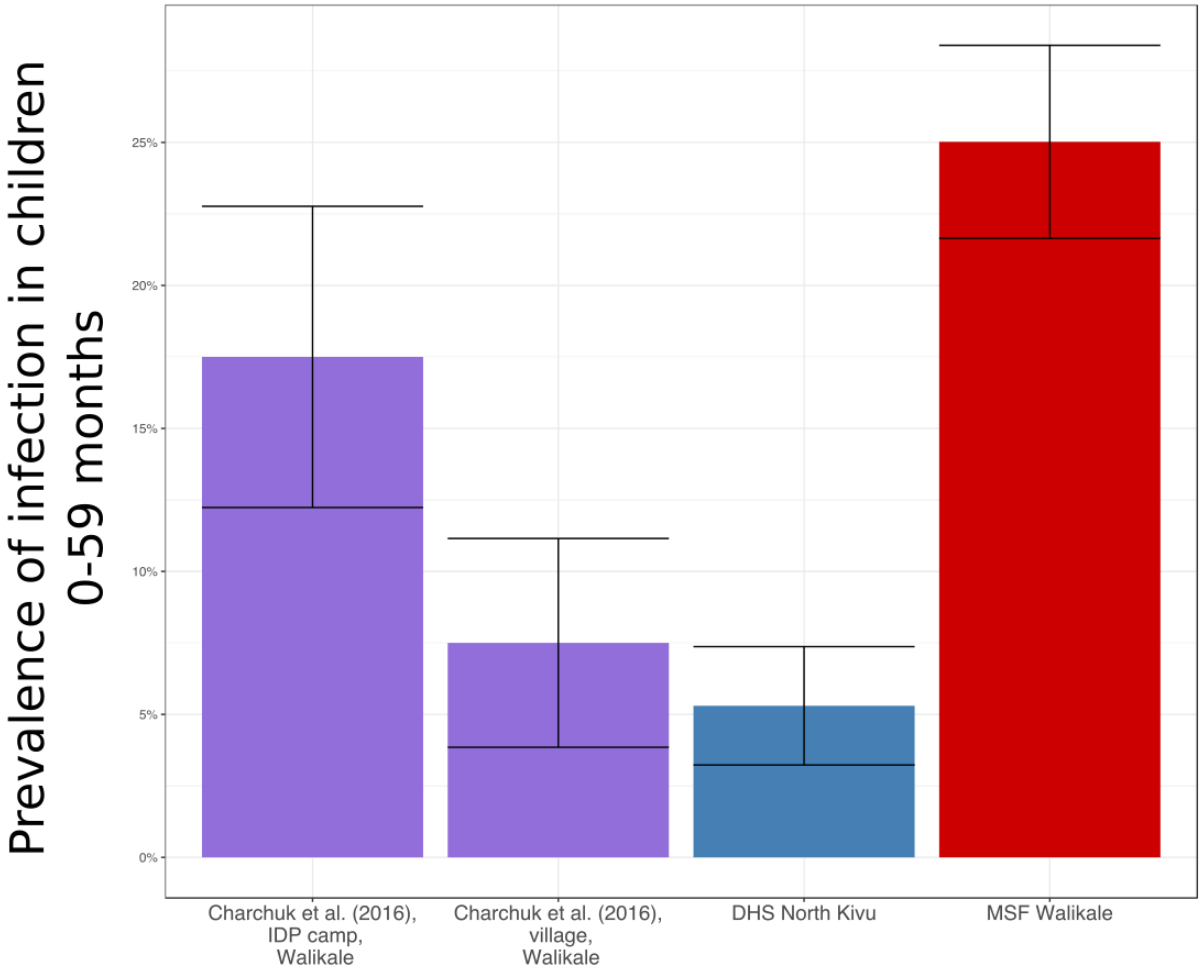

Figure 2.5: A comparison of 3 different estimates for the prevalence of infection in children in Walikale. Firstly, the estimates from Charchuk et al. (2016) testing children in a local village and an IDP camp and their corresponding 95% confidence intervals using the normal approximation method (purple). Secondly, the estimate from the DHS survey in North Kivu and corresponding 95% confidence interval using the normal approximation method (blue). Finally, the predicted prevalence of infection from the simple log-odds regression with corresponding 95% credible interval for the prediction (red).

2.4 Discussion

The estimates of the prevalence of infection in children derived from the MSF clinical data predict a substantially higher malaria burden in the areas where MSF work than the corresponding province-wide estimate calculated from the DHS dataset. The malaria burden faced by MSF could differ from the regional average because of spatial heterogeneity in malaria transmission due to environmental factors such as land use, and/or because of demographic factors that make the local population particularly susceptible to malaria. Local variations in land use support different density mosquito populations (per person) due to the availability of suitable breeding sites (Lindblade *et al.*, 2000; Paul, Kangalawe and Mboera, 2018), meaning that households in some places face far more infectious bites than the region average. Other environmental factors that might give MSF sites higher malaria burden than normal could include altitude (Bødker *et al.*, 2003), the amount of rainfall (Okuneye and Gumel, 2017), and vegetation cover (Ricotta *et al.*, 2014). However, spatial heterogeneity in transmission is

unlikely to explain why every MSF location examined here is facing a higher malaria burden that the province-wide average, since the MSF locations are also spread around the North and South Kivu regions with different environmental factors at each site. Instead, what is common to all of these areas is human displacement, with IDPs living in local communities and local communities occasionally having to leave their homes for safety reasons (MSF, 2017).

Additionally, the disparity in malaria burden between locations in North and South Kivu could reflect different levels of knowledge about malaria prevention (Obol, David Lagoro and Christopher Garimoi, 2011; Qayum *et al.*, 2012), or different abilities to afford, maintain and correctly use insecticidal bed nets (Brooks *et al.*, 2017). Bed nets are easier to hang and maintain in more permanent structures and housing quality has been shown to be a strong predictor for disease prevalence (Tusting *et al.*, 2017). It may also be due to how well serviced each community is by healthcare providers. At MSF sites, the lack of available healthcare outside of an emergency relief NGO means that people attending MSF hospitals are not provided with organised, competent healthcare in any other sense. As previously discussed, MSF have previously had to focus on case management and emergency LLIN distributions (except for LLINs handed out to pregnant women during intermittent preventative treatment in pregnancy). This policy has improved somewhat, MSF now study entomology and pyrethroid resistance to guide their LLIN choice and also consider larviciding, though the amount of disease prevention in the sites where they work is currently unreported and unclear. Some risk factors, such as net ownership and malaria prevention knowledge were highlighted as potential explanations for the large difference in the malaria burden between the village and IDP camp in Walikale in North Kivu (Charchuk *et al.*, 2016). The MSF Knowledge, Attitudes and Practice survey undertaken in North Kivu in 2013 found that 51.3% of people gave preventing malaria as their reason for using an LLIN and that 78.1% of people identified sleeping under an LLIN as a way of preventing malaria (Isidro, Martín and Arnold, 2013). The MSF burden prediction for Walikale is far closer to that observed in the IDP camp in Walikale than the DHS estimate for North Kivu as a whole. Combined with the consistent presence of IDP camps around MSF locations, this strongly indicates that the reason for the unexpectedly large malaria burden observed by MSF is due to the high malaria burden faced by IDPs who access their healthcare systems and the impact of conflict on the local communities living in that context. There could be an additional, compounding factor in that displaced communities may be forced to settle in undesirable locations, which have been historically rejected by indigenous populations due to the high malaria transmission potential.

This analysis quantified the relationship between malaria prevalence in children under 5 years and pregnant women, in line with the findings of van Eijk et al. (2015) it found that the observed prevalence of infection in children is generally higher than that found in pregnant women. A question that emerged from fitting the interaction model that stratified by malaria diagnostic is why the gap

between the prevalence of infection in each group widens as transmission increases in studies using RDTs, but not those which use microscopy. In the meta-regression by van Eijk et al. (2015) the type of test used did not significantly affect the pooled prevalence ratio, the ratio between prevalence of infection in children and prevalence of infection in pregnant women found in each study. This may be because they did not consider an interaction between the type of test used and endemicity in their model. In my analysis the slope of the regression ($\beta + \beta_2$) in the interaction model is positive and significant for studies using RDTs, but not for studies using microscopy. Therefore, when using RDTs as a diagnostic, the log-odds ratio between the prevalence in the two groups gets larger as transmission intensity increases. It has been found that RDTs are sub-optimal at detecting parasites sequestered in the placenta (Fried, Muehlenbachs and Duffy, 2012), which can help explain why the observed prevalence of infection in pregnant women is lower than that of children in general, but not why the gap between the two prevalence measures increases with transmission intensity, or why this effect only occurs when using an RDT since microscopy also struggles to detect placental infections (Uneke, 2008). A plausible explanation for why the disparity between the prevalence of infection in each group increases with endemicity more noticeably for RDTs could be due to RDTs returning a positive result for up to 4 weeks after parasite clearance (Grandesso *et al.*, 2016). As discussed above, at different levels of malaria endemicity the types of malaria infection experienced by children and pregnant women are likely to be different. At all transmission levels, any new malaria infection is more likely to result in a clinical episode for a child, whereas pregnant women are likely to carry ongoing, asymptomatic infections or placental infections that last the duration of their pregnancy. When transmission is higher, more children will have recently had clinical episodes and many of them will have been treated with anti-malarial drugs. These children will test positive by RDT because parasite proteins will remain in the bloodstream, but not by microscopy since parasites will have been cleared from their blood. Additionally, when the number of infectious bites is high, many pregnant women receiving an infectious bite will already have an ongoing infection, so these new infectious bites do not translate directly into new detectable infections by RDT or microscopy. This explains why the disparity in the prevalence of infection between the two groups grows as transmission intensity increases for RDT-based studies only. The improvement in DIC resulting from stratifying by diagnostic in the model was only modest, providing limited evidence that it is important to include these diagnostic terms. These models could be re-fit in the future when there are more RDT studies available, which could help to determine whether stratifying by diagnostic is important.

The results from this chapter agree with the studies performed in the IDP camp in Walikale, finding that, at all of the locations where MSF operate in North and South Kivu, the malaria burden experienced is far higher than would be expected from DHS data. This stark contrast highlights a potential flaw in the data collection methodology of DHS. Visiting randomly chosen villages of stable, established communities and testing children there could miss transient populations such as IDPs and refugees from other countries (although in the context of this data it is mostly the former).

Therefore, when trying to accurately estimate the malaria burden at locations where there are influxes of IDPs it is preferable to consult localised, smaller scale data sources such as the MSF clinical data rather than province-wide estimates such as the DHS survey data. Sites with IDPs act as pockets of intense malaria transmission within endemic regions; which is not just concerning due to the heavy burden on IDPs but also jeopardises the wider effort to control malaria. Transmission cannot be stopped in local indigenous populations, no matter how much effort is put into malaria control, if they are close to an IDP camp that can re-seed transmission endlessly. There have already been calls for malaria control programmes to explicitly plan how they are to help displaced populations, since failing to do so could prevent them from reaching elimination at the country level (Williams, Hering and Spiegel, 2013). The fact that DHS data underestimates the burden at MSF sites is problematic because DHS data is the current go-to source for malaria burden information for many inaccessible or politically unstable regions of Sub-Saharan Africa, which are likely to experience similar issues to the MSF sites used in this analysis.

The work has important implications for MSF. It increases the evidence base that the malaria burden in their camps in eastern DRC have a substantially higher malaria burden than the surrounding area. It is an indication that organisations working in refugee camp or conflict settings should expect higher disease prevalence than that collected by normal routine methodology which is utilised and presented by online sources such as the Malaria Atlas Project (Gething *et al.*, 2016) and the World Malaria Report (World Health Organization, 2017b). A greater level of intervention will likely be needed in these settings to achieve the same level of control even before the difficulties of working in these types of settings are considered. One drawback in the models presented in this chapter is that they do not consider the number of children that a woman has previously had before the current pregnancy. As discussed in Section 1.4, women can develop immunity to placental infections over successive pregnancy, which can make it harder for RDTs to detect low density infections (Desai *et al.*, 2007). Similarly, in the van Eijk dataset it is not known whether women attending the ante-natal appointments are doing so for the first time. If women are attending more than one appointment, they may have tested positive and had their infections cleared at the previous appointment. This could account for some of the observed variation in the observed prevalence between sites, since at sites where women are more likely to attend multiple appointments they would be more likely to have had previous treatment and test negative. Ultimately, the reason for the poor accuracy of the model for some sites is unknown. Some of the variation in observed prevalence could be due to measurement error, there are often large discrepancies between RDT and microscopy measured prevalence at the same site (Wu *et al.*, 2015).

2.5 Conclusion

The malaria burden faced at the locations that MSF work at in North and South Kivu is higher than the province average, sometimes considerably so. This chapter explored the evidence that the reason for this higher burden could be due to populations of internally displaced people living in North and South Kivu, who have a higher prevalence of malaria compared to indigenous populations. It seems likely that this is the case, given that the prevalence of infection in children at Walikale estimated from the MSF data is so similar to that observed in the IDP camp in a different study. This has implications for those monitoring the progress of malaria control efforts in areas where IDPs are living, since DHS has been shown to underestimate the true malaria burden. The next chapter builds on how MSF could use their own routinely collected data on the prevalence of infection in pregnant women, which could be used to inform themselves of malaria transmission trends around their health facilities, moving from comparing point estimates generated by the log-odds regression model to different statistical models that will capture how the prevalence of infection in pregnant women changes along with malaria transmission over time.

3 Modelling the temporal relationship between prevalence of infection in pregnant women and clinical incidence in children under 5 years old

3.1 Introduction

The last chapter derived a non-temporal relationship between the prevalence of infection in children and the prevalence of infection in pregnant women. It established that the prevalence of infection in the two groups is strongly correlated, with some of the variation between the groups due to different levels of host immunity and performance of malaria diagnostics. The prevalence surveys used to inform this relationship were infrequent and conducted over long periods of time. This chapter will explore the relationship between the prevalence of infection in pregnant women and another malaria transmission measure, clinical incidence in children under 5 years old. This will provide an understanding of how the prevalence of infection in pregnant women responds to changes in malaria transmission on a finer time scale. If the prevalence of infection in pregnant women reflects changes in malaria transmission quickly and accurately, this could allow those in charge of malaria control programmes to use the prevalence of infection in pregnant women to monitor trends in malaria transmission or to evaluate the performance of their control programme. Clinical incidence is also the most operationally useful metric for MSFs as it indicates the level of demand seen in the field sites in which they operate and will determine logistic requirements.

Currently, Africa-wide estimates of burden reduction primarily utilise cross-sectional survey data conducted by the Demographic and Health Surveys Program (USAID, 2013; Bhatt *et al.*, 2015). These surveys are undertaken at the province level, usually every 2-3 years, where children are tested for malaria in randomly selected clusters. These province-wide estimates can hide substantial spatial heterogeneity generated by local healthcare provision or local geographical, demographic or climatic differences, therefore populations in some areas face higher malaria burdens than the province-wide average (Sturrock *et al.*, 2014; Charchuk *et al.*, 2016). Finer scale estimates of burden can be collated passively using the number of malaria cases reported from local health centres. To generate meaningful incidence rates requires good estimates of the size of the health catchment population, which is unlikely to be available in many parts of Sub-Saharan Africa. The problems are exaggerated in humanitarian settings where populations may be highly transient, or size estimates hard to generate due to security concerns or resource constrains. This is especially the case in 'open' chronic conflict settings where displaced populations often live amongst the local population and not in a defined enclosed area or are frequently on the move due to insecurity. In recent years MSF has committed substantial resources into recording the number of malaria cases in health facilities in which they operate and recording the population size in the catchment their facilities serve. Though both these estimates sound simple to collect both are non-trivial and require continued investment. The results however remain under-utilised as there is scepticism whether short- and long-term changes in

incidence reflect underlying trends. Routinely collected health centre data is notoriously difficult to interpret and compare between settings. The underlying issues causing this uncertainty are exacerbated in humanitarian settings where numbers of malaria cases diagnosed will be highly dependent on the quantity of medical staff in the area, the likelihood that a person reports to the health system will depend on immediate security concerns, and treatment and use of chemoprevention will fluctuate with the frequency of diagnostic and treatment stock-outs.

A novel method for routine malaria surveillance could be the use of ante-natal care (ANC) data (Walker, 2015). Such data are used in sentinel surveillance surveys for HIV, as it corresponds well with national HIV survey data of the same catchment areas (Gregson *et al.*, 2015). For malaria, the prevalence of infection in pregnant women is strongly correlated with the prevalence of infection in children under 5 in cross-sectional survey data from across Africa (van Eijk *et al.*, 2015). During standard intermittent preventative treatment during pregnancy (IPTp) programmes, any woman that is symptomatic is tested by RDT and given Artemisinin Combination Therapy (ACT) if they test positive. Any women who are not symptomatic or are test-negative are given chemoprevention in the form of Sulphadoxine pyrimethamine (SP). Since 2011, Médecins Sans Frontières (MSF) has rolled out the model of routine Intermittent Screening and Treatment (IST) of all pregnant women combined with the IPTp-SP programme described above (the difference between the standard and MSF routines is shown in Figure 3.1). The MSF routine entails testing all pregnant women at every ANC appointment, women who are test-positive are given ACT and women who are test-negative are given SP.

Since all women are tested regardless of symptoms, this reduces under-reporting bias due to the presence of asymptomatic infections. ANC programmes run by MSF in malaria endemic countries record the number of RDTs administered and the number of positive test results during ANC appointments at each health facility or hospital every month.

In this chapter, methods are developed to predict the relationship between the prevalence of infection in pregnant women and the clinical incidence in children under 5 years old, using field data collected at five MSF field sites in the Democratic Republic of Congo (DRC). There is population denominator data available at these five field sites, which is uncommon for many of the sites where MSF work and more widely across Sub-Saharan Africa. Nested statistical models are used to investigate the relationship between ANC prevalence and clinical incidence and determine whether this association is immediate or spread out over time. The utility of routinely collected ANC data for malaria surveillance and the evaluation of control interventions is then discussed, with special regard for settings where such denominator data are not available.

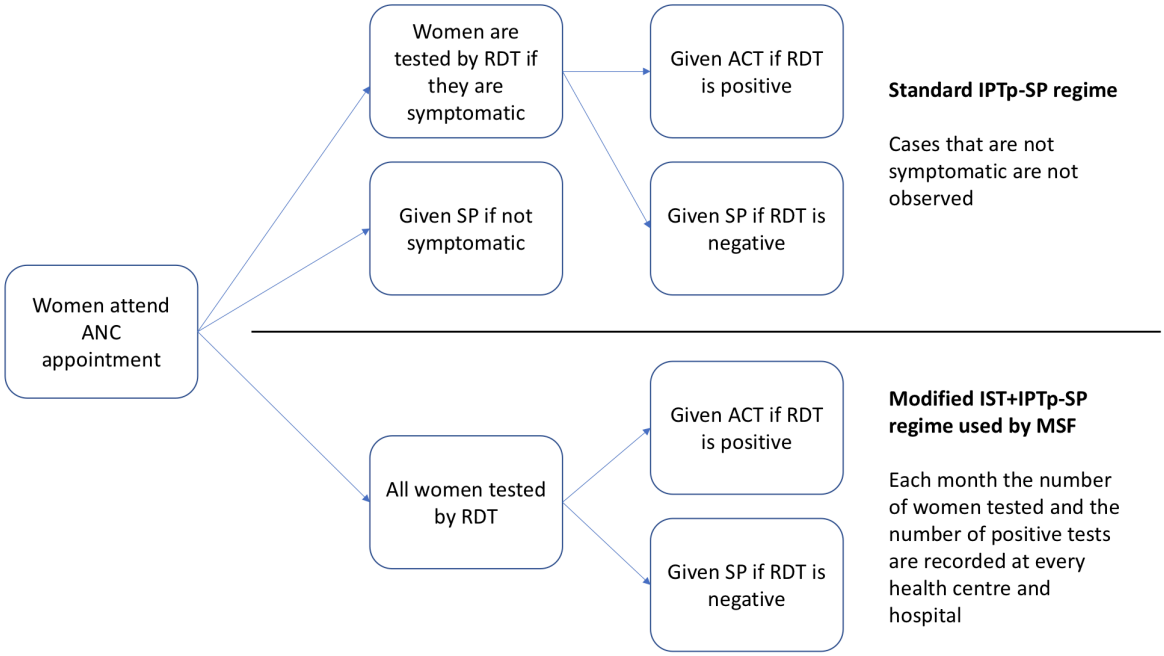

Figure 3.1: The difference between the standard IPTp-SP regimen and the expanded IST+IPTp-SP regime used by MSF in their ANC programmes in malaria endemic countries.

3.2 Methods

3.2.1 Clinical time series data

The data comprises time series from 5 different MSF health centres across the DRC for varying amounts of time between 2010 and 2016. These MSF missions vary in size and represent a mixture of hospitals, health centres and community clinics in the Great Lakes region (Figure 2.1); from North and South Kivu, close to the eastern border with Rwanda and Burundi (Baraka, Kimbi-Lulimba, Mweso and Walikale) and from the South-East province of Katanga, bordering Tanzania and Zambia (Shamwana, closed by the end of 2016). All sites are considered 'open' humanitarian settings, i.e. areas of chronic conflict mainly from the ongoing Congolese civil war, including internally displaced peoples (IDPs) and with frequent population movement due to fighting.

The ANC prevalence time series is the number of pregnant women tested for malaria using RDTs and the proportion of these that tested positive. Data is collated each month and all women that attend ANC appointments are tested for malaria regardless of whether they are symptomatic. The second time series is the monthly clinical incidence in children under 5 confirmed by RDT (i.e. symptomatic cases arriving as outpatients that tested positive by RDT). The size of the under 5 population at Mweso, Walikale and Shamwana is estimated by MSF each month using population surveys. The size of the under 5 population at Baraka and Kimbi-Lulimba, which cover larger areas, is taken from national census data conducted during the period of investigation by the DRC Department of Health.

An illustration of how the change in one metric may continue to influence another metric in the future (a lagged effect) is shown in Figure 3.2. If one metric can affect another second metric for a long period of time, then the value of the second metric will depend on the current and historical values of the first metric.

3.2.2 Vector Autoregression models

A causal framework was utilised to characterise the relationship between ANC prevalence and clinical incidence, as well as to determine the direction of the association between the two metrics. A variable X "Granger causes" Y if including past values of X in a predictive model of Y produces better predictions of Y than just using past values of Y alone (Granger, 1969). The analysis follows a two-step process. Firstly, a Granger causality test is used to determine the direction of the association (whether changes in ANC prevalence can predict future changes in clinical incidence, or vice versa) as well as the duration of any lagged effect. Secondly, this relationship is then fully characterised using more complex statistical models to determine the magnitude of the lagged effects and how the association might change with disease endemicity.

A vector auto-regression (VAR) model is used to test for Granger causality between the two metrics, determining the direction and length of potential lagged effects between two or more time series (Pfaff, 2008). A VAR model fits linked auto-regressive (AR) models to two or more time series variables at the same time, a p^{th} order AR model uses the last p observations of a time series as explanatory variables in a linear regression model to predict the current value:

$$Y_t = c + \alpha_1 Y_{t-1} + \cdots + \alpha_p Y_{t-p} + \varepsilon_t \qquad 3.1$$

Where c is a constant intercept term, α_l is the regression parameter for the observation at time $t - l$, and ε_t is a white noise term that has a standard normal distribution. An p^{th} order VAR model also includes the last p terms of each other time series featuring in the autoregressive model, capturing how changes in one of the variables are reflected in the other variables now or in the future. For two time series, Y and X, a p^{th} order VAR model has the following model formula:

$$Y_t = c_1 + \alpha_{1,1} Y_{t-1} + \cdots + \alpha_{1,p} Y_{t-p} + \beta_{1,1} X_{t-1} + \cdots + \beta_{1,p} X_{t-p} + \varepsilon_{1,t} \qquad 3.2$$

$$X_t = c_2 + \beta_{2,1} X_{t-1} + \cdots + \beta_{2,p} X_{t-p} + \alpha_{2,1} Y_{t-1} + \cdots + \alpha_{2,p} Y_{t-p} + \varepsilon_{2,t} (2)$$

The number of past observations that should be used in the VAR model (known as the order or lag order) is determined by finding the lag order that optimises some information criterion, usually the Akaike information criterion (Ivanov and Killian, 2001). Testing for the goodness-of-fit of a fitted VAR model involves performing several hypothesis tests on the model residuals. A multivariate Portmanteau test can determine whether the model residuals are correlated through time, e.g the

residual at time *t* is correlated with the residual at time *t-l* for some value *l*. An autoregressive conditional heteroscedasticity test can rule out whether the variance in the model residuals changes over time. The VAR models were fit and tested using the package 'vars' in the R statistical software (Pfaff, 2008).

To test whether X Granger-causes Y, in Equation 3.2 defined above, is equivalent to using a Wald test that has the null hypothesis that $\beta_{1,1} = \cdots = \beta_{1,p} = 0$. If the null hypothesis is rejected, then the alternative hypothesis is that X Granger-causes Y because the past values of X significantly improve predictions of Y over just past values of Y alone. This method of Granger causality testing only works if X and Y are stationary time series. A time series X is considered to be stationary if

$$F_X(x_{t_1+p}, \dots, x_{t_k+p}) = F_X(x_{t_1}, \dots, x_{t_k}) \qquad 3.3$$

For all *p, k* and times t_1 to t_k. Where F_X *is* the cumulative distribution function of the unconditional joint distribution of X. In practice the stationarity of a time series can be evaluated using an Augmented Dickey-Fuller (ADF) test (Lütkepohl, 2005).

3.2.3 Distributed lag non-linear models

Distributed lag non-linear models (DLNMs) are used to fully characterise the relationship between the two metrics, these flexible models allow a "lagged effect" as well as a "endemicity effect" of one metric upon the other. The "lagged effect" means that the effect of the explanatory metric upon the response metric happens over time (with the effect size changing with respect to time), whereas the "endemicity effect" enables the relationship between the two metrics to change according to the level of disease (the effect size varies with the value of the explanatory metric) (Gasparrinia, Armstrong and Kenward, 2010). DLNMs are specified by choosing two "basis" functions, the first basis function describes the shape of the association between the two metrics at each point in time (the transmission effect basis), the second basis function controls the shape of the lagged effects in the model (the temporal lag basis, an example being Figure 3.2B). These two functions are combined into a "crossbasis" function that describes the relationship between the value of an observation, how long ago it was observed and what its current effect will be on the response variable (Gasparrini, 2014). The crossbasis function can vary in shape depending on the two individual functions used to construct it. A crossbasis function can be written as $s(x_{t-l}, t-l; \eta)$, where x_{t-l} is the observation of the explanatory variable *l* months ago, *t-l* is the number of months since the observation, and η are the so-called "basis parameters" which are the parameters that describe the shape of the two functions combined in the crossbasis. The crossbasis function can be included as a predictor in a generalised additive model with the following form:

$$logit\big(E(Y_t)\big) = \alpha + h_i + \sum_{l=0}^{L} s(x_{t-l}, t-l; \eta) \qquad 3.4$$

where $E(Y_t)$ is the expected value of the response variable at time *t* (as determined by the Granger causality test outlined above), x_{t-l} is the value of the explanatory variable at time *t-l*, α is a parameter determining mean difference between the two metrics, h_i is the location-specific modifier of the mean difference between the metrics for location *i,* and *L* is the optimal lag order found when fitting the VAR model (and takes a value of 0 in models with no lagged effects). Different crossbasis functions ($s(x_{t-l}, t-l; \eta)$) made up of the two different basis functions are fit to the observed data and compared to determine the most parsimonious model. Two different functions are used to investigate how the relationship between metrics changes with endemicity, i.e the transmission effect basis:

- **Linear basis** - the simplest model assumes that the endemicity effect varies linearly with the explanatory metric
- **Hill function** – a function flexible enough to fit the relationship between the incidence and prevalence typically observed in non-temporal data (Cameron *et al.*, 2015)

A choice of three different basis functions are used as the temporal lag basis:

- **No lagged effect**
- **Linear basis** – the effect of a change in the explanatory metric increases or decreases linearly with respect to time
- **Non-linear basis** - a non-linear spline function that is penalised to produce a smooth curve, using penalised splines has been shown in simulations to be an effective method of reconstructing a variety of lag-exposure relationships when fitting DLNMs (Gasparrini *et al.*, 2017)

All combinations of endemicity effect and lagged effect basis functions are tested, giving a total of 6 different models. For clarity, each model is named with an acronym that represents its structure. The first two letters of the acronym represent the function used for the transmission effect basis, this can be either LE for a linear function or NE for a Hill function. The second two letters indicate the function used for the temporal lag basis, this can be LL for linear lagged effects or NL for non-linear basis spline lagged effects. If there is only one pair of letters, then the model does not have lagged effects. The names of all six models are listed in Table 2.

Models were fit using the '*dlnm*' package (Gasparrini, 2011) for the R statistical software and the most parsimonious model was identified using AIC value. The AIC value is equal to two times the number of parameters in the model minus two times the maximum value of the log likelihood function. Nested models can be compared using AIC, the model with the lowest AIC value is considered the best model. The predictive power of each model (its ability to correctly predict into the

future) was compared use a rolling origin cross-validation method. This predicted a year of unseen data at a time, with the model being fit using all previous years of data at the given location and all the data from every other location. The models can then be compared using the root mean squared error of their predictions.

3.3 Results

ANC prevalence and clinical incidence in children under 5 across the five locations are shown in Figure 3.3. Visually, it is clear that the temporal trends in the metrics are broadly the same, though the association has substantial variability over time and between different locations. Baraka and Shamwana show pronounced seasonal patterns in both transmission metrics, whereas the other sites do not show obvious seasonal variation in transmission. In Figure 3.3 the sites are ordered from the northernmost site to the southernmost site when moving from left to right along the top row and then the bottom row, there is a steep gradient in the degree of seasonality of malaria transmission when moving from north to south (Cairns *et al.*, 2015). Different sites also have differing levels of ANC prevalence despite similar incidence rates in children under 5. For example, Shamwana and Kimbi-Lulimba have median observed clinical incidence rates in children under 5 of 1.714 and 1.711 respectively, but their median observed ANC prevalence is 34.6% in Shamwana and 18.5% in Kimbi-Lulimba (Table 1). A direct cross-sectional comparison of the two metrics each month is shown in Figure 3.4.

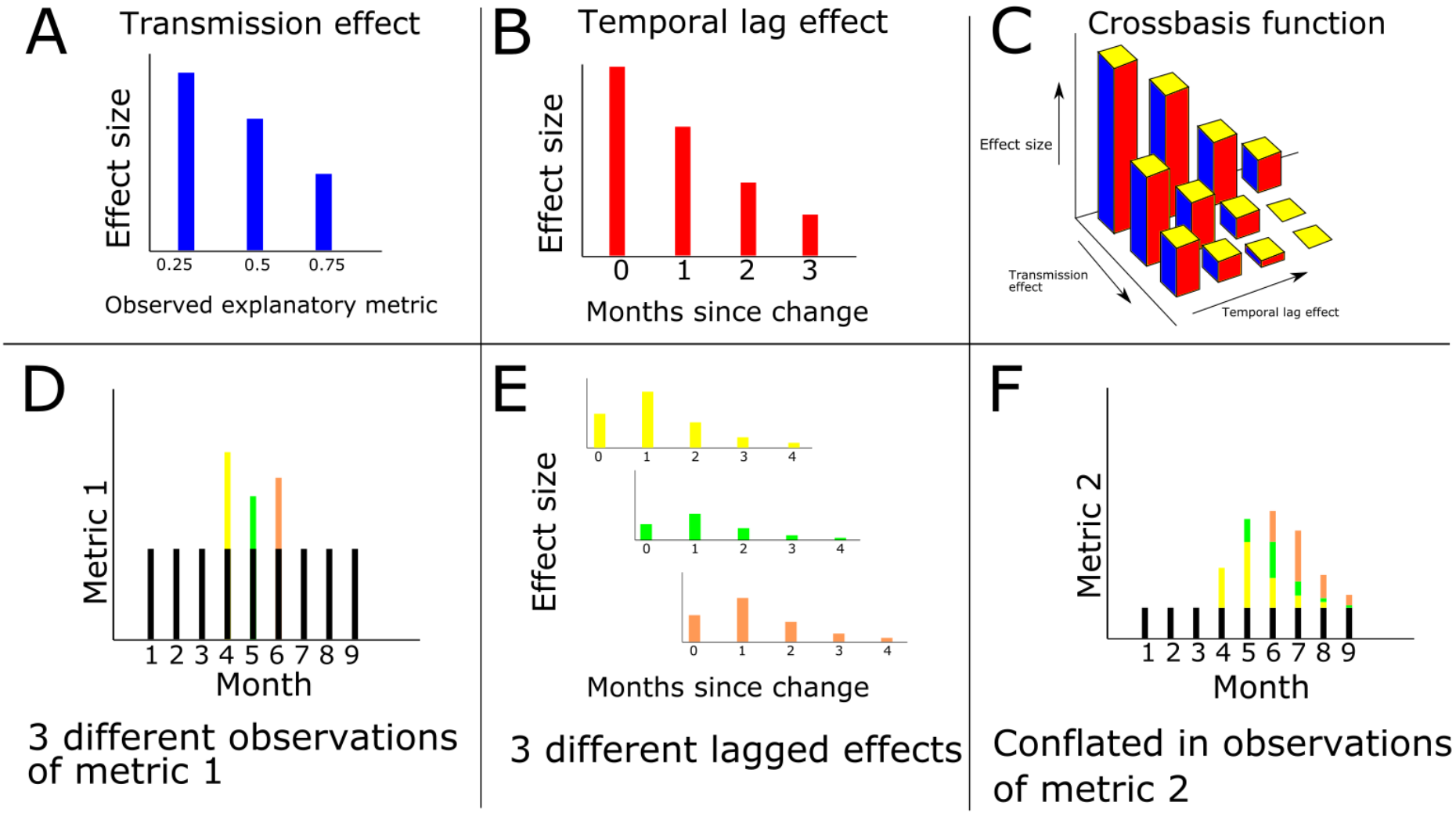

Figure 3.2: Panels A-C depict the concept of a crossbasis function in this context, in (A) the explanatory metric has corresponding effect on the response metric, the function that explains this relationship is the transmission effect basis. In (B) for a given value of the explanatory metric, this may have delayed effects on the response metric – in this plot for 3 months afterwards. This relationship is characterised by the temporal lag basis. In (C), these two basis functions are combined into a bi-dimensional plot, the shape of the crossbasis function is restricted by the choice of functions in (A) and (B). The precise shape of the crossbasis is determined during the fitting of the DLNM model. Panels D-F depict how subsequent changes in one metric (Metric 1) can cause unpredictable patterns in another metric (Metric 2). (D) shows the different changes in Metric 1 differentiated by colour (yellow for the change in month 4, green for the change in month 5 and brown for month 6). (E) Each of these changes in Metric 1 have lagged effect that may differ with the size of the observation in Metric 1 and start at different times. These lagged effects are then observed as changes in Metric (2) over multiple months (Panel F) with the lagged effects of three different changes in Metric 1 stacking up to create complex patterns in Metric 2. This is illustrated in this example where month 4 saw the greatest increase in Metric 1 whilst Metric 2 peaked in month 6.

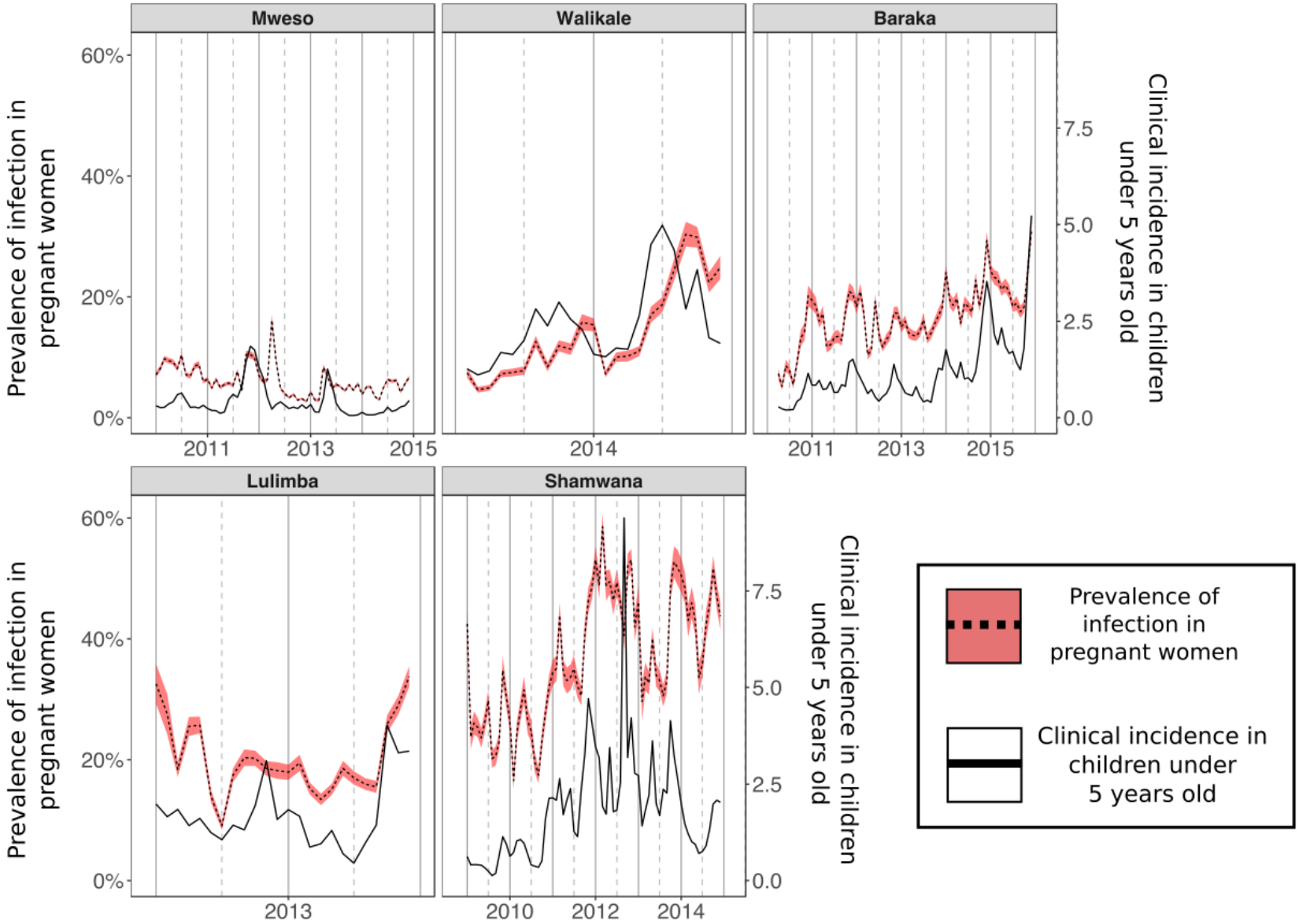

Figure 3.3: Time series data from the five different settings used in the analyses. The solid black line shows the recorded clinical incidence rate in children under 5 years old each month (cases per child per year). The dotted black line shows the recorded anti-natal clinic prevalence recorded each month with the red shaded area indicating the 95% confidence intervals using the normal approximation method. Data are available for different durations in the different settings.

Table 3.1: Summary of the time series data collected during the same month from the different DRC settings. The population size of the catchment area (used to convert case numbers into clinical incidence rates are and the number of women attending anti-natal clinics (ANC visits) are summarised using the median value. The longitudinal timeseries is shown graphically in Figure 3.3.

Location	**Number of data points in time-series**	**Median population size**	**Median monthly ANC visits**	**Median monthly ANC prevalence**	**Median incidence in children under 5 years (minimum, maximum)**
Baraka	69	71238	636	17.3%	0.929 (0.199, 5.24)
Mweso	60	65867	1074	5.7%	0.277 (0.059, 1.854)
Walikale	23	31536	437	11.3%	2.072 (1.112, 4.986)
Shamwana	72	36000	455	34.6%	1.714 (0.129, 9.397)
Kimbi-Lulimba	24	15812	582	18.5%	1.711 (0.451, 4.028)

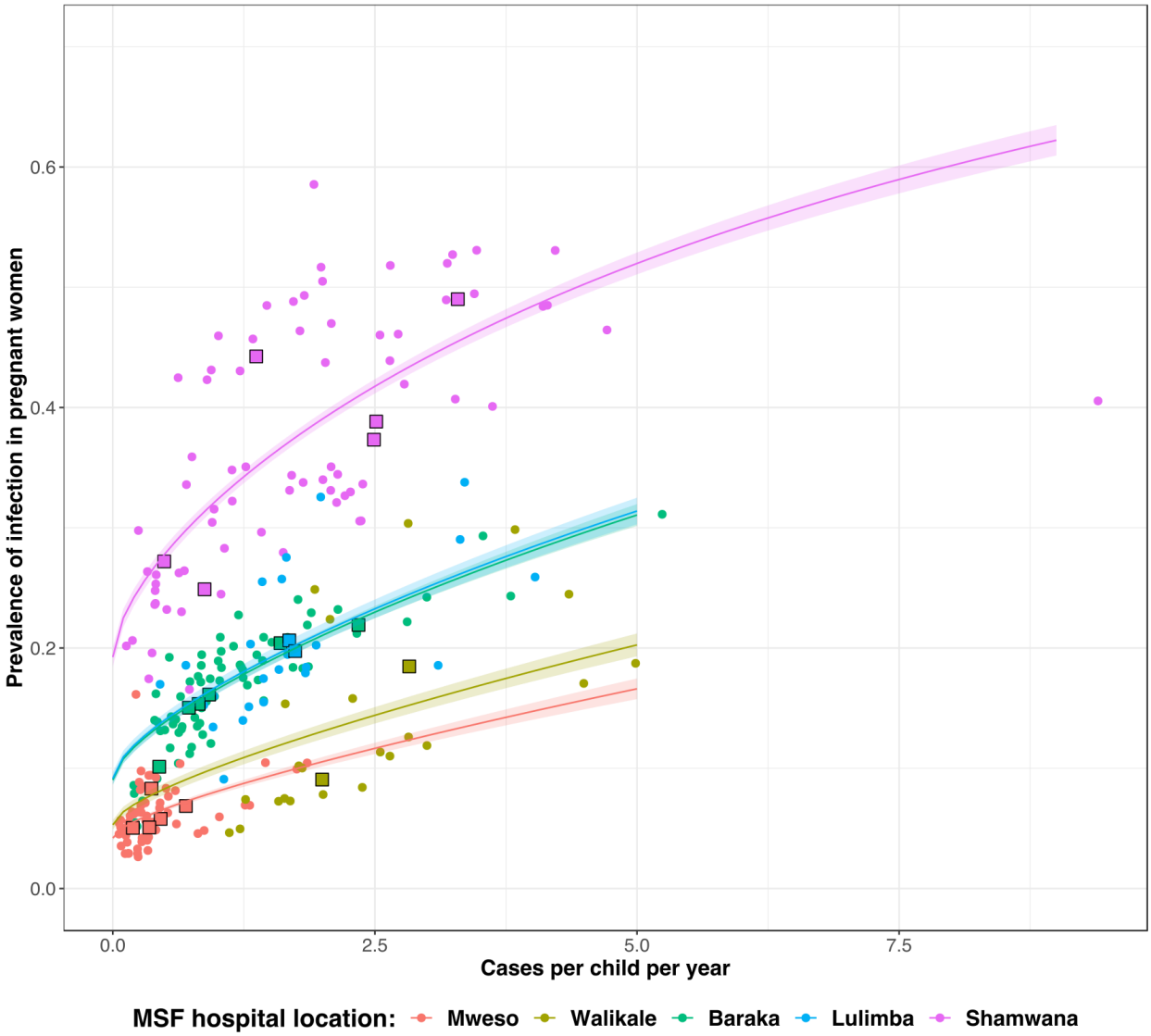

Figure 3.4: Cross-sectional relationship between prevalence of infection in pregnant women attending anti-natal clinics (ANC) and clinical incidence in children under 5 years reported at the same site. The small circular points show the raw monthly values, coloured by location. The large square points show the same data aggregated by calendar year. The coloured curves show a simple non-linear relationship between the two metrics with no lagged effects (equivalent to model NE) and corresponding 95% confidence interval.

3.3.1 DRC Ministry of Health population denominator data

The locations Baraka and Kimbi-Lulimba (also referred to as just Lulimba) cover larger areas, meaning that MSF do not conduct monthly population surveys to estimate the size of the local population. Therefore, for these two locations, the population denominator data available are infrequent census data from the DRC ministry of health. To calculate the incidence rate, the population is assumed to be constant over large periods of time, sometimes years, which is contrary to the rapid population changes that are observed at other locations. However, there is evidence in the wider clinical data that MSF collects that the population size in these locations remains relatively stable over the period of investigation. The number of hospital consultations that are not related to

malaria does not show the same general upwards trend that malaria consultations do. The conditions that form the bulk of non-malaria consultations include: acute watery diarrhoea, acute upper and lower respiratory tract infections, eye infections, severe acute malnutrition, intestinal parasites, and skin infections. If a large amount of people arrived in the catchment area of hospitals, there should be an increase in number of consultations observed for non-febrile patients, not just malaria (Figure 3.5). This is especially true if the arriving population are internally displaced people, since they lack access to healthcare across the board (Birganie, 2010). In Baraka the number of consultations that do not involve using an RDT to confirm whether a febrile patient has malaria does not have an upwards trend after 2011. The upwards trend between 2010 and 2011 represents the number of consultations increasing as the hospital sets up and people begin to go there. In Kimbi-Lulimba there is a similar pattern whereby the number of non-febrile consultations rises for around the first six months but then levels out at around 2000 per month. This provides moderate evidence that the population sizes at these two locations remains relatively stable.

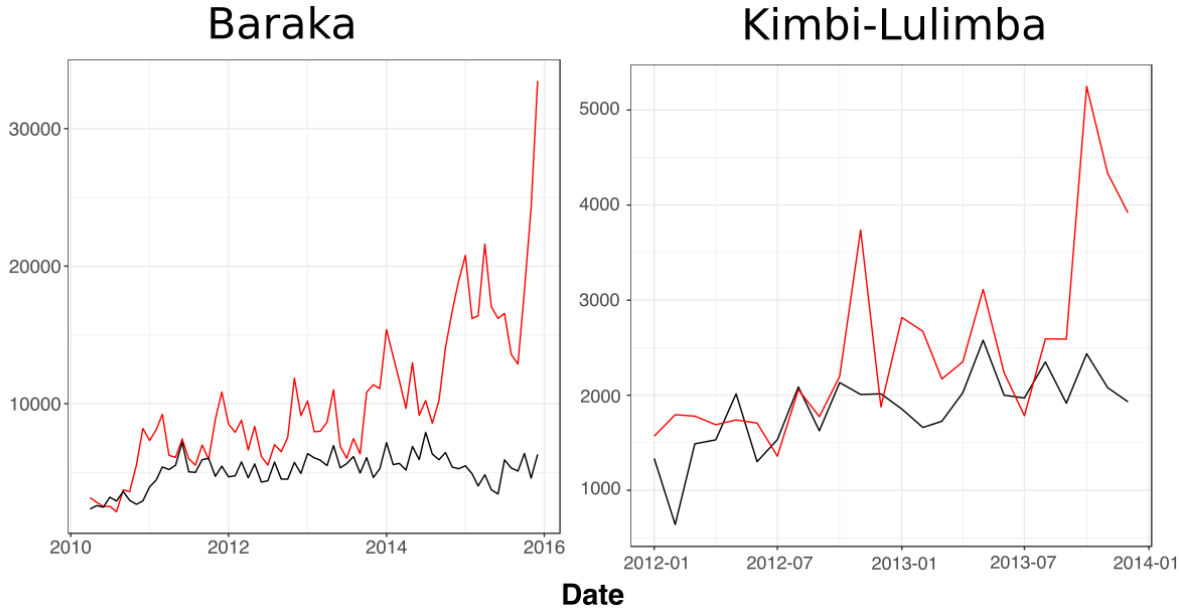

Figure 3.5: The number of RDT tests used to determine whether a febrile patient has malaria is shown in red, the black line shows the number of consultations each month that were not related to fevers.

3.3.2 Granger causality testing

The optimum lag order for the VAR model fit to the two time series was 3 months, different information criterion suggested different values (Table 3.2), but AIC was used since it has been shown to work best for selecting the lag order given reasonable sample sizes (Ivanov and Killian, 2001). The residuals of the VAR model with a lag order of 3 showed no sign of autocorrelation (H_0: autocorrelations all equal to 0, p=0.191) or autoregressive conditional heteroscedasticity (H_0: residual variance does not change over time, p=0.691).

Table 3.2: Information criterion values for different lag orders of the VAR model (Section 2.3.2)

	Lag order = 1	Lag order = 2	Lag order = 3	Lag order = 4
Akaike information criterion (AIC)	-6.53649	-6.55116	**-6.58123**	-6.57792
Bayesian information criterion (BIC)	**-6.33772**	-6.33085	-6.29626	-6.232539
Final prediction error (FPE)	0.00145	0.00143	**0.001387**	0.001392
Schwarz criterion (SC)	**-6.09881**	-6.05512	-6.02684	-5.96516

The Granger causality test indicated that past clinical incidence can significantly improve predictions of future ANC prevalence compared to past values of ANC prevalence alone (p=0.002). Conversely, ANC prevalence was unable to predict future clinical incidence with significantly more accuracy compared to using past values of clinical incidence alone (p=0.42). The subsequent analysis therefore uses clinical incidence in children under 5 years as the explanatory variable and ANC prevalence as the response variable. The optimum lag order chosen by the VAR model, 3 months, determined the length of the lagged effect in the DLNM models (how many previous months of clinical incidence in under 5s would be used to predict the current ANC prevalence).

3.3.3 DLNM fitting results

The "NENL" model provides the best fit (in terms of both AIC value and out-of-sample predictive power) indicating that changes in clinical incidence impact ANC prevalence non-linearly according to the level of endemicity, and that these effects manifest themselves (again non-linearly) immediately and over the subsequent months (Table 3.3). The 3D relationship (crossbasis function) is shown in Figure 6A, while a representation of the temporal lag basis function is depicted for various endemicity levels in 6B.

The lagged effects are significant for 3 months, with the effect size being greatest in the month that the change in incidence is observed and then decreasing over time. The best fitting model that uses

non-linear splines to model lagged effects (NENL) is an improvement, albeit a smaller one, upon the similar model the uses a linear function to model lagged effects (NELL). The non-linear lagged effects (NENL) estimate that incidence has a bigger effect on ANC prevalence with 1 and 2 months lag than the linear model (NELL) predicts (Figure 3.6B). Allowing the relationship between clinical incidence and ANC prevalence to be non-linear substantially improves model fit (Table 3.3). A graphical representation of the out-of-sample predictive power of the best "NENL" model is shown in Figure 3.7. Though the best fit model is unable to predict small changes in prevalence the overall trends are well captured. How well the model captures trends in prevalence is demonstrated both when the model is fit to all available data and when using the rolling origin cross validation technique, where predictions are made using the history of infection from the last year or more.

3.3.4 Lag order sensitivity analysis

The "NENL" model was also fit using lagged effects lasting 2 or 4 months, to see how this might change the shape of the estimated lagged effects. The fitted crossbasis function and lagged effects (Figure 3.8) correspond well with the optimum lag order of 3 months. This allows for more confidence in the chosen optimum lag order of 3 months, since the conclusions of the analysis would not change if the lag order was 2 or 4 months.

Table 3.3: Summary of the different distributed lag non-linear models (DLNMs) characterising the relationship between clinical incidence and ante natal clinic (ANC) parasite prevalence. The second and third columns indicate the shape of the basis function used to characterise how the relationship is influenced by endemicity and the lagged effect. Models are compared using Akaike information criterion (AIC, lowest value in bold indicating most parsimonious model) and root mean squared error (RMSE, lowest value in bold indicating most predictive model).

Acronym	**Endemicity effect**	**Lagged effect**	**Number of parameters**	**AIC**	**RMSE (rolling cross-validation)**
LE	Linear	No lagged effects	6	3859.2	0.0667
LELL	Linear	Linear	7	3116.6	0.0563
LENL	Linear	Non-linear	13	3116.0	0.0564
NE	Hill function	No lagged effects	8	3499.8	0.1126
NELL	Hill function	Linear	9	2982.0	0.05434
NENL	Hill function	Non-linear	15	**2978.9**	**0.05431**

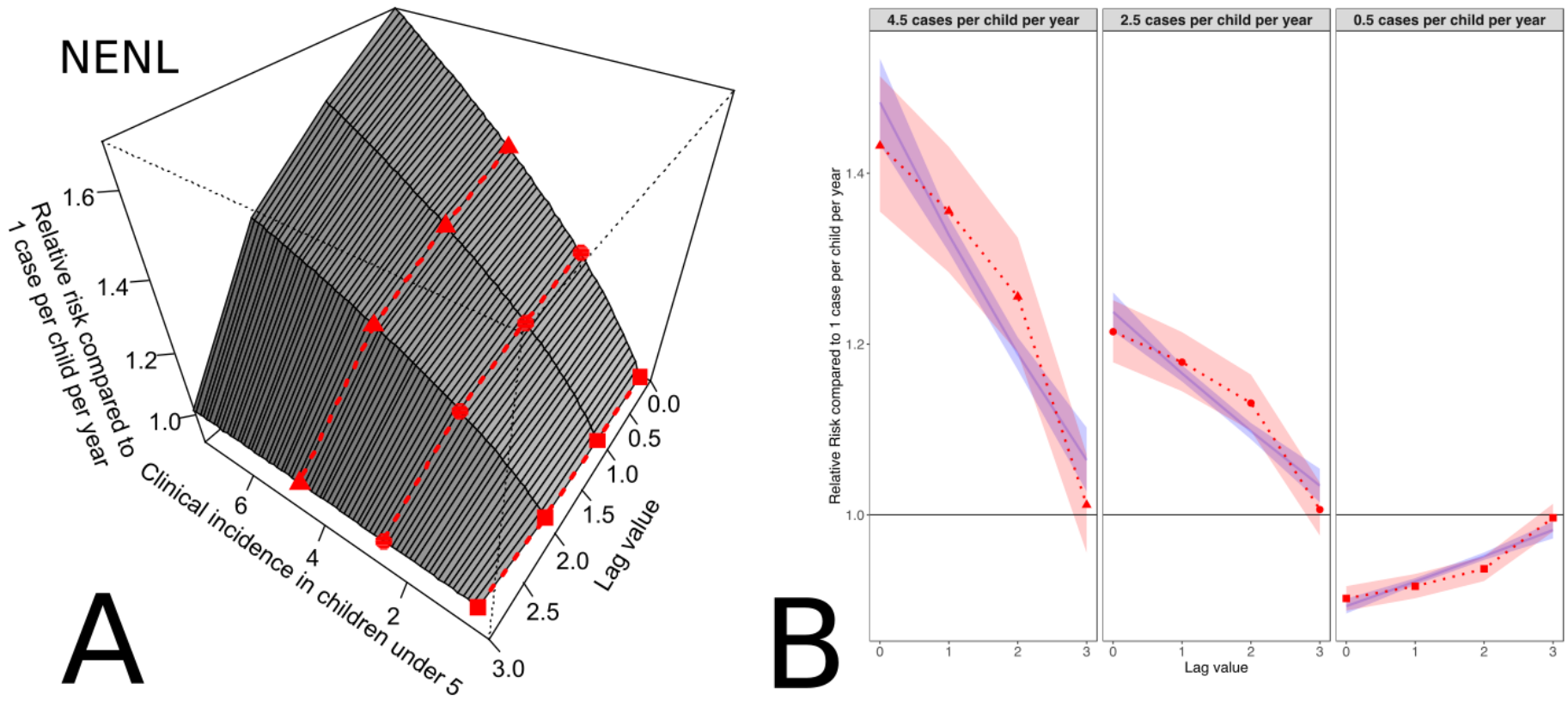

Figure 3.6: The best fit "NENL" model showing how clinical incidence over the last three months influences current anti-natal clinic (ANC) prevalence in terms of relative risk when compared to an observation of 1 case per child per year. (A) gives the full 3D relationship (the crossbasis function). Values greater than 1 indicate an increase in ANC prevalence whilst values less than one signify a decline. (B) Cross-sectional slices through the crossbasis function at three different clinical incidence values denoted by the shape of the points and corresponding lines on (A). The red shaded band shows the 95% confidence interval in the fitted lagged effects whilst the blue line and associated band show the lagged effects predicted by the model NELL (allowing a comparison between the linear and non-linear lagged effects).

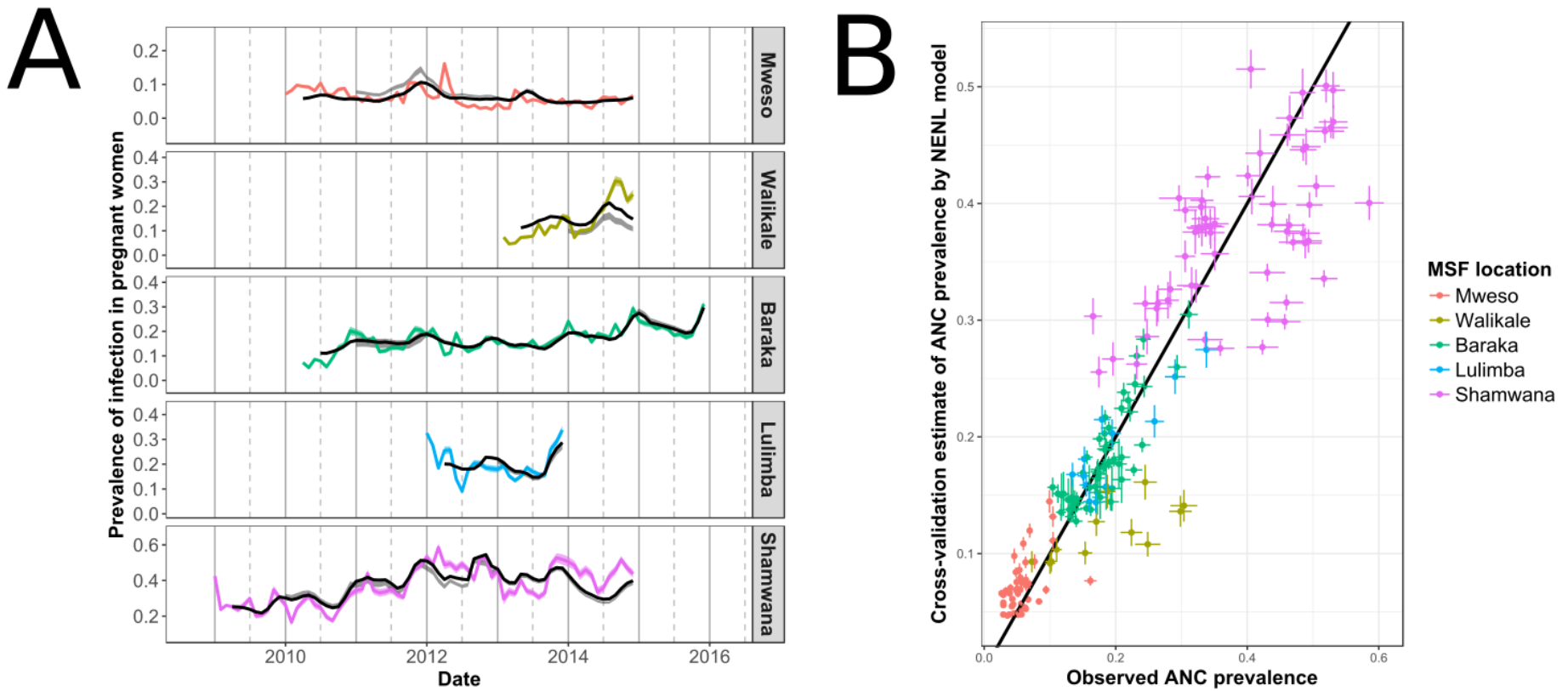

Figure 3.7: The results of the out-of-sample prediction for the best fitting "NENL" model. This uses at least one previous year of data as a training dataset before trying to make out-of-sample predictions for the subsequent years. (A) The coloured lines show the observed ANC prevalence each month at each location and their corresponding 95% confidence interval. The black line shows the model predictions of the ANC prevalence when the model was fit using all data. The grey band shows a 95% confidence interval for the rolling origin cross validation technique. (B) Points show a comparison of observed ANC prevalence and the corresponding out-of-sample predictions, coloured by site. Lines around the points show the 95% confidence interval for the observations and out-of-sample prediction. The black line shows a perfect correspondence between observation and prediction.

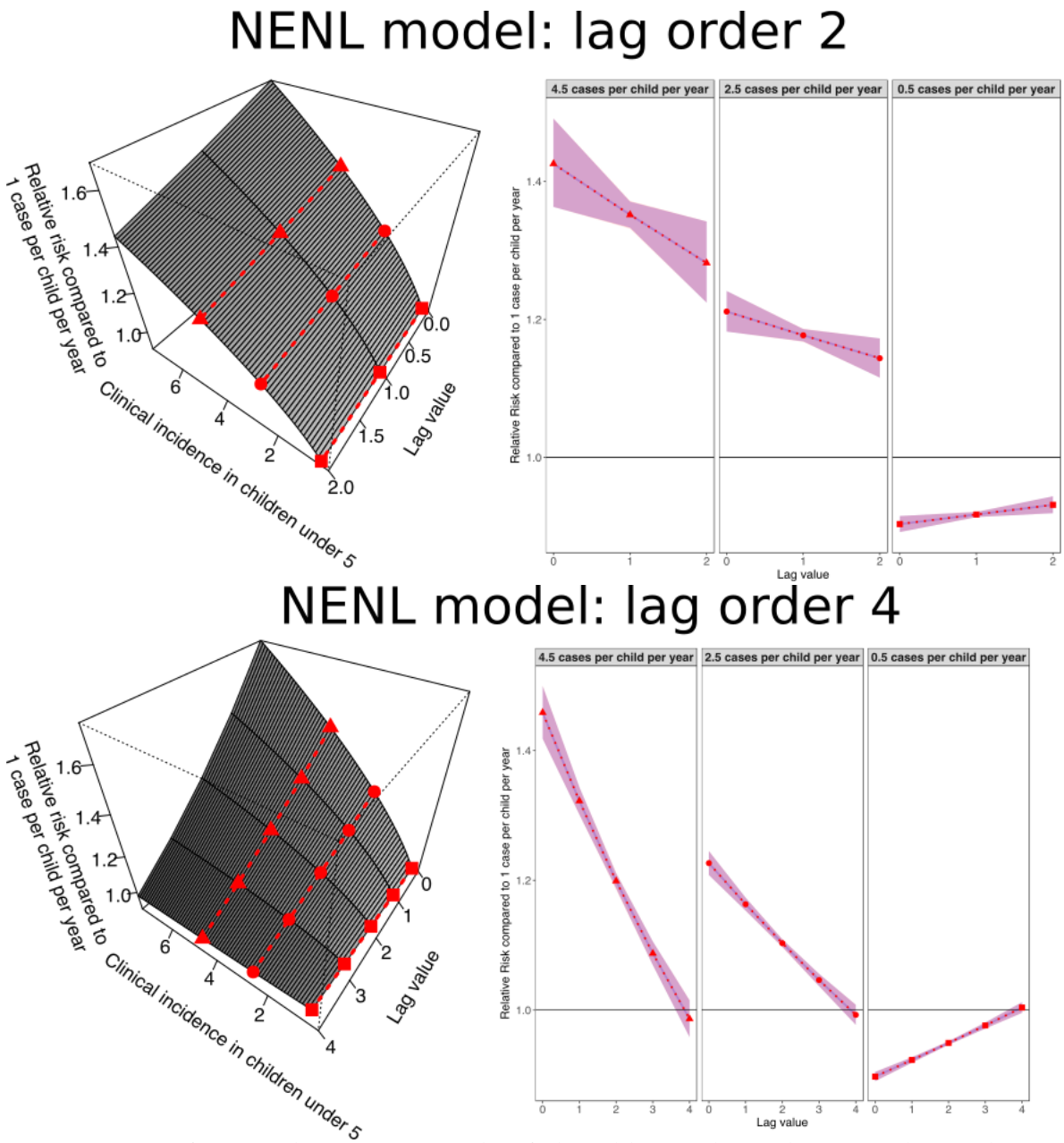

Figure 3.8: A copy of Figure 4 when using 2 or 4 months to fit the model NENL, showing the corresponding crossbasis function (left) and lag basis functions (right)

3.4 Discussion

Clinical incidence in children under 5 years old could predict ANC prevalence but not vice versa. This matches our current understanding of the epidemiology of malaria. Clinical incidence in children under 5 years, who have low levels of malaria immunity, is likely to closely reflect the incidence of new infections and thus be a good proxy for the current intensity of transmission. Conversely, in pregnant women an infection, and associated HRP-2 antigenemia, can persist asymptomatically for a prolonged period of time. Since pregnant women are being tested routinely, regardless of symptoms, ANC-based prevalence is likely to be a measure of exposure accumulated in preceding months

(Ashley and White, 2014; Grandesso *et al.*, 2016). This is consistent with our analysis where high clinical incidence rates in under 5s were associated with an increased risk of a positive RDT in pregnant women for the next 3 months, as well as a recent study demonstrating that in areas of sustained, seasonal transmission a substantial proportion of women attending ANC appointments remain infected throughout the dry season (Berry *et al.*, 2018). The models that assumed a non-linear relationship between clinical incidence in under 5s and ANC prevalence were superior in terms of AIC value and out-of-sample predictive power. The best fit function produces a curve whereby increasing clinical incidence in children under 5 is approximately linearly associated with larger effects upon ANC prevalence up until around 3 cases per child per year, where it begins to plateau. This shape has been observed in multiple cross-sectional surveys comparing malaria prevalence in the overall population with clinical incidence (Cameron *et al.*, 2015). This is likely a product of heterogeneity in mosquito biting (some people are bitten substantially more than others) leading to repeatedly infected people developing asymptomatic infections (so new infections occur in people already infected meaning that there is no change in prevalence).

Due to the changes in the model fit between sites (significantly different *h* parameter values), the model cannot currently be used to predict ANC prevalence from incidence alone. For example, the best fitting model systematically under-predicted the level of ANC prevalence in Walikale, which has similar rates of incidence in children under 5s as seen in Shamwana but much lower ANC prevalence (Figure 3.3). Some of the differences between sites may be accounted for if there was more precise ANC data on factors known to affect the epidemiology of malaria in pregnancy such as timing of gestation (Walker *et al.*, 2013) and parity. The sensitivity of malaria RDTs are known to vary depending on the number of children that a woman has already had, with more children meaning a likely history of exposure to the parasite during pregnancy and a developed placental immunity (Fried, Muehlenbachs and Duffy, 2012). Alternatively, the variation between sites could be attributable to poor incidence estimates at some locations due to sparse health systems, insecurity, inaccurate estimates of population size, or short-term population movement into areas of higher risk (e.g. forested areas). Analysis of mobile phone data in malaria endemic countries shows large-scale population movement within and between countries (Wesolowski *et al.*, 2012; Zu Erbach-Schoenberg *et al.*, 2016). The infrequency of national census surveys may therefore limit the accuracy of incidence estimates derived from these surveys. However, census data was only used for two of the sites in the MSF dataset and the incidence recorded at those two sites (Baraka and Kimbi-Lulimba) was not unusual when compared to the other locations. The work needs to be extended to use data from more locations where good population data estimates are available to confirm these results. For example, Tanzania have been testing all pregnant women attending ante-natal clinics nationally since 2015 (Willilo *et al.*, 2016). Combining these data with accurate clinical incidence estimates could substantially improve the generalisability of results. To redress some of the uncertainty in the data, the NENL model was fit using several different maximum lag values (Figure 3.8), with the general results

remaining the same for maximum lag values of 2 or 4. However, as is clear from Figure 3.7, there is still uncertainty in the data that the current model is unable to capture.

These results have practical implications for the proposed use of ANC prevalence as a tool to monitor malaria. This method has established, at these 5 sites at least, that ANC prevalence seems to be a promising, simple, and cost-effective measure of recent malaria incidence. This has important applications in humanitarian settings and beyond. Good quality population size estimates are difficult, expensive to obtain, and are only available in a small number of sites where MSF operate. ANC data is much more widely available, and this work suggests it should be used to monitor recent trends in malaria endemicity over simple case count data alone. As an illustration of its importance it was unclear from hospital case counts data whether malaria transmission was increasing in sites in Eastern DRC around Baraka or not. Case counts had risen dramatically, though this may have been because of increased investment by MSF (for example the use of mobile malaria teams to diagnose and treat the wider population) or a true increase in disease transmission. The spectra of mosquitoes resistant to pyrethroid insecticide and the possibility of the spread of drug resistant parasites means that local control interventions need to monitor secular trends in transmission regularly and tailor their programmes to maintain good levels of control. Examination of ANC data in these sites during this period would have provided a simple, unbiased method of raising concerns over recent increases in transmission. This method also provides a way of singling out changes in incidence that should be matched by a corresponding change in ANC prevalence, but this does not happen. For example, a change in reporting capacity or surveillance may induce an increase in incidence, but this would not cause an increase in ANC prevalence so those responsible for monitoring malaria can be confident that the increase in incidence was not due to increase in overall transmission.

Humanitarian organisations and other bodies are regularly trialling new methods of malaria control in specific areas to try and meet local needs. For example, MSF have used mobile malaria teams, community-based malaria management and different models of health centre support in different areas of the DRC. They are also considering deploying non-standard vector control tools which are thought to be easier to deploy than current methods recommended by WHO. The evidence-base to support these interventions is lacking due to the huge expense and infeasibility of conducting large RCTs in some areas. The full effect of a sustained decrease in transmission due to an intervention may not be observable in ANC prevalence measurements until several months after it begins, therefore availability of routine ANC data from a strategy of IST alongside IPTp in area where the intervention is introduced, combined with the model outlined here, could provide a low-cost measure of triaging new interventions to see which should go on for more thorough investigation.

ANC prevalence was found not to be useful for predicting future short-term changes in clinical incidence in children under 5 years old, so there is no evidence to support its use in predicting future

malaria trends from this work. However, it may be that combining ANC prevalence with other data such as the amount of rainfall may allow for models with better predictive power, though this analysis is beyond the scope of this work. In the future, it would be beneficial to invert the relationship used in this work to use ANC prevalence to predict past trends in incidence, useful in many of humanitarian contexts discussed where cases or denominator populations cannot be reliably recorded.

3.5 Conclusions

The work in this chapter shows that time-series data of clinical incidence in children under 5 years predicts future short-term prevalence of infection in pregnant women, but not the other way around. Increases in clinical incidence were associated with increased risk of a positive RDT in a pregnant woman for the next three months, with the opposite being true for decreases in incidence. This helps us to understand the role that ANC prevalence can play as a tool for retrospectively examining how malaria transmission has changed in a location over time. Though ANC prevalence derived from routinely collected clinical data may not directly reflect clinical incidence rates calculated from accurate population data, this analysis establishes that it does correspond to recent trends in malaria transmission and provides an easily collected metric in situations where good malaria data is sparse, such as chaotic, rapidly changing humanitarian crises. The next chapter will consider why long-lasting insecticidal nets might not be a feasible malaria intervention among internally displaced people, and how emanators that passively release pyrethroids might provide an alternative. The impact of these emanators will be considered in terms of the prevalence of infection in pregnant women. This would enable me to explore whether the predicted epidemiological impact of this potentially new vector control tool could be evaluated using routine data as organisation such as MSF who are monitoring the prevalence of infection in pregnant women each month. This links the performance of interventions that have a humanitarian focus to a source of low-cost routine data available in the locations where it is likely to be used.

4 Modelling airborne pyrethroid emanators as a back-up malaria control intervention in humanitarian crises

4.1 Introduction

This chapter explores the impact of pyrethroid emanators used at night in areas of humanitarian crises, where long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS), usually the mainstay of malaria control, are not an option.

LLINs and IRS are less effective or feasible in areas of acute humanitarian crisis for two broad reasons. The first reason concerns distribution. Net distribution or house spraying campaigns require large amounts of organisation and co-ordination. Many malaria endemic countries have managed, over time, to achieve reasonable or high LLIN and IRS coverage in their indigenous populations (World Health Organization, 2017b). However, it remains to be seen whether this existing campaign infrastructure could procure and distribute a large amount of LLINs or spraying equipment in a humanitarian crisis in a timely manner (Rowland and Nosten, 2001; White, Conteh, *et al.*, 2011). Irrespective of a country's capacity to distribute nets, populations of internally displaced people (IDPs) and refugees are often not directly considered when planning malaria control programmes and are therefore missed out during net distribution or spraying campaigns (Williams, Hering and Spiegel, 2013). People fleeing conflict are often forced to leave most of their belongings behind, including LLINs. There is significantly less LLIN ownership in refugee and IDP camps compared to indigenous populations (Spencer *et al.*, 2004; Carrión Martín *et al.*, 2014; Charchuk *et al.*, 2016). The combination of all of these factors means that people living in IDP or refugee camps are unlikely to already own a net and are unlikely to be given another one.

The second reason concerns correct use. LLINs and IRS are considered by some to be impractical for humanitarian crises because the type of housing that IDPs live in is frequently of too low quality to allow for proper use of each intervention. Insecticide treated nets need to be hung from the ceiling, stored carefully, and kept off the floor to prevent holes (Hakizimana *et al.*, 2014; Kilian *et al.*, 2015). Living in tents or cheaply constructed temporary shelter can make it difficult or impossible to meet any of these requirements, meaning that nets are either not usable at all or quickly deteriorate and become less effective (Spencer *et al.*, 2004; Gnanguenon *et al.*, 2014). In the same manner, IRS relies on insecticides soaking into the walls of a house and being released over time. Different building materials initially absorb different amounts of insecticide, as well as being able to retain the compound for different amounts of time. Therefore, IRS can be far less effective depending on the material that is being treated (Mutagahywa *et al.*, 2015). Malaria control campaigns that have focused specifically on people in humanitarian crises living in low quality housing have sought ways around this problem by impregnating tents (Graham *et al.*, 2004), clothes (Kimani *et al.*, 2006), bed sheets (Rowland *et al.*, 1999) and wall linings (Messenger, Miller, *et al.*, 2012; Ngufor, Tungu, *et al.*, 2014;

Messenger and Rowland, 2017) with insecticide. None of these alternative methods present a physical barrier between the human and mosquito (like an LLIN), but instead rely on mosquitoes contacting the insecticide-treated surface at some point before or after biting (like IRS).

A newly emerging method for vector control is the use of emanators. These products constantly release sub-lethal doses of pyrethroids into the air around them to deter mosquitoes away from users near the emanator and prevent bites from occurring (Ogoma, Moore and Maia, 2012). Various types of emanator are currently under development both as commercial products or home-made varieties (Matsuo *et al.*, 2005; Ishiwatari *et al.*, 2009). Home-made emanators (from herein simply referred to as emanators) do not face the same problems with mass distributions as LLINs do, because they are can be manufactured outside of a factory environment (e.g. by soaking hessian cloth in transfluthrin or metafluthrin and attaching it to a metal frame) and the materials are easy and inexpensive to procure in bulk (Ogoma *et al.*, 2017). Since emanators release insecticides into the air passively, they do not require any human compliance to prevent bites and will work in any type of housing situation, including outdoors. Field testing of emanators has been undertaken with volunteers sat near emanators collecting data outdoors at night in Tanzania (Ogoma *et al.*, 2017). Volunteers sat within a mosquito trap between 2 and 40 metres away from the emanator to measure the size of the area of protection. The trial found that the transfluthrin treated strips averted between 71 and 91% of bites from *An. arabiensis* mosquitoes for users within one metre of the emanator, as well as providing lesser protection for others sat up to five metres away. Emanators still provided statistically significant protection after two and a half years of use, averting ~20% of bites within one metre.

When considering the effects of pyrethroids other than mortality, it is important to distinguish between spatial repellency (repulsion away from the source of pyrethroid) and a longer-lasting effect, where pyrethroids interfere with odorant receptors on the sensory organs of mosquitoes, temporarily preventing them from responding to signals that they use to locate humans (Bohbot and Dickens, 2010; Bohbot *et al.*, 2011). Only in experiments that gave mosquitoes a chance to take a blood meal after pyrethroid exposure can researchers distinguish between these two effects. Emanator products treated with the volatile pyrethroid metofluthrin almost completely inhibit biting in Aedes mosquitoes in laboratory and semi-field conditions (Ritchie and Devine, 2013; Buhagiar, Devine and Ritchie, 2017). After exposure to smoke from a burning transfluthrin coil (in a small chamber), only 43% of anopheline mosquitoes fed 12 hours after exposure when given the opportunity to compared to 81% of control mosquitoes (Ogoma, Ngonyani, *et al.*, 2014). This suggests that at least some of the biting prevented by airborne pyrethroids is due to mosquitoes being unable to feed after exposure, rather than spatial repellency. This effect, which I will be referring to as temporary feeding interruption (TFI), is not directly observed during the field trial in Tanzania for the emanator product considered in this work. There was no increase in the mosquito biting rate on volunteers sat 80 metres away from the emanator user (both sitting outside) (Ogoma *et al.*, 2017). If mosquitoes were only repelled away

from the emanator user, we expect that some of the bites would be deflected onto the non-user sat nearby, since this is not the case this suggests that mosquitoes were not able seek hosts after pyrethroid exposure. Together these studies show that mosquito exposure to enough airborne transfluthrin causes a TFI effect, and that airborne transfluthrin released at the concentration given off by an emanator does not deflect bites onto nearby users. What remains unclear is whether those free-flying mosquitoes exposed to emanators in the field trial definitely had a TFI effect and were not instead repelled away.

Since the Tanzanian field trial for transfluthrin emanators measured exposure to mosquito biting outside, it was not possible to determine whether there was any mosquito mortality. Airborne pyrethroids such as burning transfluthrin coils cause considerable mosquito mortality indoors in experimental hut trials (Ogoma, Lorenz, *et al.*, 2014). However, the concentration of insecticide, which will determine the level of mortality caused, is likely to be far lower for spatial repellents that evaporate from the emanator than for other methods such as burning coils. Ogoma *et al.* (2017) placed their emanator in a sealed room for 24 hours and measured the concentration of transfluthrin in the air to be 0.13±0.06 $\mu g.m^{-3}$. A study monitoring the concentration of transfluthrin released by a burning transfluthrin coil in a sealed room was 0.0045 parts per million (ppm), 0.0003ppm and <0.0001ppm after 30 minutes, 2 hours and 8 hours (Ramesh and Vijayalakshmi, 2001). Converting these measurements to their equivalent values in micrograms per metre cubed gives 68.3 $\mu g.m^{-3}$ (30 minutes), 4.6 $\mu g.m^{-3}$ (2 hours), and <1.51 $\mu g.m^{-3}$ (8 hours). Therefore, the concentration of transfluthrin in experimental huts due to burning coils would be far in excess of that generated by using emanators indoors. Hence, I have chosen not to include a mortality effect of transfluthrin emanators even when used indoors.

This chapter will explore emanators as an alternative method of malaria control. Special consideration shall be given to the use of emanators in humanitarian settings, it is important to predict whether they could ease the burden of malaria cases on strained healthcare systems. The potential impact of emanators will be estimated using the Imperial College malaria transmission model, which will be expanded to include emanators as an intervention method. The vector compartments in the model will also be expanded to create a TFI effect on vectors after airborne pyrethroid exposure. This will allow me to disentangle the importance of potentially two different actions of the emanator: (1) its ability to deter mosquitoes away from those using a device and (2) quantify the impact of any TFI effect. Epidemiological impact will depend on how the emanators are used alongside other malaria control interventions. Here we will initially investigate the use of emanators as the sole vector control intervention as they may be used in settings in which MSF operate. Potential public health benefit will be assessed using a sensitivity analysis reflecting the uncertainty in our understanding of the duration of the emanator-caused TFI effect.

4.2 Methods

4.2.1 Proportion of bites prevented by emanators within their effective range

Evidence indicates that the percentage of mosquito bites averted by an emanator decreases as the user moves away from the intervention (Ogoma *et al.*, 2017), since the concentration of transfluthrin is likely to be highest nearer the emanator. People are unlikely to remain in close proximity to an emanator at all times, so the efficacy at preventing bites (outside of experimental conditions) will be determined by how close people stay to the emanator and how effective the emanator is at different distances. The size of the radius around the emanator where it prevents mosquito bites is termed the effective range. It is characterised on a continuum by assuming that efficacy declines at a constant rate as a person moves away from the device. This results in the proportion of bites prevented by the emanator (denoted D) being described by the following equation,

$$D(x) = Ae^{-Yx}, \qquad 4.1$$

Where x is the distance between the person being protected and the emanator (in metres), Y is the rate of decay, and A is the level of protection immediately next to the emanator. This exponential curve was fitted to the Tanzanian data of Ogoma et al. (2017) using a simple least-squares method to give a smooth, plausible effective range function. Goodness of fit is estimated using a coefficient of determination (R^2). This study tested emanators outside and is likely to be a conservative estimate of efficacy if the emanator is used inside where lower airflow may increase pyrethroid concentration. Data were only available for *An. arabiensis* mosquitoes, since this is the only vector species observed in the Tanzanian experiment.

Emanator users will spend different durations of time away from their emanators. I am currently unaware of any published data which tracks the proximity of people to homesteads (or places where emanators are likely to be placed) either in the Tanzanian site used to parameterise the effective range of emanators or in sites in which MSF operate. Since emanators are being used at night while people are asleep it is reasonable to assume that the population will spend the majority of their time stationary and quite close to an emanator. For simplicity, this analysis assumes an exponential distribution $C(x)$ for the proportion of time a user spends at a distance of x metres from their emanator. The parameter λ indicates the mean distance a person spends away from an emanator, e.g. $\lambda = 2$ indicates that on average the person spends half of their time within two metres of their emanator and half of their time further than two metres from their emanator. Currently, it is also assumed that emanator use is consistent between people, i.e. that no person goes near to the emanator of another. The two distributions just defined, $C(x)$ and $D(x)$, are used to estimate the probability that a bite will be prevented, considering the combination of emanator effectiveness and time spent at each distance from the emanator over all distances (r_{EM}), which is given by:

$$r_{EM} = \int_0^\infty C(x)D(x) \qquad 4.2$$

The parameter r_{EM} is incorporated into the Imperial College malaria transmission model to determine the probability that a mosquito successfully bites a person who is using an emanator (Griffin *et al.*, 2010; White, Griffin, *et al.*, 2011; Griffin, Ferguson and Ghani, 2014; Griffin, 2015). It is assumed that r_{EM} decays exponentially over time as emanators becomes less effective. The rate of decay was estimated by fitting an exponential curve to the Tanzanian data (Ogoma *et al.*, 2017) which tested how many bites emanators prevented within one metre using new (recently dipped) and aged emanators (dipped two years ago and hung outside when not in use).

If emanators are being used inside the home, then their overall effectiveness will depend on the percentage of bites taken when people are indoors. This will depend on the mosquito biting time (as assessed using methods such as human landing catches) and human behaviour. Letting Φ_I denote the proportion of all bites received while indoors, the probability that a mosquito is deterred away from a human using an emanator, z_{EM}, is given by:

$$z_{EM} = r_{EM}\Phi_I \qquad 4.3$$

As discussed in the introduction, there was currently no evidence from the experimental site in Tanzania to suggest that emanator exposure would cause elevated mortality in mosquitoes (Ogoma *et al.*, 2017). The probability that a mosquito feeds on an emanator user and survives is therefore the same as the probability that the mosquito feeds, w_{EM}. The probability of a mosquito successfully feeding on an emanator user is given by:

$$w_{EM} = 1 - z_{EM} \qquad 4.4$$

4.2.2 Vector temporary feeding interruption modelling framework

The framework outlined above characterises how mosquitoes may be deterred away from biting someone protected by an emanator. The following section investigates what would happen if the process of being deterred causes a sub-lethal effect on mosquito feeding.

A TFI effect was incorporated into the existing framework of the vector model by introducing three parallel compartments that correspond to the original categories (see Section 1.3.3.2): susceptible (S_v), latently infected, (i.e. infected but not infectious, E_v) and infectious (I_v). Subscript *v* signifies a

normally biting mosquito. Mosquitoes have a chance of entering these parallel compartments when they try to take a blood meal on a human near an emanator (i.e. S_{vTFI}, E_{vTFI} or I_{vTFI}, with subscript *TFI* indicating being in the *TFI* state). Some mosquitoes will be deterred away from biting by the emanator and a proportion of these mosquitoes (f_{TFI}) experience TFI. A value of 0 indicates that mosquitoes have no TFI and immediately can go on to start another feeding attempt. A value of 1 would indicate that all mosquitoes being deterred away from the emanator experience TFI and do not immediately attempt to re-feed. While in the TFI compartments mosquitoes continue to age, die at a natural background rate, and develop parasites in their midgut if they are latently infected. Crucially, no mosquitoes move from the susceptible TFI compartment into the latently infected TFI compartment, since they are not biting (due to TFI) and so cannot become infected. Mosquitoes leave the TFI compartments at a rate (l_{TFI}) determined by the mean length of the TFI effect. The rate of blood-feeding mosquitoes moving into TFI compartments is determined by the following formula:

$$f = \Phi_I \times a_{vEM} \times z_{EM} \times f_{TFI} \qquad 4.5$$

where Φ_I is the proportion of all biting that happens whilst hosts are indoors, a_{vEM} is the average mosquito biting rate on emanator users, z_{EM} is the probability that a mosquito is deterred by an emanator, and f_{TFI} is the probability that a mosquito goes on to have TFI given that it has been deterred. Since the probability z_{EM} decays over time, this also reduces the TFI effect of emanators as they age. The actual proportion of the vector population that has TFI at any one time depends on how quickly the mosquitoes leave the TFI state (the parameter l_{TFI}). A schematic of the compartment structure for the vector model including the TFI effect is shown in Figure 4.1. The differential equations for mosquito movement between the compartments are as follows:

$$\frac{dS_v}{dt} = \beta - \Lambda S_v - \mu S_v - f S_v + l_{TFI} S_{vTFI} \qquad 4.6$$

$$\frac{dE_v}{dt} = \Lambda S_v - \Lambda(\mathrm{t} - \tau)\mathrm{S_v}(t-\tau)\mathrm{P_v} - \mu E_v - f E_v + l_{TFI} E_{vTFI}$$

$$\frac{dI_v}{dt} = \Lambda(\mathrm{t} - \tau)\mathrm{S_v}(t-\tau)\mathrm{P_v} - \mu I_v - f I_v + l_{TFI} I_{vTFI}$$

$$\frac{dS_{vTFI}}{dt} = f S_v - \mu S_{vTFI} - l_{TFI} S_{vTFI}$$

$$\frac{dE_{vTFI}}{dt} = f E_v - E_{vTFI}(t-\tau)\mathrm{P_v} - \mu E_{vTFI} - l_{TFI} E_{vTFI}$$

$$\frac{dI_{vTFI}}{dt} = f I_v + E_{vTFI}(t-\tau)\mathrm{P_v} - \mu I_{vTFI} - l_{TFI} I_{vTFI}$$

where β is the rate of new mosquitoes being born, Λ is the force of infection on mosquitoes, P_v is the probability that a mosquito survives the time period from latent infection through to becoming infectious, τ is the extrinsic incubation period, and μ is the death rate of mosquitoes given interventions.

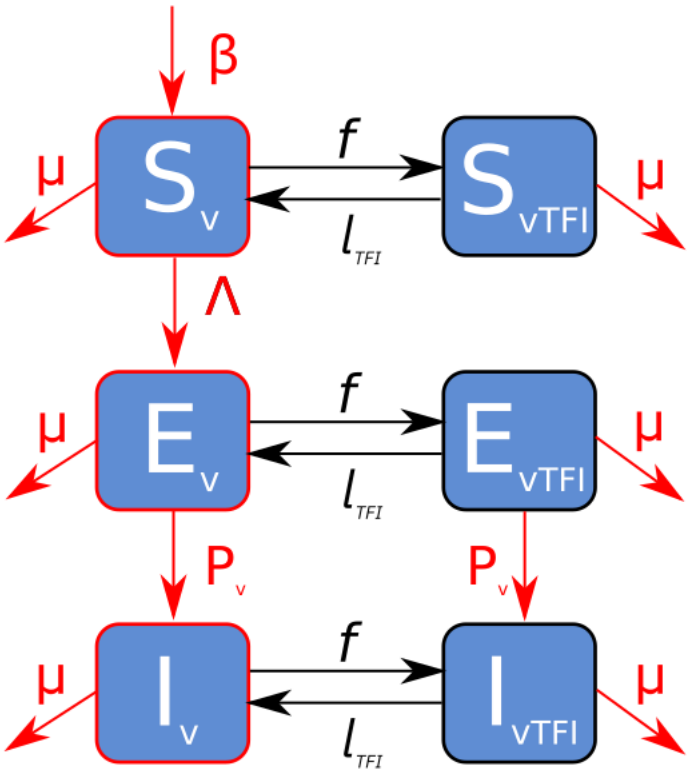

Figure 4.1: A schematic of the updated vector population structure including a temporary feeding interruption (TFI) effect. Compartments and parameters in red are from the original model, compartments and parameters in black are my additions. Vectors move from the original susceptible (S), latently infectious (E), and infectious (I) compartments into parallel versions (S_{vTFI}, E_{vTFI} and I_{vTFI}) where they do not feed because of the TFI effect. Vectors die from all compartments at a constant background rate μ. New susceptible vectors are born at a rate β determined by the number of newly maturing larvae, which varies with the size of the mosquito population (White, Griffin, *et al.*, 2011).

Table 4.1: Parameter descriptions and values for all new parameters added to the Imperial College malaria transmission model as well as the descriptions and values of relevant old parameters not added during this analysis.

Notation	**Description**	**Equation/(range of) value(s)**	**Reference**
r_{EM}	Probability of an emanator preventing a bite on someone currently using an emanator	Depends on C(x) and D(x)	
Φ_I	Proportion of all bites that take place indoors	0.89	(Griffin *et al.*, 2010)
z_{EM}	Probability of a mosquito being prevented from biting someone that owns an emanator (in the Imperial College model)	$r_{EM}\Phi_I$	

w_{EM}	Probability that a mosquito successfully bites someone that owns an emanator	$1 - z_{EM}$	
a_{vEM}	Average mosquito biting rate on someone that owns an emanator	Depends on number of successful bites on emanator users	
f_{TFI}	Probability that a mosquito that has been deterred by an emanator goes on to develop TFI	0-100%	
β	Birth rate of new, susceptible mosquitoes	Varies depending on mosquito population density	(Griffin *et al.*, 2010) (White, Griffin, *et al.*, 2011)
τ	Extrinsic incubation period (days)	10	(Griffin *et al.*, 2010)
μ	Mosquito death rate	0.132	(Griffin *et al.*, 2010)
f	Rate at which mosquitoes enter TFI compartments	$\Phi_I \times a_{vEM} \times z_{EM} \times f_{TFI}$	
l_{TFI}	Rate at which mosquitoes recover from TFI (hours)	12-72	(Bohbot *et al.*, 2011; Ogoma, Ngonyani, *et al.*, 2014)
Λ	Force of infection on mosquitoes	Varies depending on number of infectious humans	(Griffin *et al.*, 2010)
P_v	Probability that a mosquito survives its extrinsic incubation period	Calculated using the probability of a mosquito surviving 10 days given the current rate of death	(Griffin *et al.*, 2010)
S_v	Susceptible mosquitoes compartment	Equation 4.6	(Griffin *et al.*, 2010)
E_v	Latently infected mosquitoes compartment	Equation 4.6	(Griffin *et al.*, 2010)
I_v	Infectious mosquitoes compartment	Equation 4.6	(Griffin *et al.*, 2010)
S_{vTFI}	Susceptible mosquitoes with TFI compartment	Equation 4.6	
E_{vTFI}	Latently infected mosquitoes with TFI compartment	Equation 4.6	
I_{vTFI}	Infectious mosquitoes with TFI compartment	Equation 4.6	

4.2.3 Intervention scenarios

In this chapter, the impact of emanators is not modelled in conjunction with LLINs or IRS, as it is assumed that emanators are being used in locations where these two interventions are deployed separately. Instead, it is imagined that emanators are distributed to users who will sleep near them and

use them for personal protection, or that the emanators will be deployed in a communal area providing a radius of protection around many people (Masalu *et al.*, 2017). The emanator coverage in the population is the percentage of the population that are initially given an emanator during distribution. I undertake an intention to treat analysis, assuming that there is no drop out of people using emanators over time.

The modelling exercise will first focus on the entomological impacts of TFI, performing sensitivity analyses for the proportion of mosquitoes experiencing the volatile pyrethroid, the proportion of mosquitoes that go on to have TFI after being experiencing the volatile pyrethroid, emanator coverage, and the mean length of the TFI effect. After this, the epidemiological impact of emanator distribution in the human population will be explored in two settings with different baseline prevalences of infection in under-fives of 30% and 5% respectively. These values are chosen to roughly correspond to the minimum and maximum prevalence in under-fives predicted or observed in North and South Kivu at locations where MSF work (Figure 3.3). The added benefit of a TFI effect is then quantified in terms of how protective it is of the human population.

Finally, the impact of emanator distribution will be measured in terms of the ANC prevalence (the prevalence of infection in pregnant women), by converting the mean incidence rate in under-fives into a prediction of ANC prevalence using the best fitting DLNM model specified in Chapter 3. This will give a monthly prediction of how ANC prevalence will change over the course of emanator impact. A simplified hypothesis test will be used to determine whether MSF would be able to detect that changes in the ANC prevalence were significant and not due to variance introduced by sampling a finite population. Here we assume a very simple study design comparing disease prevalence in a community before and after emanators are distributed to a population coverage of 80%. Sampling pregnant women in two different months will give two different prevalence estimates, p and p_0. For given Type 1 and Type 2 error rates α and β, and standard normal quantile function z, the minimum sample size required to detect the difference between p and p_0 is as follows (Chow, Shao and Wang, 2008):

$$n = p(1-p)\left(\frac{z_{1-\alpha/2} + z_{1-\beta}}{p - p_0}\right)^2 \qquad 4.7$$

This test makes some simplifying assumptions because MSF are unlikely to sample exactly the same number of women each month, but the calculated minimum sample size will give some indication of whether MSF sample enough women to have the power to measure changes in ANC prevalence caused by emanator distribution.

4.3 Results

4.3.1 Emanator deterrence for different distributions of time spent nearby

Emanators provide protection against mosquito bites for on average 5.37 metres around the device and the estimated half-life of emanators is 355 days. The exponential function, $D(x)$, fitted to emanator effectiveness at different distances from the Tanzanian trial data, describes the data well, with an adjusted $R^2 = 0.926$ (Figure 4.2A). Therefore, it is reasonable to assume that emanator effectiveness decreases exponentially with increased distance of the user from the emanator. Four potential choices of the mean proportion of time spent at each distance from an emanator (parameter λ in the exponential distribution $C(x)$), are shown in Figure 4.2B. The distributions $D(x)$ and $C(x)$ are then combined using Equation 4.2 to give the proportion of bites that are successful (or alternatively are prevented) by emanators. The proportion of bites that emanators prevent over all distances depends corresponds strongly with how close people stay to them (λ). Since emanators are most effective at close range they avert a higher proportion of bites over all distances when people spend their time closer to them (when λ is small, Figure 4.2C). In the best-case scenario (λ = 1 metre), emanators prevent just over 60% of all bites from occurring within its effective radius. If people spend more time further away from the emanator this proportion drops. However, some bites would still be prevented; emanators would stop a large proportion of bites happening during the small amount of time that people were close to the emanator, and a small proportion of bites during the larger amount of time that people were further away from the emanator.

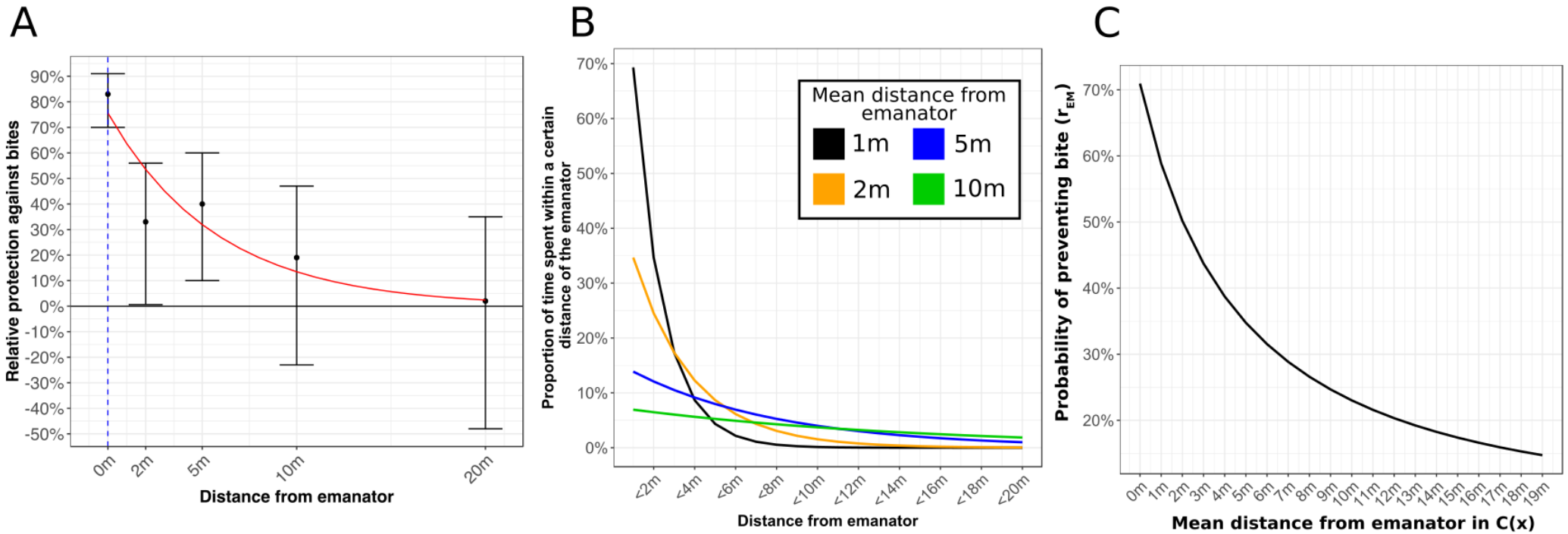

Figure 4.2: (A) The proportion of bites averted at each distance from the emanator (function E(x), shown in red) fitted to data from the Tanzanian trial Ogoma et al (2017) (black points with corresponding 95% confidence intervals). Solid red line indicates the best fit exponential function (B) Theoretical relationship charting the percentage of time spent within a certain distance of an emanator. Four different example exponential distributions C(x) characterised by their mean parameter λ. are shown which are later used in predictions (C) The proportion of preventing a bite (r_{EM}) by an emanator depending on the fitted relationship shown in (A) and different predictions of the average distance spent from an emanator (choice of λ in the distribution C(x) shown in B) which are plotted on the x-axes. For example, a person with a movement profile of the blue line in (B) is predicted to have 35% of mosquito bites averted by the emanator

4.3.2 Entomological modelling outcomes of TFI

The proportion of the vector population that have TFI is determined by four key factors. Firstly, the movement profiles of people around their emanators, which determines the probability that their emanators will prevent bites from occurring. Secondly, the proportion of mosquitoes that develop the TFI effect after exposure to the volatile pyrethroids. Thirdly, emanator coverage in the population, which changes the number of mosquitoes exposed to volatile pyrethroids. Finally, the duration of the TFI effect in mosquitoes. These four factors all change the mean percentage of the mosquito population with TFI at any one time over the year following emanator distribution (Figure 4.3).

When people spend more time closer to their emanators (λ is small), more mosquitoes have TFI because emanators are more likely to prevent bites when users are sat closer to them (Figure 4.3A). Varying the mean distance that users spend from their emanators between one metre and ten metres causes the probability that a mosquito takes a bite on an emanator user (r_{EM}) to vary between just under 30% and just over 75% (Figure 4.2C). Understandably, increasing the likelihood that mosquitoes develop TFI after exposure to transfluthrin (f_{EM}) leads to more mosquitoes having TFI in general. When a greater percentage of the human population are given an emanator, mosquitoes are more likely to try and bite a human sleeping near an emanator and, therefore, are more likely to develop TFI. Therefore, when population coverage increases, more mosquitoes have TFI (Figure 4.3B). Finally, varying the rate at which the TFI effect wanes in mosquitoes causes large changes in the percentage of the mosquito population with TFI. If the vectors recover more slowly (l_{TFI} is small), then a far higher proportion of mosquitoes have TFI at any one time (Figure 4.3C).

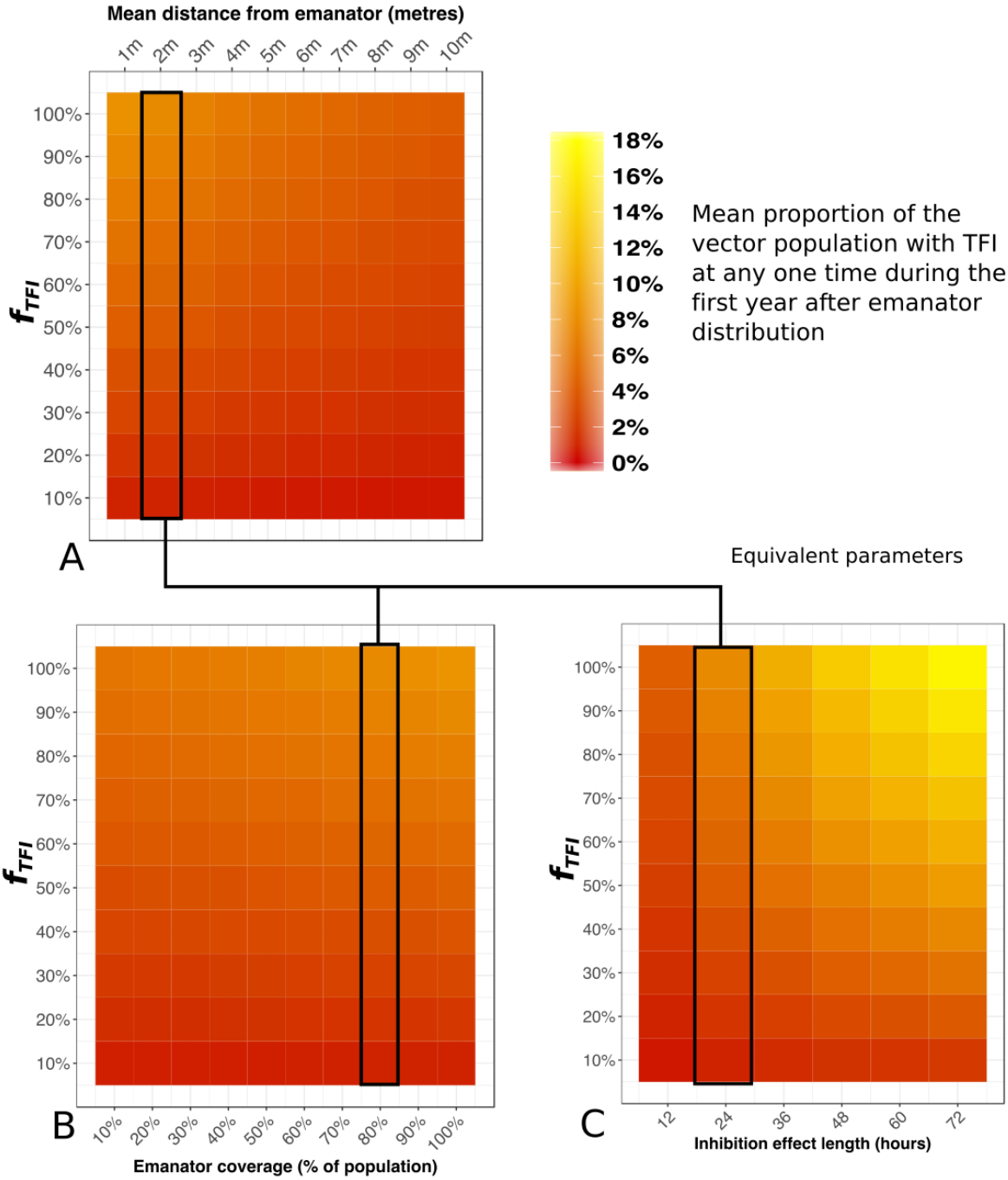

Figure 4.3: Impact of (A) distance from emanator (B) emanator population coverage and (C) duration of inhibition effect on the mean proportion of the total vector population with temporary feeding interruption (TFI) over the first year following emanator distribution. The y-axis in each panel shows the probability that a mosquito goes on to have TFI given that it has been dissuaded from biting (f_{TFI}). The resulting value (colour of the panel) is the mean proportion of the total vector population that have TFI at any point during the first year after emanator distribution. The 3 linked columns highlighted in black denote model runs where the parameter values are equivalent: 80% emanator population coverage, a 24-hour TFI effect length, and the value of the mean parameter for the distribution of time spent at different distances from the emanator is 2 metres.

4.3.3 Epidemiological outcomes of emanator distribution

Emanators deterring mosquitoes away from humans and preventing them from feeding on further hosts due to TFI will prevent some new infections from happening. This causes a moderate reduction in the prevalence of infection in under-fives as a result of emanator distribution in both of the transmission settings considered (Figure 4.4). As expected, emanators cause a modest reduction in disease burden compared to LLINs. The reduction in prevalence from a single emanator distribution is greatest at around the first year after introduction but has nearly returned to the baseline prevalence value by 4 years after introduction. The magnitude of the reduction in prevalence depends greatly on the proportion of deterred mosquitoes that go on to have TFI (denoted f_{EM}). Increasing f_{EM} from 0 to 100% causes nearly double the reduction in prevalence when all deterred mosquitoes have TFI compared to no TFI effect (Figures 4.4A & 4.4B). This indicates that the TFI actions of the emanator are equally important as its ability to simply deter mosquitoes. Furthermore, emanators cause a proportionally greater and longer-lasting reduction in prevalence in the setting with a lower baseline prevalence of 5%. In this setting, emanators reduce the prevalence from 5% to a minimum of just over 2.5% (approximately 50% reduction), whereas in the higher prevalence setting emanators reduce the prevalence from 30% to a minimum of 21% (approximately 33% reduction). This pattern of emanator impact depending on baseline endemicity is visible in Figure 4.4C. The lines denoting the prevalence before and after emanator introduction are slightly curved, meaning emanators (like other methods of vector control) cause proportionally bigger reductions in prevalence when the prevalence is lower to begin with. Similarly, absolute reductions in malaria prevalence will be higher in a moderate transmission setting (Figure 4.4A).

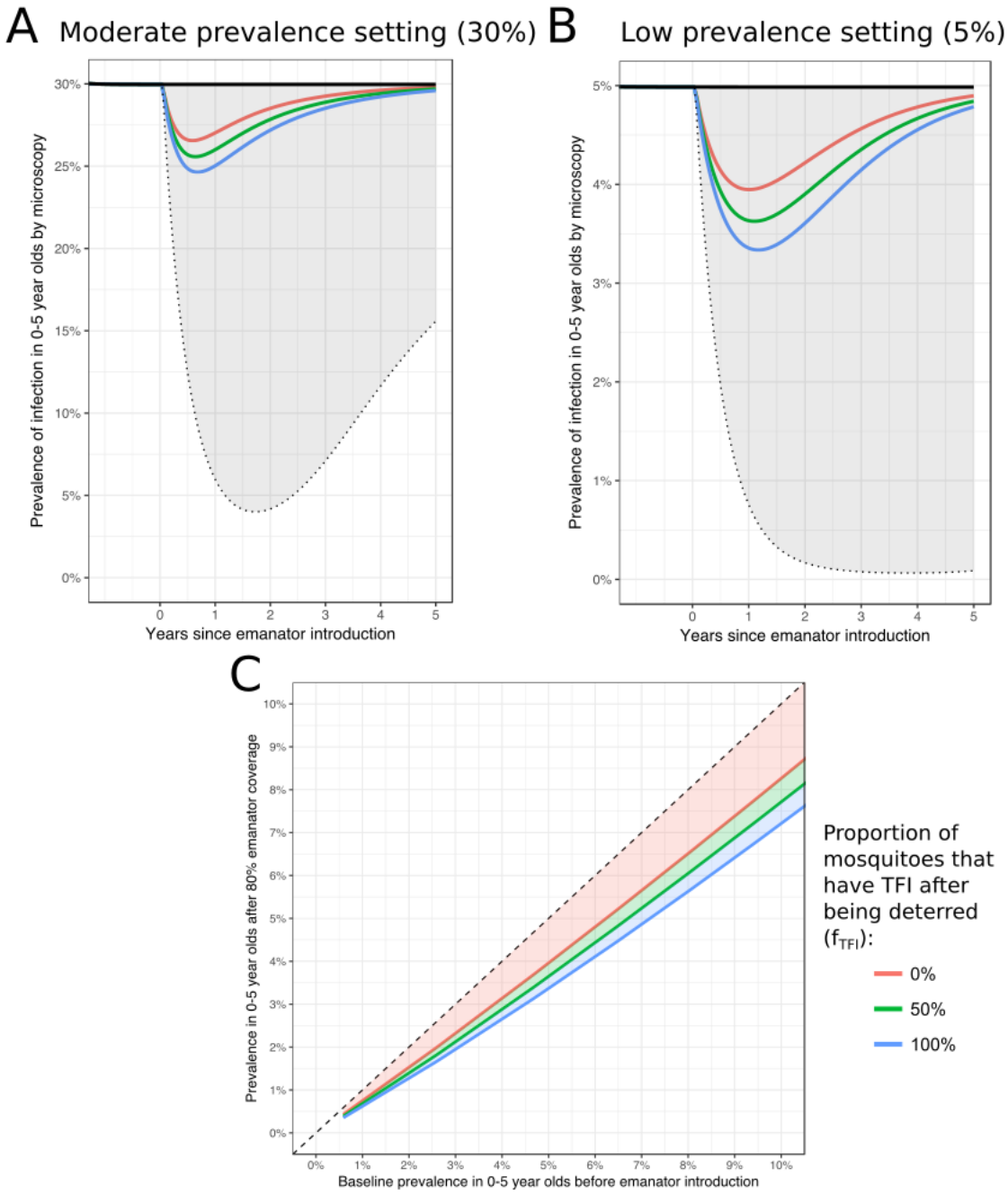

Figure 4.4: The impact of emanator distribution reaching 80% population coverage on the prevalence of infection in under-fives in two different transmission settings with a baseline prevalence of 30% (A) or 5% (B). The red, blue and green lines denote different assumptions regarding the probability that a deterred mosquito has TFI for 24 hours. The grey shaded areas denote the impact achieved by 80% ITN coverage in each transmission setting. For simplicity it is assumed that transmission is perennial with the same baseline prevalence of the disease throughout the year. (C) shows the reduction in the prevalence of infection in under-fives after one year due to 80% emanator population coverage expected for all transmission settings with a baseline prevalence of infection below 10%, as well as how this reduction changes with the assumption about the probability of TFI occurring. Dashed black line indicates where the intervention would have no epidemiological impact.

Emanator impact is favourable when considering the number of cases that they would prevent from occurring that would otherwise have to be treated by health services operating in humanitarian crises. The number of cases averted per 1000 under-fives per year increases as the likelihood of TFI occurring increases. Estimating the cases averted when the probability of a TFI effect occurring is 0% or 100% gives a range within which the true impact of emanators falls. In the 5% baseline prevalence scenario, with a baseline of 178 cases per 1000 children per year, achieving 80% emanator population coverage prevents between 46 and 70 of these cases per 1000 children in the first year (Figure 4.5B). This is between 26% and 39% of total cases in children prevented; the range corresponds to a probability of TFI upon deterrence (f_{TFI}) between 0% and 100%. In the 30% baseline prevalence scenario there are 1181 cases per 1000 children per year and 80% emanator population coverage prevents between 244 and 376 of these, i.e. between 21% and 32% of total cases (Figure 4.5A). As with the impact that emanators have on prevalence (Figure 4.4), when emanators are distributed once the number of cases prevented is greatly reduced by 3 years after introduction (Figures 4.5A and 4.5B). When emanators are handed out yearly (Figures 4.5C and 4.5D) rather than once (Figures 4.5A and 4.5B), the number of cases prevented per year increases year upon year in the 5% prevalence setting but decreases year upon year in the 30% prevalence setting. This is likely because of human immunity and interruption of transmission in low transmission settings (discussed further in Section 4.4).

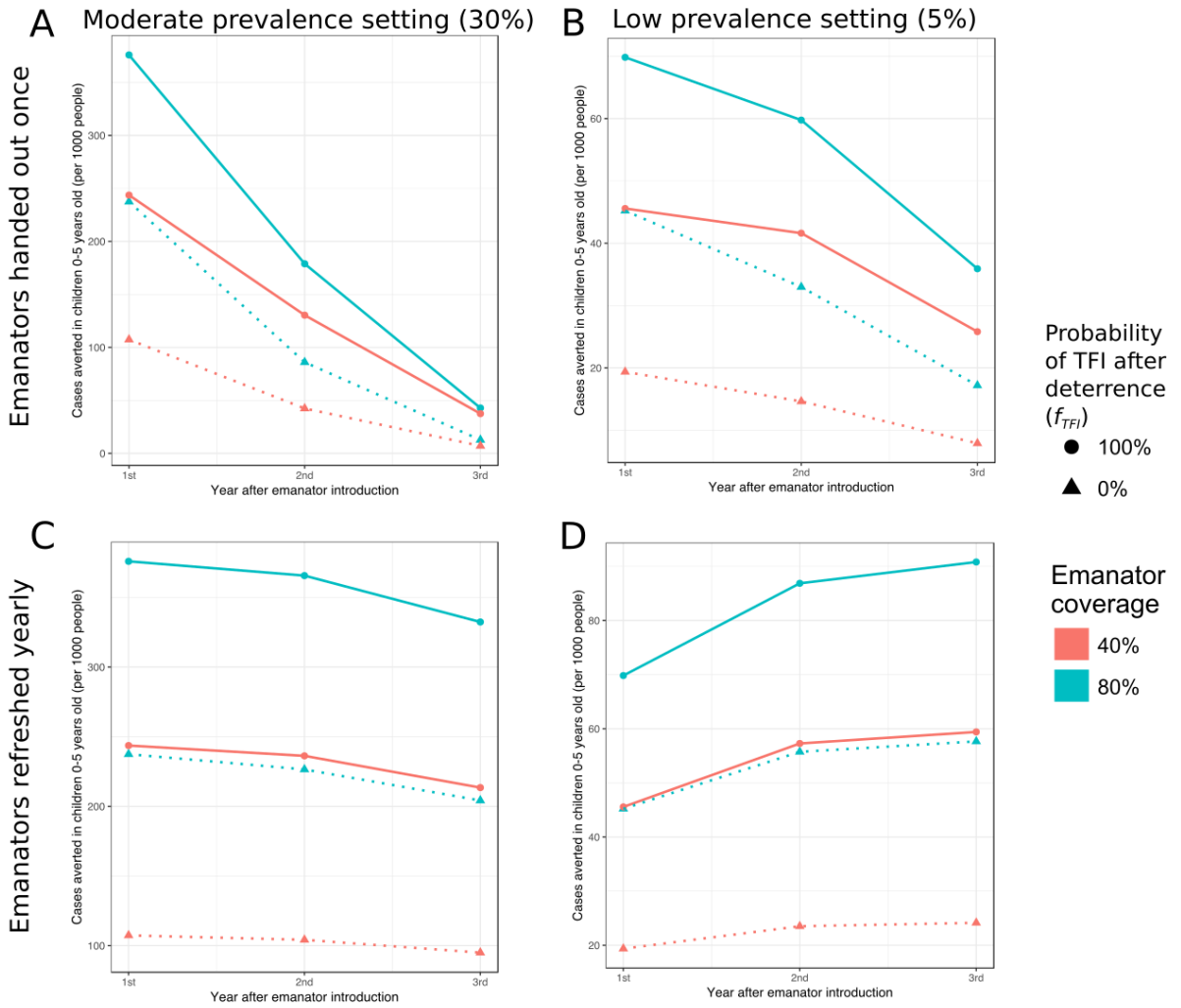

Figure 4.5: The number of cases averted in under-fives in the first 3 years of emanator impact, across 2 different transmission settings (A and C a moderate transmission setting, B and D a low transmission setting) and emanators being distributed once (A and B) or refreshed yearly (C and D). Line colour denotes emanator coverage, point shape and line type denotes the assumption regarding probability of feeding inhibition in deterred mosquitoes, with dashed line indicating lower TFI efficacy.

4.1.1 Impact measured through routine testing of pregnant women

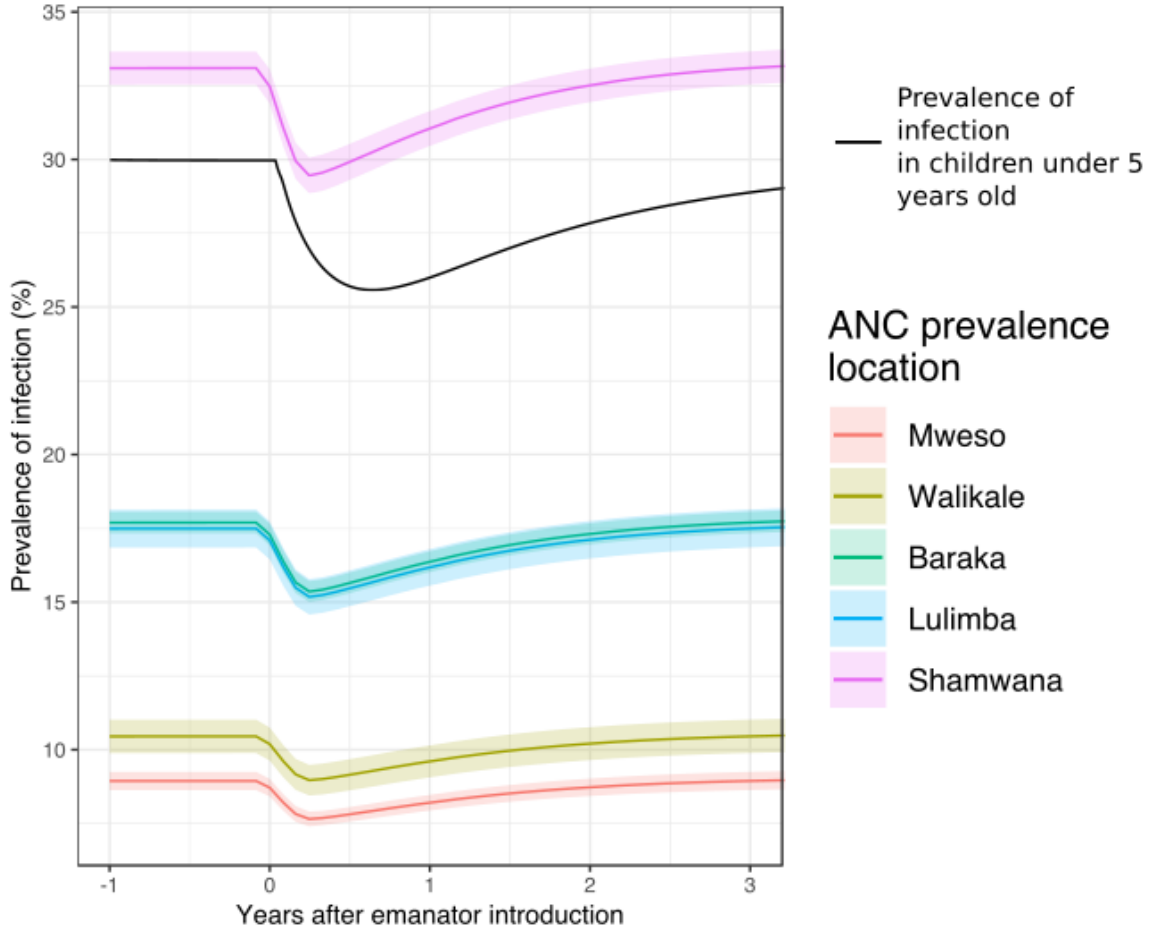

Figure 4.6: Comparison between how the prevalence of infection in children and the prevalence of infection in pregnant women change during emanator use. The black line shows the prevalence of infection in children between 0 and 5 years old with 80% emanator population coverage at year 0. The coloured lines show the corresponding predicted prevalence of infection in pregnant women at each of the MSF locations introduced in Chapter 3. The coloured bands around each line shows the 95% confidence interval from the predictions of the best fitting DLNM model in chapter 3.

When measuring the impact of emanators in terms of ANC prevalence (Figure 4.6) instead of the prevalence of infection in under-fives (Figure 4.4), the reduction in ANC prevalence caused by emanators is predicted to be smaller than for children. This reflects the relationship between the two groups assessed in Chapters 2 and 3. For example, in the community of Shamwana there is predicted to be a 3.0% reduction in malaria prevalence in children under 5 years old one year after emanator distribution at 80% coverage, whereas the prevalence of infection in pregnant women is predicted to only reduce by 1.45%.

The relatively small magnitude of the effect size predicted here means that, on their own at least, none of the MSF locations introduced in the previous two chapters have sufficient women attending each month to have enough statistical power to be able to discriminate between the small, real changes in ANC prevalence due to emanators and the monthly fluctuations in observed ANC prevalence caused by sampling noise (Table 4.1). For example, the 3.64% reduction in prevalence prediction in the

community of Shamwana would require 1232 women before and after the introduction of emanators to detect a significant difference. In this location MSF is currently treating 455 women each month, so data from multiple months or from different communities combined would be required to have enough power to detect the predicted epidemiological impact of the intervention.

Table 4.2: The results of the simplified hypothesis test specified in Section 4.2.3 to determine whether MSF would be able to detect small changes in the prevalence of infection with the number of women sampled at ANC centres each month with 95% confidence and 80% statistical power. Column 2 shows the maximum difference in true ANC prevalence before emanator impact and at the peak of emanator impact (the values correspond to Figure 4.6). Column 3 shows the minimum sample size of pregnant women required to detect the effect size shown in column 2. Column 4 shows the median number of pregnant women that were actually sampled monthly at each MSF location.

Location	**Maximum effect**	**Necessary sample size**	**Actual median sample size**
Baraka	2.33%	1873	636
Mweso	1.27%	3351	1074
Walikale	1.48%	2915	437
Shamwana	3.64%	1232	455
Kimbi-Lulimba	2.31%	1890	582

4.4 Discussion

The work in this chapter shows that pyrethroid emanators could be used to prevent a considerable proportion of malaria cases when used at night in humanitarian settings where IRS or LLINs cannot be used. This would reduce the malaria burden on overloaded health care systems, freeing up capacity to tackle other public health issues that are often exacerbated during humanitarian crises (Carrión Martín *et al.*, 2014). However, my predictions of the public health impact of emanators alone indicate that they do not cause substantial enough reductions in the prevalence of infection in a population and therefore should not be considered as competing with LLINs or IRS as ways of pushing malaria transmission down to zero. The small impact of emanators in terms of ANC prevalence is an issue, because it means that MSF would struggle to detect that emanators had reduced malaria burden unless they tested a large number of women each month. Meanwhile, the clinical incidence in under-fives would be significantly reduced by emanator distribution, but this data is not so easily collected by MSF (as discussed in Chapter 3).

The reduction in disease burden associated with emanators is modest compared to LLINs primarily because LLINs kill mosquitoes that try to get through the net. This killing effect reduces the infectious vector population by killing mosquitoes that are infectious or would have gone on to become infectious. Since emanators simulated here only deter mosquitoes temporarily the mosquitoes will live on to try and successfully feed again. Adding the temporary feeding interruption (TFI) effect to the vector model improves emanator effectiveness substantially because it reduces the size of the infectious vector population, even though it does not kill mosquitoes. A complete TFI effect (i.e. all mosquitoes deterred away from the emanator develop the TFI effect) on average doubles the effectiveness of the intervention compared to the deterrence effect alone in the situations examined

here. Susceptible mosquitoes with TFI are prevented from becoming latently infectious, and infectious mosquitoes with TFI are prevented from contributing to transmission. The end result is that fewer mosquitoes become infectious and less of the infectious mosquito population is able to bite. The longer the duration of the TFI effect, the greater the reduction in transmission (Figure 4.3C), with a TFI effect that prevents a mosquito from ever feeding again being epidemiologically equivalent to having killed the mosquito.

The length of the TFI effect is as important as the likelihood that a mosquito acquires the TFI effect. Only a small proportion of the vector population will have TFI at any one time if mosquitoes recover from it quickly. Accurately parameterising the TFI effect presents some challenges, since the likelihood of a mosquito getting TFI and how long it lasts is likely to depend on the length of exposure, concentration of the pyrethroid in the air, level of pyrethroid resistance in the mosquito, and the species of mosquito. The pyrethroid concentration in the air will further depend on what is releasing pyrethroid and where it is being released. How long the TFI effect lasts varies by mosquito, but seems to have finished within 24 hours (Siegert, Walker and Miller, 2009), with some studies finding that about half of mosquitoes have recovered within 12 hours after exposure (Ogoma, Lorenz, *et al.*, 2014). A mosquito that has TFI for 6 to 8 hours will in effect be prevented from biting for the rest of the night and will have to wait even longer until their nightly feeding window begins again. Since the Imperial College malaria model does not take into consideration the time of day, the TFI effect length parameter should reflect that many mosquitoes will not be able to immediately resume feeding after they recover from TFI. If the true TFI effect length is 12 hours, the TFI effect length used in the model could well be in the region of 24 hours to incorporate the fact that mosquitoes will not be able to feed again until the next night. Further studies that attempt to quantify the length of the TFI effect due to pyrethroid exposure would be very welcome, given what a difference the TFI effect makes to the number of cases prevented by emanators and presumably other spatial repellents. As shown in this chapter, low emanator population coverage with a high likelihood of TFI is preferable to high population coverage with a low likelihood of TFI (Figure 4.5).

Emanators caused a larger and more sustained proportional reduction in the prevalence of infection in under-fives in the low prevalence setting compared to the high prevalence setting (Figure 4.4). This finding corresponds with other modelling work that finds that malaria interventions are associated with larger percentage decreases in disease burden for lower pre-intervention prevalence levels (Cameron *et al.*, 2015). Emanators reduce the EIR by the same proportion in both transmission settings, but this corresponds to a greater proportional decrease in new clinical cases and therefore a greater drop in prevalence in the lower baseline prevalence setting. The reduction in prevalence in the higher baseline setting lasts a shorter amount of time because there is a higher rate of infectious bites, meaning that people who are clear of infection due to cases averted by emanators will quickly acquire new infections.

Another difference in emanator impact between prevalence settings is that the impact of repeated emanator distributions increases year upon year in the low prevalence setting and decreases year upon year in the high prevalence setting (Figures 4.5C and 4.5D). The number of cases averted by emanators decreases year upon year in the high prevalence setting because much of the population have asymptomatic or sub-microscopic infections (in the Imperial College malaria transmission model they are in compartments A or U, see Section 1.3.3.1). When emanators prevent bites from occurring, some of these infections gradually clear and people move into the susceptible compartment over time. This means that the clinical incidence increases slightly year upon year because now more people are susceptible to a new infection than before emanators were introduced. Therefore, the number of cases averted by emanators (equal to the number of cases that would have happened without emanators minus the number of cases that happened with emanators) decreases slightly. This effect plateaus when the susceptible, asymptomatic infection, and sub-microscopic infection compartments reach a new equilibrium (assuming that emanators keep being handed out each year).

The modelling approach used here has limitations that will prevent the accuracy of the predictions of emanator impact. For example, the effectiveness of emanators at different distances is parameterised using only one study. Due to this we cannot discern how mosquito responses to emanators using volatile pyrethroids would change due to resistance to lethal doses of pyrethroids. The model also assumes that human movement patterns around their emanators are homogeneous and that they will continue to use them for a long time after distribution. Both of these assumptions could be considered slightly unrealistic in the chaotic context of humanitarian crises where people have been displaced and are moving from place to place.

4.5 Conclusions

This chapter showed that emanators used near people that are sleeping could avert around a quarter to a third of malaria cases. However, they should not be considered an alternative to LLINs and should only be considered when it is not feasible to use LLINs. Emanator distribution would not cause detectable reductions in ANC prevalence at MSF's sites unless MSF were sampling a large number of women each month. This is therefore something that MSF should consider going forward, if they are to use ANC prevalence to ascertain whether their malaria control efforts are working. The next chapter will consider how emanators could be used in a different context to prevent outdoor biting in the evenings, when LLINs cannot prevent biting from occurring.

5 Closing the coverage gap: emanator use to prevent outdoor biting in the evening

5.1 Introduction

This chapter will build upon the previous chapter by considering whether emanators could be distributed alongside LLINs to prevent residual malaria transmission. Residual malaria transmission is defined as transmission that takes place even when there is universal coverage of LLINs and IRS, such as biting taking place outside the house in the early evening (Durnez and Coosemans, 2013). The rate of infectious bites required to maintain ongoing transmission is low enough that in many places ITN and IRS campaigns cannot permanently interrupt transmission, even if they do manage to reduce the disease burden in the human population (Killeen, 2014). Therefore, to move beyond suppressing the disease in humans and towards malaria elimination, control methods must be used that target residual transmission. The contribution that residual transmission will make to the local disease burden, and the best methods to prevent it, will depend on human behaviour and the interaction with the feeding behaviours of local mosquitoes (Kiware *et al.*, 2017).

The mass LLIN distribution campaigns since the turn of the century means there are now many countries in sub-Saharan Africa where LLIN use is widespread, thus protecting many people from infectious mosquito bites while they are in bed (World Health Organization, 2017b). Studies in various countries have observed some mosquitoes biting outdoors in the early evening, before people have gone to bed (Bradley *et al.*, 2015; Cooke *et al.*, 2015; Dambach *et al.*, 2018). It is unclear whether this is due to differences in the local mosquito population or an evolutionary change induced by the selection pressure of LLIN and IRS. Outdoor evening biting is observed in the common anthropophagic *Anopheles* species, which are the primary vectors of malaria, as well as in some zoophagic species that are less effective malaria vectors but still contribute to residual transmission by biting at different times of the day (Killeen, 2014). In addition to general species-level feeding preferences, mosquitoes are less likely to feed indoors when it is hotter and drier relative to outside (Ngowo *et al.*, 2017). This Chapter will refer to residual transmission taking place outdoors during the evening as “the evening coverage gap”. There is no widespread vector control tool used to prevent biting during the evening coverage gap, though alternative control methods are currently being developed to target mosquitoes whose feeding behaviour reduces the effectiveness of current vector control tools (Müller *et al.*, 2010; Menger *et al.*, 2014; Killeen *et al.*, 2017).

Spatial repellents operate by releasing chemicals into the air that either cause mosquitoes to move away from the source of the chemical (excito-repellency), prevent a mosquito from finding a host within an area around the source of the repellent (Maia *et al.*, 2018), or kill them. Both of these modes of action reduce the amount of mosquito bites on humans near the source of spatial repellents. Previous modelling work using the Imperial College malaria transmission model has focused on using

LLINs or IRS to prevent people indoors or in bed from being bitten, since this is primarily where most malaria transmission happens in countries starting malaria control programmes. Spatial repellents such as emanators have only been included in less complex malaria transmission models, which have found that outdoor spatial repellents would complement LLIN use to reduce EIR (Killeen and Moore, 2012; Kiware *et al.*, 2017). The work in this Chapter builds upon this previous modelling efforts by using mosquito biting data to establish an estimate of the amount of biting that takes place during the evening coverage gap and what proportion of clinical cases these bites are responsible for. This quantifies how much malaria transmission could be reduced by preventing biting during the evening coverage gap, and how biting during the evening coverage gap can prevent local malaria elimination. Using data on the performance of emanators in the Tanzanian field trial (Ogoma *et al.*, 2017), the Imperial College malaria transmission model is used to predict the reduction in disease burden associated with emanator use. This will give a more detailed picture of the public health impact of emanators as the change in disease burden is more complex than simply the change in EIR because of the actions of human immunity on the malaria parasite.

Finally, emanator use will be explored in scenarios where the local mosquito population is resistant to lethal doses of pyrethroids on LLINs. It is currently unclear how pyrethroid resistance in vector populations, as measured by bioassay mortality, will mitigate the deterrent effect of spatial repellents. Experimental hut trials of LLINs in areas with pyrethroid resistant anopheline mosquitoes show little to no deterrence effect from the pyrethroids on LLINs (Strode *et al.*, 2014; Churcher *et al.*, 2016). However, a deterrent effect is still maintained when pyrethroid-treated clothing is used against *Aedes aegypti* mosquitoes with genetic mutations that are associated with pyrethroid resistance (Bowman *et al.*, 2018). Insensitivity to transfluthrin in the air has been selectively bred *in vitro* into *A. aegypti,* and has been shown to be associated with reduced insecticide susceptibility (Wagman, Achee and Grieco, 2015a). Of relevance for this particular analysis is that vector resistance to many pyrethroids is widespread in Tanzania, where the field trial for emanators took place (Kabula *et al.*, 2012, 2014; Kisinza *et al.*, 2017). Therefore, the emanators added to the Imperial College malaria transmission model in the last chapter are based upon data measuring emanator effectiveness in areas with reasonable levels of pyrethroid resistance, suggesting that the deterrence effect of volatile airborne pyrethroids is maintained against pyrethroid resistant mosquitoes which have reduced susceptibility to pyrethroids incorporated into LLINs. If this is the case then they can be explored as a temporary solution to the loss of lethality in existing LLINs. This Chapter ends by investigating where using emanators during the evening coverage gap could offset the increased malaria burden due to pyrethroid resistance. Emanators could be an affordable method of controlling excess malaria cases due to pyrethroid resistance, since it may be some time before new non-pyrethroid-based net products, such as the Interceptor G2 net that uses chlorfenapyr, will be available for mass distribution (Hemingway *et al.*, 2016).

5.2 Methods

5.2.1 Amount of outdoor biting in the evening

A systematic search of the published literature was used to identify studies in Africa measuring mosquito biting rate using human landing catches placed indoors and outdoors throughout the night. For full details of the search terms employed see Skarp 2016 (a copy of the search terms used is presented in Table 5.1). This meta-analysis identified twenty-four studies on mosquito biting times for predominantly *An. arabiensis, An. funestus* and *An. gambiae s.s*, (giving a total of 61 days of observation). Results from this analysis were used in this thesis to estimate the proportion of all bites that are received during the evening coverage gap, denoted Φ_E (Equation 5.1). The Imperial College model has previously only considered when people are indoors or in bed, so the percentage of bites received while outdoors in the evening needs to be estimated from available data.

Table 5.1: "Search strings for a systematic meta-analysis of Anopheles mosquito biting behaviour in African countries". Copied from Skarp, Janetta., BSc project Imperial College London (2016) "Investigating the impact of Anopheles mosquitoes' biting time on the efficacy of bednets against malaria."

Search	Query	Items found
Web of Science search string		
	Search: TOPIC: (Anopheles AND man AND biting AND rate AND Africa)	391
	Search: TOPIC: (malaria AND (transmission OR exposure)) *AND* TITLE: ((Anopheles OR mosquito* OR vecto*) AND (behavio* OR outside OR outdoor OR inside OR indoor OR patter* OR season*))	502
PubMed search string		
1	anopheles [Title/Abstract]	11313
2	mosquit* [Title/Abstract]	36776
3	vector [Title/Abstract]	138515
4	#1 OR #2 OR #3	168236
5	transmission [Title/Abstract]	277118
6	exposure [Title/Abstract]	640631
7	#5 OR #6	6339591
8	behavio* [Title/Abstract]	946966
9	outside [Title/Abstract]	110239
10	outdoor [Title/Abstract]	13555

11	inside [Title/Abstract]	91278
12	indoor [Title/Abstract]	18354
13	patter* [Title/Abstract]	1022271
14	season* [Title/Abstract]	110037
15	#8 OR #9 OR #10 OR #11 OR #12 #13 OR #14	2153475
16	malaria [Title/Abstract]	65457
17	Blood-feed* [Title/Abstract]	2158
18	Bit* [Title/Abstract]	56120
19	Feed* [Title/Abstract]	321958
20	#17 OR #18 OR #19	375578
21	#4 AND #7	48294
22	#21 AND #15	6569
23	#22 AND #16	1227
24	#23 AND #20	436

Table 5.2: List of the studies used to estimate outdoor biting rates of mosquitoes at over 24 hours (as collated from the meta-analysis search string presented in Table 5.1). Some of the studies had several sites, rounds, or locations.

Number	**Source**	**Location**
1	(Bayoh *et al.*, 2014)	Kenya
2	(Bradley *et al.*, 2015)	Equatorial Guinea
3	(Cooke *et al.*, 2015)	Kenya
4	(Fontenille *et al.*, 1997)	Senegal
5	(Geissbühler *et al.*, 2007)	Tanzania
6	(Githeko *et al.*, 1996)	Kenya
7	(Huho *et al.*, 2013)	Burkina Faso, Tanzania, Zambia, Kenya
8	(Kabbale *et al.*, 2013)	Uganda
9	(Killeen *et al.*, 2006)	Tanzania
10	(Mendis *et al.*, 2000)	Mozambique

11	(Moiroux *et al.*, 2014)	Benin
12	(Moiroux *et al.*, 2012)	Benin
13	(Mourou *et al.*, 2012)	Gabon
14	(Ojuka *et al.*, 2015)	Uganda
15	(Overgaard *et al.*, 2012)	Equatorial Guinea
16	(Owusu *et al.*, 2016)	Ghana
17	(Quiñones *et al.*, 1997)	The Gambia
18	(Russell *et al.*, 2011)	Tanzania
19	(Tchouassi *et al.*, 2012)	Ghana
20	(Tanga and Ngundu, 2010)	Cameroon
21	(Tanga, Ngundu and Tchouassi, 2011)	Cameroon
22	(Tuno *et al.*, 2010)	Ghana

Letting $\lambda_I(t)$ and $\lambda_O(t)$ be the rate at which a person is bitten at hour *t* (0-24) when they are indoors or outdoors, respectively. The value of Φ_E relies upon the proportion of the population outside during the evening coverage gap, let the proportion of the population outside at time *t* be denoted $p_E(t)$. The formula for Φ_E is as follows:

$$\Phi_E = \frac{\sum_t p_E(t)\lambda_O(t)}{\sum_t((1 - p_E(t)\lambda_I(t) + p_E(t)\lambda_O(t))} \quad 5.1$$

This formula gives 61 estimates of Φ_E for a given choice of $p_E(t)$, one estimate for each day of observation in the dataset. This range of values for Φ_E captures the variation in observed biting rates across different days and different countries. In this analysis I make the simplifying assumption that the same proportion of the population are outside continuously between 6pm and 10pm. I do this by setting the proportion of people outside at a given time ($p_E(t)$) to a certain value (that I vary between 10% and 100% for sensitivity analysis) between 6pm and 10pm. The rest of the time the proportion of

population outside is set to zero. This choice of $p_E(t)$ then gives corresponding values of Φ_E that I use in the model. This assumption also implies that people who leave the house early in the morning are not protected by emanators as they will be leaving the vicinity of the house to work.

5.2.2 Adding emanators as an intervention in the model

The Imperial College malaria transmission model is expanded to incorporate emanators in a similar manner to the previous chapter, except now emanators are used outdoors rather than when people are in bed. For a given proportion of the population outside during the evening coverage gap, the model calculates the proportion of all bites that take place during the evening coverage gap (Figure 5.1A). For these, mosquitoes that attempt to bite an emanator user have a given probability to be deterred and subsequently experience a temporary feeding inhibition (TFI) effect (Figure 5.1B). The TFI effect is presumed to prevent mosquitoes from biting for an average of 24 hours (see Section 4.4 for why this length was chosen). Mosquitoes that have a TFI effect are moved into parallel vector compartments where they do not feed, reducing the effective infectious mosquito population because susceptible mosquitoes with TFI do not become infectious and infectious mosquitoes with TFI do not bite (Figure 4.1). For the bites happening while people are in bed, mosquitoes that try to bite someone using an LLIN either die or are deterred, as specified in the original model (see Section 1.3.3).

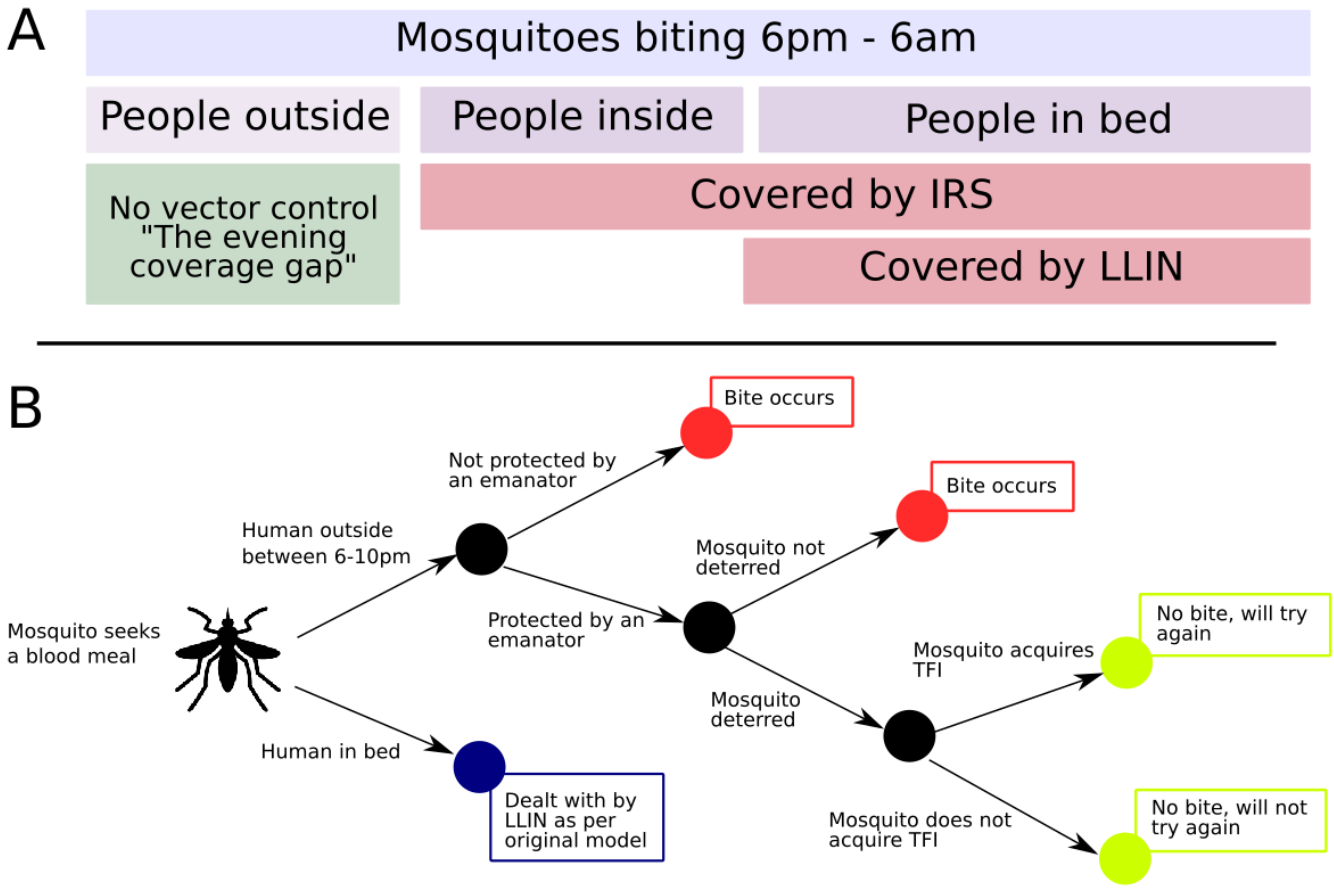

Figure 5.1: (A) Graphical depiction of the time of day being defined as "the evening coverage gap" in this chapter. (B) Flowchart of the different effects that LLINs and emanators have on vectors in the expanded Imperial College malaria transmission model.

Now that users can have emanators, LLINs, or both, the formulas for the probability of a mosquito successfully biting a human has changed (Table 5.2). In this chapter it is assumed that emanators are used exclusively during the evening coverage gap and act completely independently from LLINs used at night. For an emanator, r_{EM}, is the proportion of bites that will be prevented across all distances (see Section 4.2.1). For LLINs, s_{LLIN} is the probability of a mosquito successfully biting an LLIN user and Φ_B is the proportion of bites that happen while a person is in bed.

Table 5.3: Probabilities of successful feeding and being repelled for combinations of emanator and LLIN use.

	Emanator only (w_{EM})	**LLIN only (w_{LLIN})**	**Emanator and LLIN ($w_{EM+LLIN}$)**
Probability of successful feeding (w_i) (feeds and survives)	$1 - r_{EM}\Phi_E$	$1 - \Phi_B + s_{LLIN}\Phi_B$	$1 - r_{EM}\Phi_E - \Phi_B + s_{LLIN}\Phi_B$
Probability of repellency (z_i)	$r_{EM}\Phi_E$	$r_{LLIN}\Phi_B$	$r_{EM}\Phi_E + r_{LLIN}\Phi_B$

5.2.3 Pyrethroid resistance

Pyrethroid resistance is introduced into the vector population in the model using the framework from Churcher et al (2016) where resistance is characterised using the proportion of mosquitoes that survive a simple pyrethroid bioassay. Increasing pyrethroid resistance reduces the maximum mortality and repellent effects of the LLINs, as well as the length of time for which the LLINs are effective. The vector species used in the model is *An. gambiae sensu stricto.* since this is a key vector species in Tanzania, where the emanator field trial was undertaken. *An. gambiae s.s.* also features extensively in the mosquito data used to approximate outdoor biting rate (Table 5.1) and the data used to parameterise the relationship between pyrethroid resistance on LLIN effectiveness in Churcher et al. (2016). There insufficient data available to characterise how emanator effectiveness changes with the level of pyrethroid resistance. Therefore, for simplicity it is assume that emanator efficacy is the same irrespective of the level of resistance against pyrethroid LLINs. As mentioned above, the data used to parameterise emanators in this model was collected in a region with reasonably high pyrethroid resistance (Tanzania, 2017) so the effect of moderate pyrethroid resistance on emanator effectiveness (should it exist) is already implicit in the data.

5.2.4 Intervention scenarios

Emanator use during the evening coverage gap was modelled in a scenario where 80% of the population already uses a LLIN with new LLINs distributed every three years. The LLINs have already considerably reduced the number of infectious bites that the population experience, reducing the prevalence in children under five from 46.6% to 36.7% and the clinical incidence in children under five from 1673 to 928 cases per 1000 child under five per year. Malaria transmission was kept constant year round, removing seasonality, to help identify changes in burden associated with emanator use. After the model had run for a long enough time to reach equilibrium (see Section 1.3.3.3), everyone that had an LLIN was given an emanator, which was replaced each year. Including the systematic compliance assumption may give more conservative predictions of emanator impact, since using emanators and LLINs at the same time may have an antagonistic relationship if the emanator-caused TFI effect stops mosquitoes from dying on nets later in the night.

In measuring the impact of emanator distribution it is important to distinguish between residual cases and remaining cases. Residual cases are those that occur despite 100% LLIN coverage (i.e. initially everyone is using a LLIN and there is no drop out over time). Remaining cases are those that occur after more realistic coverages of are assumed. Here I assume that 80% LLIN coverage is a more realistic target, again assuming that there is no loss in LLIN use over time during the period of investigation. Some of the remaining cases would be prevented by higher LLIN coverage, but not all. Remaining cases can be split into two components: cases due to 20% of the population lack an LLIN, and residual cases (which would still occur even at 100% LLIN coverage).

The proportion of remaining cases that are due to biting during the evening coverage gap is calculated by measuring the change in the number of cases averted between a scenario with 80% LLIN coverage where no outdoor biting is prevented, and a scenario with 80% LLIN coverage where all outdoor biting is prevented. The difference between the number of remaining cases in these two scenarios is the number of cases that emanators could hypothetically prevent if they were perfect and prevented all biting from taking place during the evening. Emanator impact can then be measured in two ways: the proportion of cases due to biting during the evening coverage gap that they prevent, and the proportion of remaining cases that they prevent. Measuring the impact in two ways helps to distinguish between situations where emanators perform very well at closing a small coverage gap (a small number of overall cases prevented) and situations where emanators perform poorly at closing a large coverage gap (a large number of overall cases prevented). Throughout the chapter effects of emanator use were modelled using two different assumptions regarding the proportion of all biting taking place during the evening coverage gap (Φ_E). As described above, choosing a value for the proportion of the population that is outdoors during the evening coverage gap provides a range of estimates for Φ_E. The *best guess* estimate uses the median of this range of values (Figure 5.2B, solid blue line), whereas the *optimistic* estimate uses the upper 95th percentile (Figure 5.2B, dashed blue line). The likelihood of TFI occurring in deterred mosquitoes (f_{TFI}) was set to 100% when the TFI effect was included in the model. It is assumed that while people are sat outdoors, they are an average of 2.5 metres away from the emanator.

5.3 Results

5.3.1 The proportion of remaining malaria cases due to biting during the evening coverage gap

There is a large amount of variation in the observed biting rate between locations and on different days, with some locations experiencing no biting outdoors during the evening coverage gap and some locations reporting that the outdoor biting rate was comparable to the indoor biting rate by around 10pm (Figure 5.2A). Examining the median biting rate across all locations and study days, outdoor

biting rises steadily from around 6pm through to 10pm, assuming that 10% or 100% of the population are outdoors during the evening coverage gap gives a range of estimates of Φ_E, with 1.6% to 12.2% of all bites happening during the evening coverage gap (Figure 5.2B, black line). The estimates of Φ_E are much larger when using the top 95th percentile of observed outdoor biting rates, the value of Φ_E changes from 11.2% to 56.9% when 10% or 100% of the population are outdoors during the evening coverage gap (Figure 5.2B, green band).

In a population using LLINs, most of the attempted bites will happen at night will be prevented except for situations where mosquitoes enter through holes in the net. Even though a minority of attempted bites happen during the evening, more of these bites will be successful. As a result, the majority of malaria cases occurring will be due to successful bites happening during the coverage gap. Even assuming that only 10% of the population are outside during the evening, up to 35% of the remaining malaria cases could be due to biting during this time. Assuming that 50% of the population are outside at this time, which is not unreasonable, this could account for just over 75% of remaining malaria cases after good LLIN coverage (Figure 5.2C).

5.3.2 The amount of remaining cases prevented by emanators

The impact of emanator use depends on the amount of biting happening during the evening coverage gap. Emanators are able to prevent a higher proportion of remaining cases if more of the remaining cases are due to bites happening during the evening coverage gap. Comparing the best guess estimate and optimistic estimate of the percentage of cases occurring in the evening (Φ_E) (Figure 5.2B) shows how the theoretical impact of emanators on top of LLIN use changes depending on the size of the evening coverage gap. Firstly, LLINs cause a smaller reduction in clinical incidence in under-fives when more bites happen during the evening coverage gap. This can be seen in the smaller decline in malaria cases in Figure 5.3E than Figure 5.3A. Similarly, the percentage of remaining cases after 80% LLIN coverage that are due to the evening coverage gap increases with higher evening biting, so there are more cases that emanators could prevent (Figure 5.3B&F). In the best guess scenario, emanators prevent ~55% of cases due to the coverage gap in the first year, increasing to ~60% in the third year

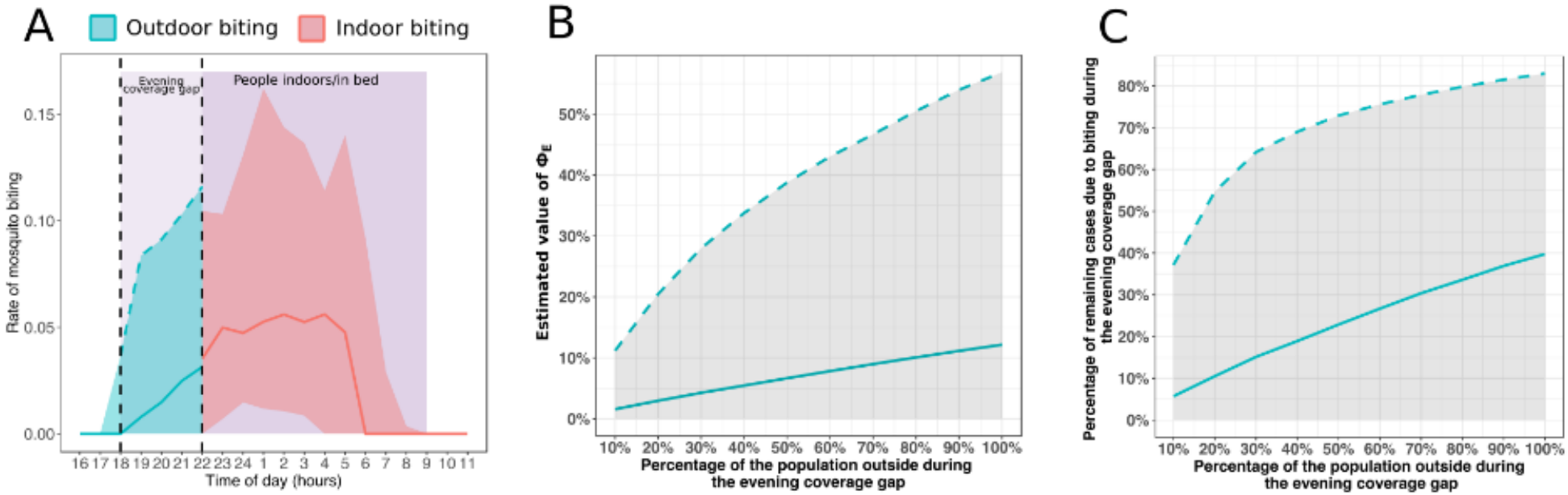

Figure 5.2: Observed variation in outdoor biting during the evening coverage gap and how much this biting contributes to residual malaria transmission. In all plots the solid blue line represents the best guess estimate (median) and the dashed blue line represents the optimistic estimate (upper 95th percentile, see Section 5.2.4) (A) observed rates of outdoor (blue) and indoor (red) biting over the day from the collated mosquito studies. Solid lines denote median observed biting rate over all studies. The intervals show 95% confidence intervals for observed biting rate over all studies. (B) the percentage of all bites that happen during the evening coverage gap (Φ_E) for different assumptions regarding the proportion of the population that are outside during the evening coverage gap combined with the observed biting in (A) (using Equation 5.1) (C) Model estimates of the percentage of all cases remaining after 80% ITN coverage that are due to biting happening the evening coverage gap, using the estimates of Φ_E shown in (B).

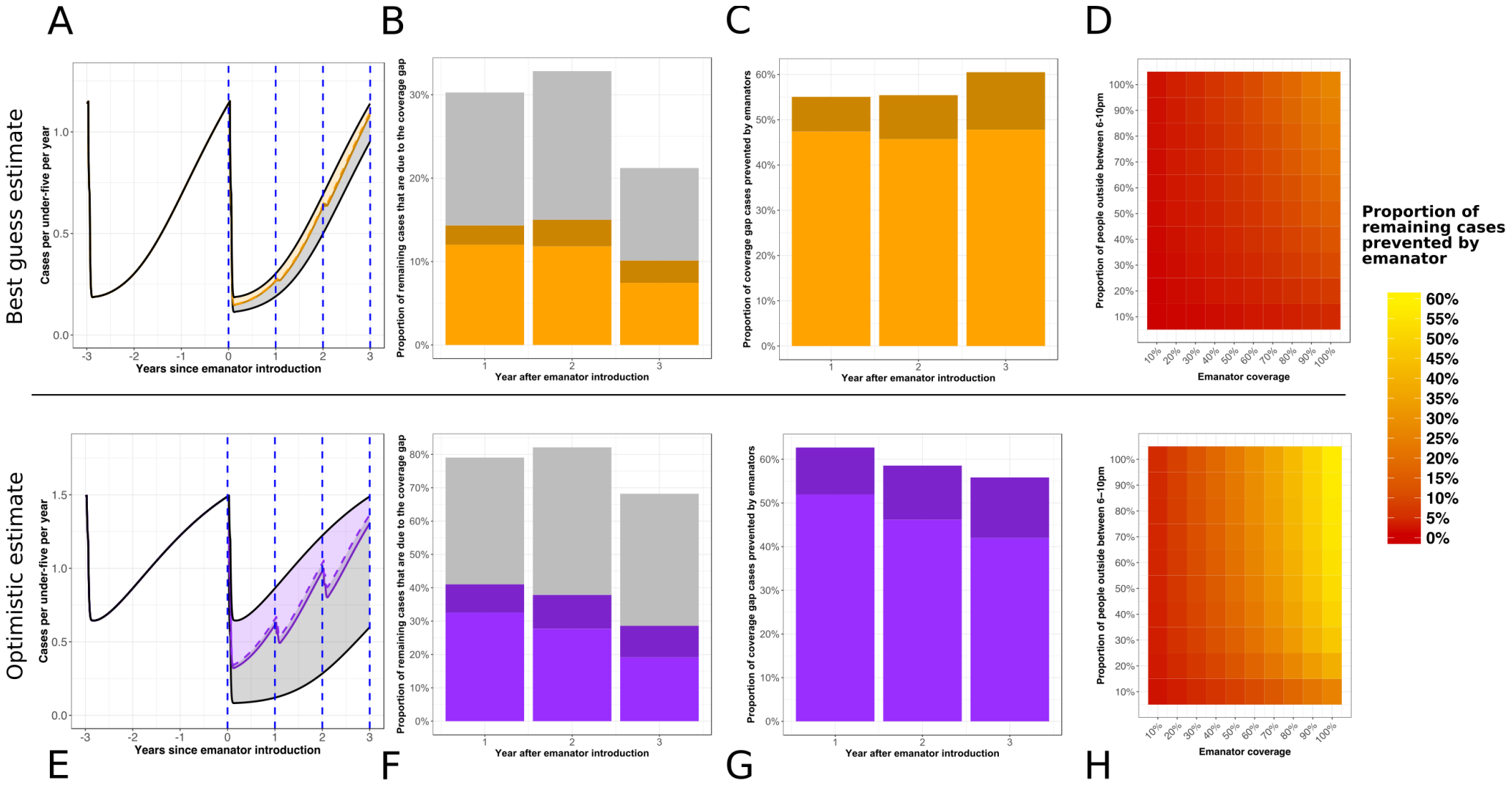

Figure 5.3: How emanator effectiveness varies with the size of the evening coverage gap. Top row indicates scenarios where the percentage of bites taken in the evening matches current best guess estimates whilst bottom row shows a scenario where a high percentage of bites occur in the evening coverage gap. (A and E) show the reduction in incidence of malaria in under-fives due to emanator use. Grey area between the two black lines shows cases that could be averted by emanators. Top black line shows incidence reduction from 80% LLIN coverage alone. Lighter coloured orange/purple band shows cases averted by emanators with deterrence effect only, darker coloured orange/purple band shows additional cases prevented by TFI effect. Blue vertical lines show yearly boundaries which are used in panels B,C,F and G. (B and F) Proportion of remaining cases due to the evening coverage gap (grey), those prevented by emanator with deterrence alone (light orange/purple) or with emanators with TFI (dark orange/purple). (C and G) Impact of emanators as a proportion of the cases during the evening coverage gap, light or dark orange/purple as above. (D and H) How proportion of remaining cases prevented by emanators changes with population coverage and assumption about percentage of population outside during the evening coverage gap.

(Figure 5.3C). In the optimistic scenario, emanators prevent just over 60% of cases due to the coverage gap in the first year, decreasing to ~55% in the third year (Figure 5.3G). Finally, in both scenarios emanators prevent a higher proportion of remaining cases when emanator population coverage is higher and when more of the population are outside during the evening coverage gap (Figure 5.3D&H). In summary, emanator use will prevent approximately the same proportion of bites during the evening coverage gap irrespective of the size of the coverage gap, though the absolute number of cases averted is higher when the coverage gap is larger (when LLINs are less effective).

In low transmission settings my optimistic estimate (with 80% population coverage and 100% likelihood of inducing TFI in deterred mosquitoes) is that emanator use can cause around a 30% reduction in the entomological inoculation rate, around 20% reduction in the prevalence of infection in under-fives, and around 40% reduction in the clinical incidence in under-fives (Figure 5.4). It is unlikely that emanators alone will reduce transmission low enough to cause local elimination, except for in locations where transmission is already at very low levels. In the scenarios explored, emanators were distributed to the 80% of the population already using LLINs. Changing this assumption of full correlation between emanator and LLIN use had very little impact on the results shown in this chapter (not shown).

5.3.3 Emanator use where vectors are pyrethroid resistant

The impact of LLINs and emanators used in combination in the presence of resistance to lethal doses of pyrethroids was compared to the expected impact of LLIN use alone if there were no pyrethroid resistance (Figure 5.5A&C). This explores how useful emanators could be at preventing excess mortality due to pyrethroid resistance in the event that airborne transfluthrin still maintains a deterrent and TFI effect on mosquitoes that are otherwise resistant to lethal doses of pyrethroids on LLINs. How well emanators can offset this excess mortality again depends on the size of the evening coverage gap. Using the optimistic estimate for Φ_E shows that emanator coverage compensated for reduced LLIN effectiveness in settings with up to 80% bioassay survival when it was assumed that 100% of the population were outside (Figure 5.5D). When using the best guess estimate of Φ_E, emanators could compensate for reduced ITN effectiveness only when bioassay survival was under 20% and when over 60% of the population were assumed to be outside during the evening coverage gap.

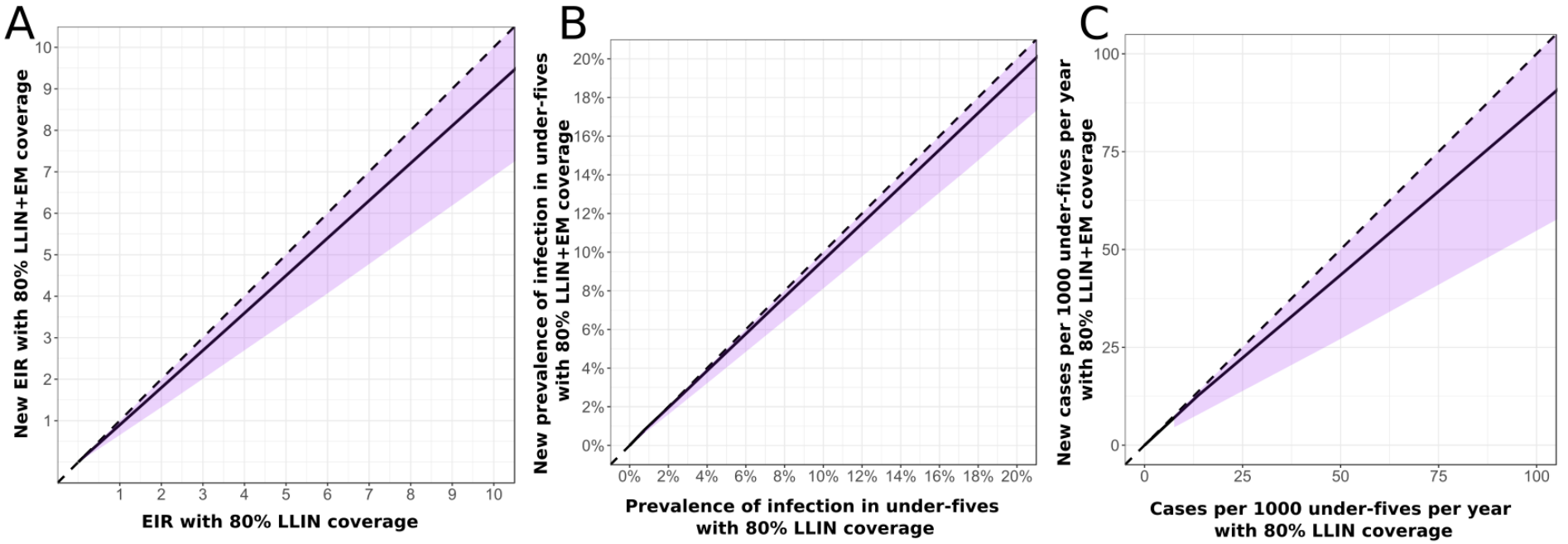

Figure 5.4: The impact of additional emanator use on 3 different epidemiological outcomes, where 80% of the population have an LLIN and are then also given an emanator. Solid black line shows the epidemiological impact of emanator use when using the best guess estimate for outdoor biting (Figure 5.2A). The purple segment corresponds to the 95% confidence interval for outdoor biting rates, showing the variation in emanator impact that would be due to different outdoor biting rates.

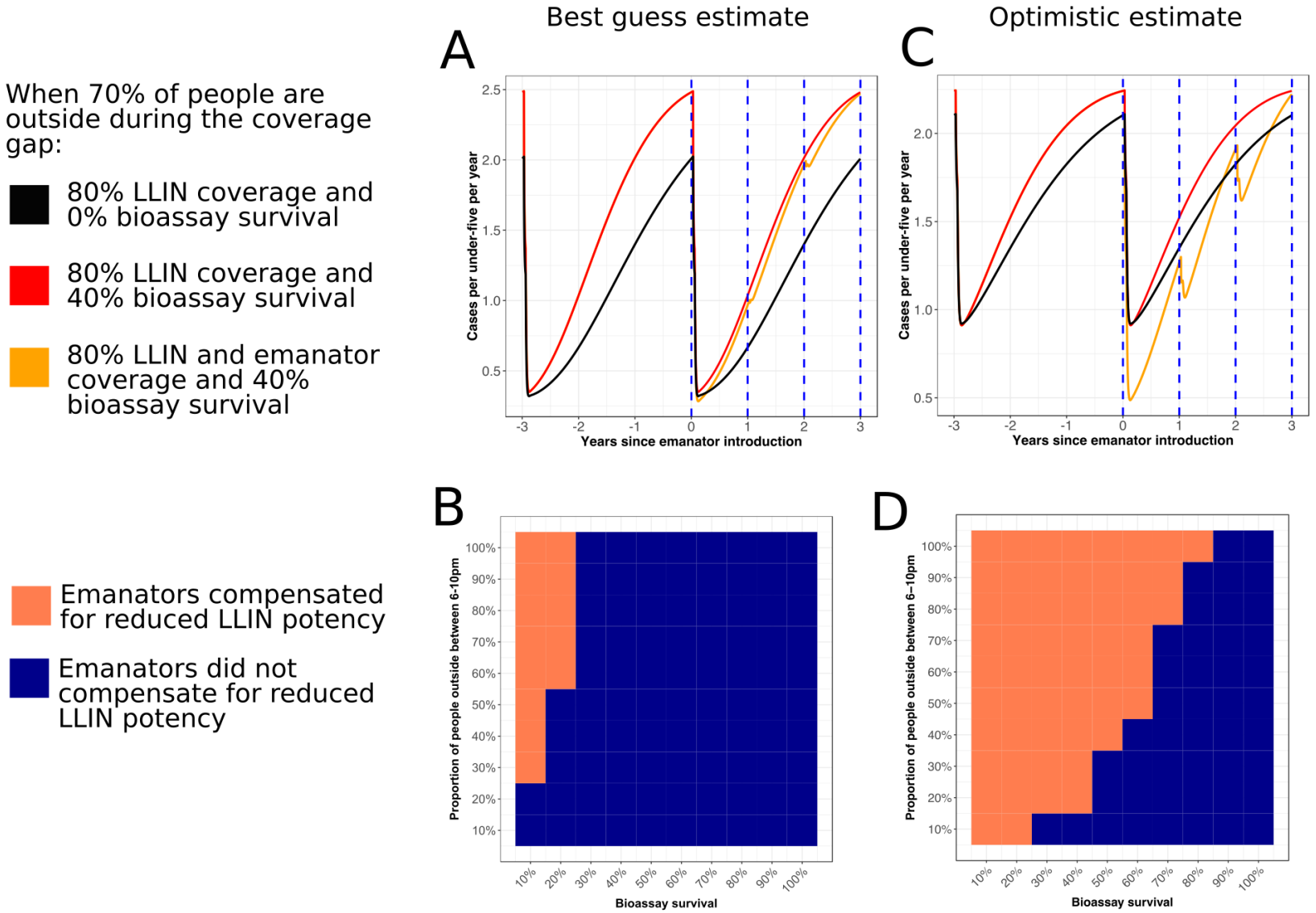

Figure 5.5: How well emanator use compensates for reduced LLIN effectiveness across a range of resistance settings. Left hand panels indicates scenarios where the percentage of bites taken in the evening matches current best guess estimates whilst right panels shows a scenario where a high percentage of bites occur in the evening coverage gap. (A and C) comparison of 80% LLIN coverage with no pyrethroid resistance (black), 80% LLIN coverage with 40% bioassay survival (red), and 80% LLIN+emanator coverage with 40% bioassay survival (orange). (B and D) Shows scenarios where emanators compensate for resistance-caused reduction in LLIN potency. Orange squares denotes that this is the case (total number of cases over three years is the same or greater than LLINs working optimally, orange line is near or below black line as in C), blue squares denote that this is not the case (as in A). Predictions in A and C are directly comparable to predictions made in 5.3A and E. All scenarios assume a TFI effect.

5.4 Discussion

In this chapter I have shown that biting occurring during the evening coverage gap could account for a large portion of the cases that remain after achieving a reasonable LLIN coverage in the population. The precise size of the evening coverage gap will depend on how much of the population is exposed during the evening coverage gap and the outdoor evening biting rate. The number of people outside during the evening coverage gap will vary geographically and over the course of the year as the temperature and weather changes. The number of bites people face during the evening will depend on the local mosquito species and when, who, and where they prefer to bite. This work estimated the biting rate during the evening coverage gap using data collated from three primary malaria vectors: *An. arabiensis*, *An. funestus*, and *An. gambiae s.s*. It is clear from this analysis that even a small number of people sitting outside during the evening could be responsible for up to a third of remaining cases, depending on the biting rate (Figure 5.2C). The Imperial College malaria transmission model has previously assumed that just under 90% of biting happens whilst people are indoors, an assumption that is shared more or less with many other malaria transmission models (specifically the ones described in Section 1.3). Hopefully this work will encourage a more location-specific approach to parameterising when and where bites take place in a model, using data on the mosquito and human behavioural factors discussed above.

An interesting result emerges when measuring emanator impact in terms of the proportion of cases that they prevent out of all cases that are due to biting during the coverage gap (Figure 5.3C&G). Emanator impact increases year upon year when using the best guess biting rate estimate but decreases when using the optimistic biting rate estimate. This is because of three things that change as the LLINs that were handed out at year 0 age and become less effective. When LLINs are less effective, due to aging or pyrethroid resistance, the proportion of mosquitoes that are infectious is larger. When there are more infectious mosquitoes the TFI effect of emanators has a greater impact and the deterrence effect of emanators has a lesser impact. Furthermore, reducing LLIN effectiveness changes the proportion of remaining cases due to the evening coverage gap, since now LLINs are failing to stop bites at night. These three things: the TFI effect, emanator effect, and proportion of bites remaining due to biting due the evening coverage gap, will increase or decrease emanator impact separately to create an overall increase or decrease on emanator impact as LLIN potency wanes. Whether the overall effect is an increase or a decrease will depend on many factors, including: the biting rate during the evening coverage gap, the proportion of the population outside during the evening coverage gap, the rate at which the concentration of insecticide on LLINs decays over time, and LLIN or emanator population coverage.

In general, the TFI effect causes much smaller reductions in transmission than observed in the last chapter, when emanators were used indoors at night. This is because the amount of biting that is

estimated to take place when users are near an emanator is usually far smaller than the amount of biting taking place indoors, even when using updated mosquito biting data from this analysis. This means that far fewer mosquito biting attempts take place near an emanator when they are used exclusively during the evening coverage gap, hence despite the fairly strong assumption that all deterred mosquitoes experience TFI, only a maximum of around 10% of the vector population will have TFI at any one point. The TFI effect in this context also has the added effect of preventing mosquitoes from trying to bite users under LLINs later on in the night. This could be a problem when LLINs are still potent, since it may prevent mosquitoes from coming into contact with the LLIN and dying. However, it is particularly useful when the mortality effect of LLINs has been reduced, because it will prevent night time feeding attempts by some mosquitoes that would have successfully navigated past the LLIN. In this analysis there was no evidence that the TFI effect preventing some mosquitoes from dying on LLINs was a cause for concern. This may have occurred because we assumed that people using emanators already had LLINs, so there was perfect correlation between the two interventions.

If the TFI effect of an emanator is greater when LLIN potency is reduced, this is of particular important where LLIN potency is reduced due to pyrethroid resistance. As discussed earlier, it has not been confirmed whether resistance to lethal doses of pyrethroids also confers some protection against TFI. However, if the deterrence and TFI effects of emanators can still occur to vectors that are unlikely to die due to pyrethroid exposure, as seems to be the case given the results of the Tanzanian trial in Kilombero valley where bioassay mortality due to Permethrin is only 58% (Kisinza *et al.*, 2017), then emanators have the potential to be a cheap, additional malaria control measure in areas with mild pyrethroid resistance. Depending on the proportion of bites that occur during the evening coverage gap and the level of pyrethroid resistance, emanators could maintain the malaria burden at pre-resistance levels and avoid excess deaths and strain on healthcare systems.

There are several assumptions used in the model for this analysis that could significantly alter the predicted impact of emanators. Firstly, I assume that the average amount of time that people spend close to their emanator during the evenings. In Chapter 4, the mean distance from the emanator was big factor in overall emanator effectiveness (Figure 4.2C). I don't know of any reliable data of how close people would remain to their emanator outside. Anecdotal evidence suggests that people outside would stay close to their buildings to cook or socialise in chairs beside their houses. If emanators were placed in social areas, this could avert a large proportion of bites. This sort of data could be collected by giving people a GPS tag or using their mobile phones to track their proximity to their house throughout the day. This data would also reveal more about the size of the morning coverage gap, there is still some biting taking place between 6-9am (Figure 5.2A) and anyone outside during these hours would not be protected by current vector control tools. The model also assumes that exposure during the evening is homogeneous across age groups, whereas it is probable that children go to bed

earlier than adults. In the future the model could be parameterised so that biting happening at different times happens in different places depending on age. This is important since infectiousness to mosquitoes and susceptibility to clinical disease is heavily dependent on age.

5.5 Conclusions

Emanators were predicted to reduce the number of cases that were due to biting during the evening coverage gap, preventing between 15 and 40% of cases remaining after widespread LLIN coverage. This solidifies the place of emanators within a package of vector control strategies aimed at tackling residual transmission (Killeen, 2014). Given the observed reductions in EIR, prevalence of infection in under-fives, and clinical incidence in under-fives, it is unlikely that emanators alone will eliminate malaria (Figure 5.4). This finding is supported by other modelling work that examined packages of vector control tools for use after good LLIN coverage has been achieved (Kiware *et al.*, 2017). This analysis built upon previous modelling work by utilising the more complex Imperial College malaria transmission model, with its age-related immunity structure, and by parameterising emanator impact using real data on mosquito biting rates and emanator performance in the field. The next chapter will model an extension of the TFI effect for pyrethroid-resistant vectors that contact with LLINs but do not die. It is motivated by two observations that emerge from the TFI modelling undertaken so far. Firstly, that the TFI effect causes larger decreases in burden when emanators are used at night, because this is when the majority of biting still takes places at most locations. Secondly, that the TFI effect causes a bigger decrease in burden when LLINs are not as deadly to mosquitoes. Therefore, a TFI effect caused by a LLIN used at night in a setting with pyrethroid resistance has the potential to cause a large impact on transmission.

6 Estimating the epidemiological effects of a sub-lethal temporary feeding interruption effect caused by pyrethroid-treated bed nets

6.1 Introduction

This chapter explores the impact of a temporary feeding interruption (TFI) effect caused by pyrethroids on long-lasting insecticidal nets (LLINs). Resistant mosquitoes that no longer die upon contact with the treated net may be prevented from feeding for a period of time afterwards. I will utilise data from experimental hut trials that compared treated and untreated nets to parameterise a TFI effect for LLINs that varies by resistance level. The TFI effect could help explain why some people believe that the public health impact of pyrethroid resistance has not been as great as initially thought (Kleinschmidt *et al.*, 2018).

The severity of pyrethroid resistance in a location is measured in terms of the percentage of local mosquitoes that survive exposure to doses of pyrethroid that would be lethal to susceptible mosquitoes. This is termed the discriminating dose bioassay and there are three main methods used to measure it; the WHO tube susceptibility bioassay (World Health Organization, 2016), CDC bottle assay (Cdc, 2012) and the WHO cone test (World Health Organization, 2013a) doses of pyrethroids in a bioassay. Bioassay survival is associated with decreased mosquito mortality and deterrence due to LLINs in experimental hut trials (Churcher *et al.*, 2016). The Imperial College malaria transmission model uses a mixed effects regression model to specify the link between observed bioassay survival and the parameters that determine LLIN potency. Greater pyrethroid resistance not only reduces both the overall mortality and deterrence probability of a net, but also increases the rate of decay of these parameters over time. The regression model captures the strong association between bioassay survival and LLIN effectiveness in experimental hut trials, but the relationship between bioassay survival and LLIN effectiveness in randomised controlled trials is less predictable (Lindblade *et al.*, 2015; Mathanga *et al.*, 2015). Many such trials have found that LLINs continue to function well across a range of locations with pyrethroid resistant mosquitoes, suggesting LLINs have an effect on mosquitoes beyond merely killing or deterring them (Strode *et al.*, 2014).

Such “sub-lethal” effects of LLINs may not be explicitly observable in the outcomes of pyrethroid bioassays or experimental hut trials. For example, a delayed mortality effect has been found in mosquitoes that are repeatedly exposed to pyrethroids on LLINs (Viana *et al.*, 2016). Highly resistant mosquitoes have significantly shorter lifespans after pyrethroid exposure every few days, which would happen as they took blood meals on people sleeping under LLINs. This delayed mortality would reduce the vectoral capacity, since it increases the likelihood of them dying before becoming infectious to humans. However, this effect would not be observed in an experimental hut trial where only immediate 24 hour mosquito mortality is typically recorded. Some studies do record the percentage dying after 48 and 72 hours, though this is normally only done for insecticides known to

have a slower mode of action (Agossa *et al.*, 2018). Similar circumstances may obscure the occurrence of an LLIN-caused TFI effect, which would not be observed directly through measuring bioassay survival, the number of mosquitoes killed in experimental huts, or a reduced rate of mosquito entry into the hut.

Evidence for an LLIN-caused TFI effect would need to link mosquito willingness to feed to recent LLIN exposure. Siegert *et al.* (2009) counted mosquito landings on a hand under a mitten made of treated or untreated nets to monitor whether mosquito landing behaviour changed in the presence of insecticides. Mosquitoes initially landed on treated and untreated nets at the same rate, but over time the rate of landings on treated nets was severely reduced while remaining constant throughout the experiment on the untreated nets. The fact that mosquitoes did not return to the treated nets was taken to imply that the mosquitoes were not mere repelled (since then they would try again) but had instead stopped responding to host cues for the hand under the net. A similar observation was made by Parker *et al*. (2015) when they tracked mosquito behaviour towards LLINs with people underneath using infra-red tracking software. There was no significant difference between mosquito activity at treated or untreated nets initially, with activity at the treated nets reduced to almost nothing by around 30 minutes. Unfortunately, neither of these experiments could offer the recently exposed mosquitoes a blood meal to see whether they were willing to feed. Glunt *et al*. (2018) exposed mosquitoes to treated or untreated nets in bottle assays (rather than the free-flying conditions in the experiments above), finding that mosquitoes exposed to treated nets were significantly less able to host-seek up to 24 hours after exposure. The effect was severe 1 hour after exposure, with a 90% reduction in host seeking in mosquitoes exposed to treated nets, dropping to a 30% reduction after 24 hours. To definitively prove the existence of a LLIN-induced TFI effect, an experiment would need to test the willingness to feed of mosquitoes that have been exposed to LLINs in free-flying conditions and had subsequently stopped approaching the net after initial contact.The statistical relationship between TFI and the level of pyrethroid resistance has never been rigorously characterised nor have the epidemiological consequences been explored.

This analysis will use data from experimental hut studies conducted in a range of resistance settings to quantify how the excess blood feeding inhibition prevented by treated nets (on top of that observed for untreated nets) changes with the level of resistance as measured by LLIN mortality. The excess blood feeding inhibition refers specifically to blood feeding prevented by an effect other than the physical barrier of the net as both treated and untreated nets are assumed to have physical integrity (untreated nets are meant to have the same dernier as the LLIN under investigation and the same number of standard man-made holes). Untreated net arms are typically included as a control arm in every experimental hut trial. Assuming that this excess blood feeding inhibition is due to an LLIN-caused TFI effect, adding such an effect to the Imperial College malaria transmission model, in a

similar vein to the previous two chapters, will give a clearer picture of any epidemiological role of TFI.

In Chapter 4, I showed that the TFI effect from emanators used at night drastically increased emanator effectiveness, because the majority of mosquito biting usually happens while people are in bed. An LLIN-caused TFI would also benefit from this fact; the loss of LLIN mortality could be offset if most mosquitoes that would have died experience a TFI effect instead. The TFI effect reduces the size of the infectious mosquito population, causing a community protection effect by preventing mosquitoes from biting other people for a short while. Comparing the effects of pyrethroid resistance with and without an LLIN-caused TFI effect will show to what extent the expected increase in malaria transmission due to pyrethroid resistance will be mitigated by TFI. The adapted model will also be used to predict the outcome of a randomised controlled trial in a location with severe pyrethroid resistance, demonstrating how modelling TFI as a sub-lethal effect of LLINs can improve the predictions of LLIN impact in places with pyrethroid resistance. Finally, there will be a discussion of how measuring the severity of pyrethroid resistance could develop beyond bioassay survival, as well as the types of data which could be collected from future experimental hut trials to facilitate the understanding of the sub-lethal effects of LLINs.

6.2 Methods

6.2.1 Measuring excess blood feeding inhibition and how it changes with pyrethroid resistance

To characterise the relationship between pyrethroid resistance and the excess blood feeding prevented by treated nets, experimental hut trials that directly compared treated and untreated nets were selected from a previous systematic meta-analysis of bioassay survival and experimental hut trial outcomes (Churcher *et al.*, 2016). The dataset comprised of a selection of studies comparing standard pyrethroid-only LLINs and those with pyrethroid and the synergist chemical piperonyl butoxide (PBO). Treating nets with PBO is thought to inhibit the enzymes that allow pyrethroid resistance in mosquitoes, making the pyrethroid on the net lethal again to resistant mosquitoes (Gleave *et al.*, 2017). PBO LLINs have recently been recommended for widespread use by the WHO following demonstration of public health value over standard pyrethroid only LLINs (World Health Organization, 2015; Protopopoff *et al.*, 2018). The actions of TFI can therefore be assessed in the two main classes of LLIN currently in use and widely distributed by national malaria control programmes.

Table 6.1: The LLIN studies selected from the meta-analysis of Churcher et al. (2016) used in this analysis, comparing standard LLINs and PBO LLINs.

Country	**Species Composition**	**Study**
Cote D'Ivoire	An. gambiae s.s.	(Koudou *et al.*, 2011)

Benin	An. gambiae s.s.	(N'Guessan *et al.*, 2010)
Benin	An. gambiae s.s.	(Corbel *et al.*, 2010)
Tanzania	An. gambiae s.s.	(Kitau *et al.*, 2014)
Nigeria	An. gambiae s.s.	(Adeogun *et al.*, 2012)
Togo	An gambiae s.l.	(Kétoh, 2016)
Tanzania	An gambiae s.l.	(Malima *et al.*, 2008)
Tanzania	An gambiae s.l.	(Tungu et. al. *Unpublished)*
Benin	An. gambiae s.s.	(Ngufor, N'Guessan, *et al.*, 2014)
Benin	An. gambiae s.s.	(Pennetier *et al.*, 2013)

For each trial, the blood feeding inhibition caused by treated nets on top of untreated nets (referred to here as the 'excess blood feeding inhibition') was calculated as follows:

$$b = \frac{b_t - b_u}{1 - b_u} \qquad 6.1$$

where b_t and b_u are the proportions of alive mosquitoes that had not been able to feed, collected from the hut with a treated or untreated net, respectively. The mortality observed in the hut with a treated net, m_t, is converted into expected bioassay survival, $surv$, using the relationship for *An. gambiae s.s.* mosquitoes derived by Churcher et al. (2016):

$$surv = 1 - \left(\frac{logit(m_t) - 0.634}{4.497}\right) \qquad 6.2$$

A logistic mixed effects regression model was fitted to the data to explain excess blood feeding inhibition with other variables as fixed effects: bioassay survival, the number of times that the net has been washed, and treatment with PBO. Nets were either not washed or washed 20 times, with the number of washes chosen to mimic natural net deterioration. This is based on WHO guidance which states that washing a net 20 times is equivalent to reducing the concentration of pyrethroid on the net to the level that would be expected at the end of its useful life (Atieli *et al.*, 2010a). The random effect component allowed the intercept to vary by study according to a normal distribution with mean zero and fitted variance parameter. This accounts for variation in excess blood feeding inhibition between studies. The regression model is as follows:

$$logit(b) = \beta_0 + \beta_1 surv + \beta_2 PBO + \beta_3 (PBO \times surv) + \beta_4 washes + \varepsilon_{study} \qquad 6.3$$

Where *b* is the amount of excess blood feeding inhibition, *surv* is the percentage of mosquitoes that survive a discriminating dose bioassay, *PBO* is an indicator variable for PBO treatment, *washes* indicates the amount of times that a net has been washed (takes the value of 0 or 20), and $\varepsilon_{\text{study}}$ is the random effects term that captures variations in excess blood feeding inhibition between studies.

6.2.2 Feeding inhibition in the malaria transmission model

Experimental hut trials can measure blood-feeding inhibition in mosquitoes which enter the hut. However, LLINs prevent susceptible mosquitoes from entering huts with a treated LLIN (here referred to as deterrence). It seems likely that these mosquitoes are detecting the presence of the pyrethroid in the hut and taking avoidance action, but it is unclear whether the exposure of pyrethroid outside of the hut is sufficient to induce TFI. Given the lack of evidence here we make the conservative assumption that only those mosquitoes which enter the hut are exposed to high enough concentrations of the insecticide to induce TFI and that all mosquitoes being deterred away from huts with LLINs are able to refeed immediately.

The probability that a mosquito acquires TFI after trying to bite someone under an LLIN for a given proportion of bioassay survival, $surv_{bio}$, is as follows:

$$f_{LLIN} = r_{LLIN} \times b \times surv_{bio} \times decay_{LLIN} \qquad 6.4$$

Where r_{LLIN} is the probability that the mosquito is repelled away from biting an LLIN user. This probability scales independently with bioassay survival, as parameterised in Churcher et al. (2016). The value of b (i.e. the scale of the TFI effect for a given level of resistance) is determined by the regression model in Equation 6.3 above. When the level of resistance is high, fewer mosquitoes will die but more mosquitoes will instead acquire the TFI effect. The function is scaled by bioassay survival so that when there is no resistance LLIN efficacy will remain unchanged. The $decay_{LLIN}$ parameter reduces f_{LLIN} as LLINs age at a rate determined by the regression model. The rate will be chosen by setting the effect of 20 washes on excess blood feeding inhibition as occurring exponentially over 2 years. If $\overline{av}_{LLIN}$ is the average mosquito biting rate on LLIN users, the rate at which mosquitoes move into the parallel TFI compartments is:

$$f = \overline{av}_{LLIN} \times f_{LLIN} \times \Phi_B \qquad 6.5$$

The equations that determine mosquito movement to and from the TFI compartments are the same as in Section 4.2.2, except now the value of the parameter f is calculated using Equation 6.5 above rather than Equation 4.4.

6.2.3 Predicting a randomised controlled trial outcome

The randomised controlled trial used in this analysis was undertaken by Protopopoff et al. (2018) in Tanzania. Protopopoff et al. (2018) undertook an RCT in Tanzania with four trial arms of different vector control combinations: standard LLINs, LLINs treated with PBO (PBO LLINs), standard LLINs in conjunction with a single round of IRS (Actellic), and PBO LLINs in conjunction with single round of IRS (Actellic). The primary aim of the study was to test the effectiveness of PBO LLINs compared to standard LLINs in a setting with considerable pyrethroid resistance. The local mosquito population was sampled used entomological surveys, showing that it was overwhelmingly composed of *An. gambiae sensu lato* (94.5% of all mosquitoes caught). These mosquitoes had considerable pyrethroid resistance, with 91.2% mosquito survival in resistance bioassay tests. The Imperial model can predict the outcome of this RCT by replicating the trial conditions as closely as possible. Annual EIR in the model was scaled to produce the equivalent prevalence of infection observed in the baseline survey for the standard LLIN and PBO LLIN trial arms. The LLIN population coverage in each arm was adjusted to match the level recorded in the trial, such that the model predictions can be compared to the observed prevalence in the four follow-up surveys (collected over 2 years). The model used the seasonality profile for Kagera, Tanzania that was previously fitted to rainfall data at the admin 2 (province) level (Griffin *et al.*, 2010). The model ran 1000 times for each arm, using random draws from the previously fitted posterior distributions for other model parameters to account for variability in other parts of the model (see Section 1.3.3.3).

6.3 Results

6.3.1 Excess blood feeding inhibition from treated nets

The uncorrected blood feeding rates in the experimental hut trials ranged from 0-56% for standard LLINs and 0-46% for PBO LLINs (Figure 6.1). Pyrethroid treated nets prevent a significant amount of blood feeding on top of that prevented by untreated nets across all resistance levels (Figure 6.2). The proportion of excess blood feeding prevented significantly decreases as bioassay survival increases, and this decrease is significantly slower for PBO LLINs than it is for standard LLINs (Table 6.2, β_1 and β_3, both p values <0.001). The mean level of excess blood feeding inhibition is also slightly higher for PBO LLINs than standard LLINs (Table 6.2, β_2). There was no evidence that nets that had been washed 20 times prevented less excess blood feeding than nets that had not been washed at all (Table 6.2, β_4, p value = 0.741). Nets are washed 20 times before use in an experimental hut trial to emulate the decay in the concentration of pyrethroid on the net that would be expected

over its lifetime. This result suggests that the excess blood feeding prevented by both PBO and standard nets does not significantly decay over the life of the net in contrast to the level of induced mortality and deterrence which both decrease substantially between unwashed and washed LLINs. The value of the $decay_{LLIN}$ parameter in Equation 6.4 was therefore set to 1 for the rest of the analysis.

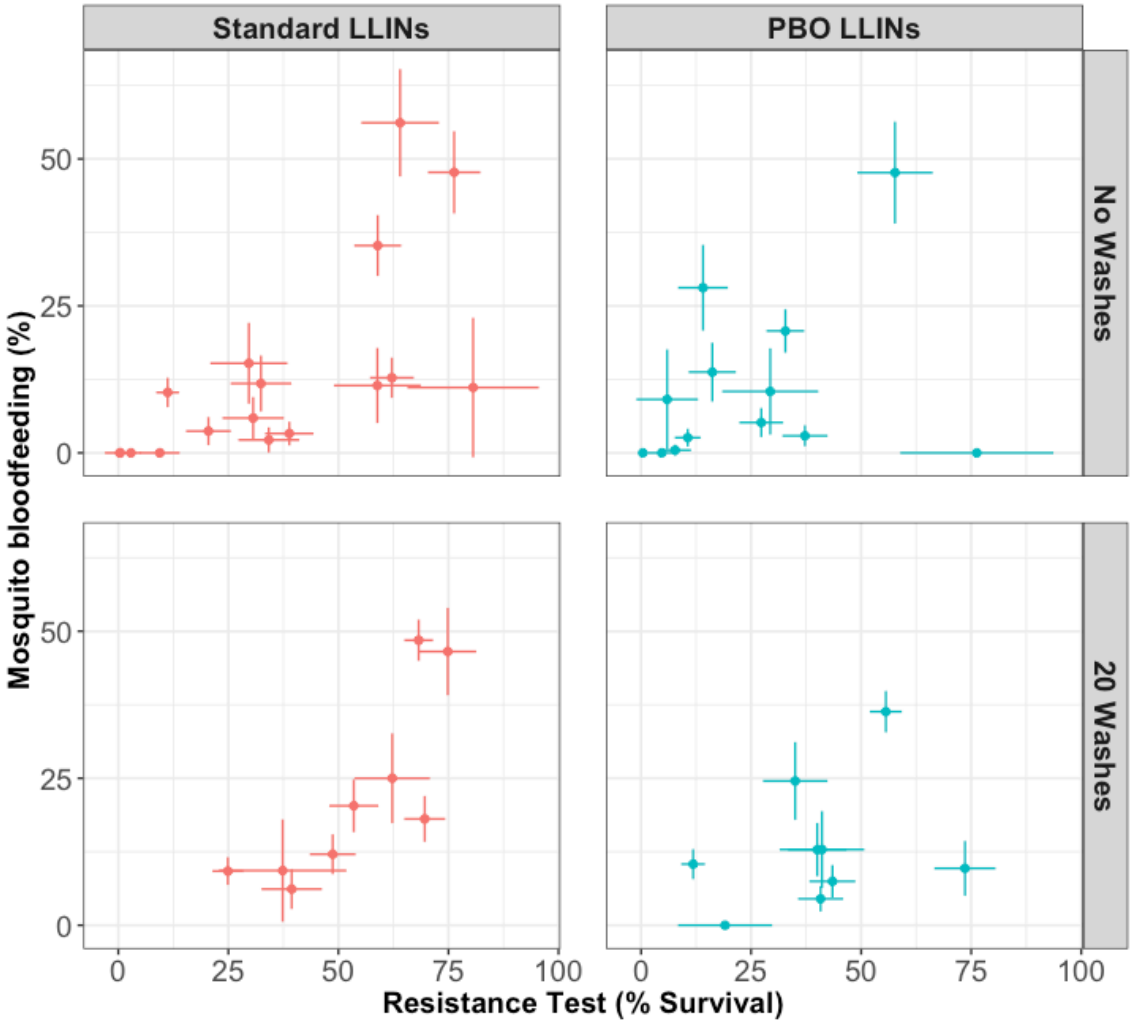

Figure 6.1: Raw experimental hut data for bioassay test survival and mosquito blood feeding rates split by standard and PBO LLINs as well as the number of times that they have been washed.

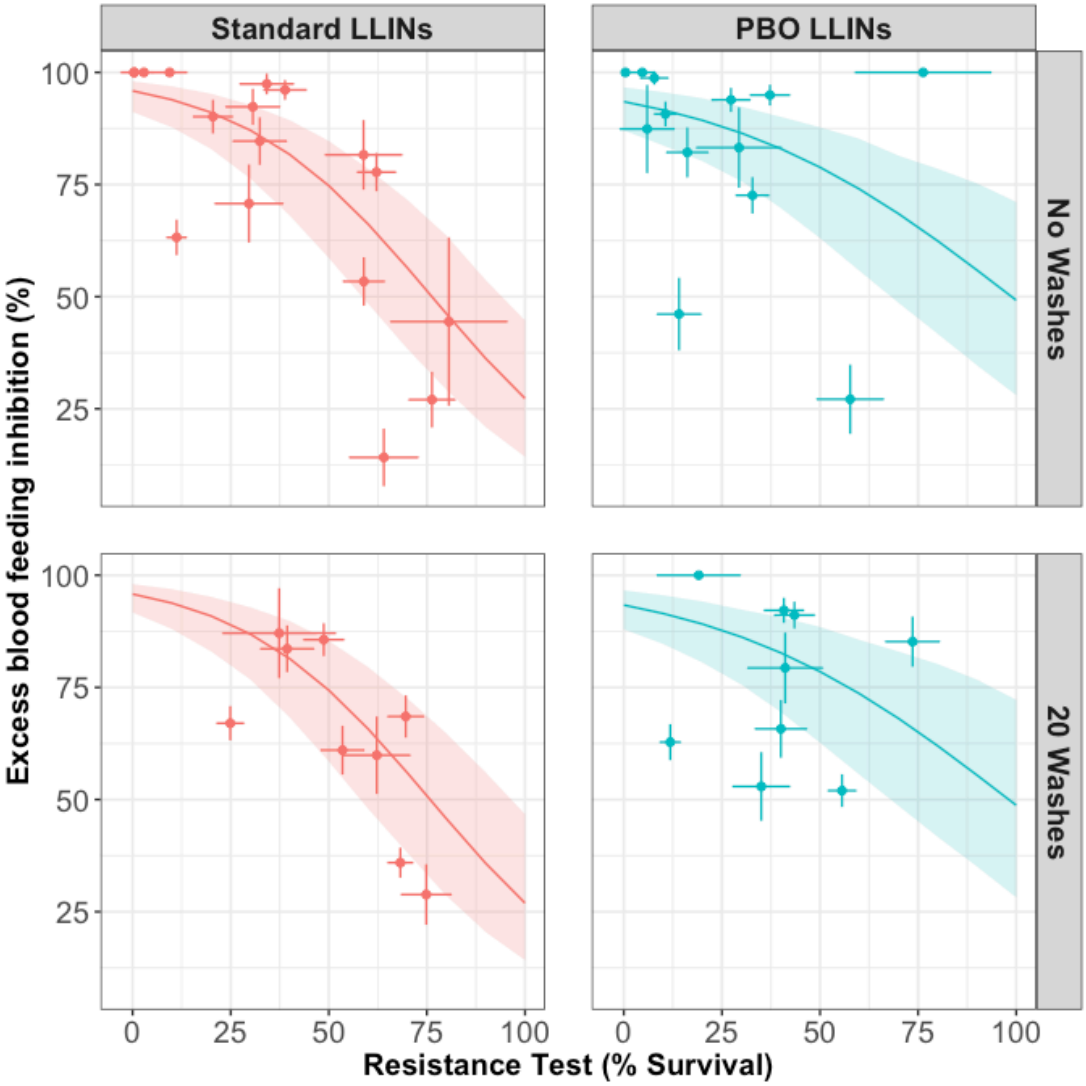

Figure 6.2: The excess blood feeding inhibition caused by treated LLINs at different levels of pyrethroid resistance. Excess blood feeding inhibition is the proportion of mosquitoes that did not feed due to the chemical effects of LLINs rather than the barrier effect alone, as measured in untreated nets. Points show the observations from experimental hut trials and their corresponding 95% confidence intervals. The coloured lines and shaded areas show the model predictions using the mean parameter estimates (lines) and 95% confidence intervals (shaded area).

Table 6.2: Fitted parameter values for the fixed and random effects in the logistic mixed effects regression model described in Section 6.2.1. (: significant at 95% confidence level)*

Parameter	Description	Fitted value	Standard error	P-value
β_0 (Intercept)	Mean level of excess blood feeding inhibition	3.148 *	0.309	<0.001
β_1 (Bioassay survival)	How excess blood feeding inhibition changes with bioassay survival	-4.127 *	0.252	<0.001
β_2 (PBO effect)	Modifies the mean level of excess blood feeding inhibition for PBO LLINs	Standard LLINs: baseline PBO LLINs: -0.486 *	0.105	<0.001
β_3 (PBO-bioassay interaction)	Modifies how excess blood feeding inhibition changes with bioassay survival for PBO LLINs	1.433 *	0.229	<0.001
β_4 (Washes)	Modifies the mean level of excess blood feeding inhibition for washed nets	No washes: baseline 20 washes: -0.0193	0.229	0.741
ε (Normally distributed random effect)	Accounts for variation in observed blood feeding inhibition between trials	Mean: fixed at 0 Fitted variance: 0.709		

The probability of a mosquito acquiring the TFI effect after contact with an LLIN increases with pyrethroid resistance (Figure 6.3). At the same time, mosquito mortality and deterrence due to nets becomes less likely, while successful blood feeding and the TFI effect become more likely. For PBO LLINs the probability of a mosquito acquiring TFI rises only when mosquito bioassay survival is around 70%, PBO LLINs still cause significant mortality and deterrence up until this point (Figure 6.3). Moving from the experimental hut data to the corresponding parameters in the malaria transmission model, the probability of the TFI effect occurring is largest at around 50% bioassay survival for standard LLINs and 90% for PBO LLINs (Figure 6.4). For both LLINs this is the point where the probability of the TFI effect has increased with resistance, but the probability of a mosquito being repelled has not been heavily reduced due to resistance yet. In the transmission model, as the LLINs age the mortality and deterrence effects decay, but the proportion of mosquitoes that acquire

the TFI effect remains the same after 20 washes (equivalent to 3 years of use) (Figure 6.2). This means that the TFI plays a bigger role relative to killing and repelling as the LLIN ages (Figure 6.5).

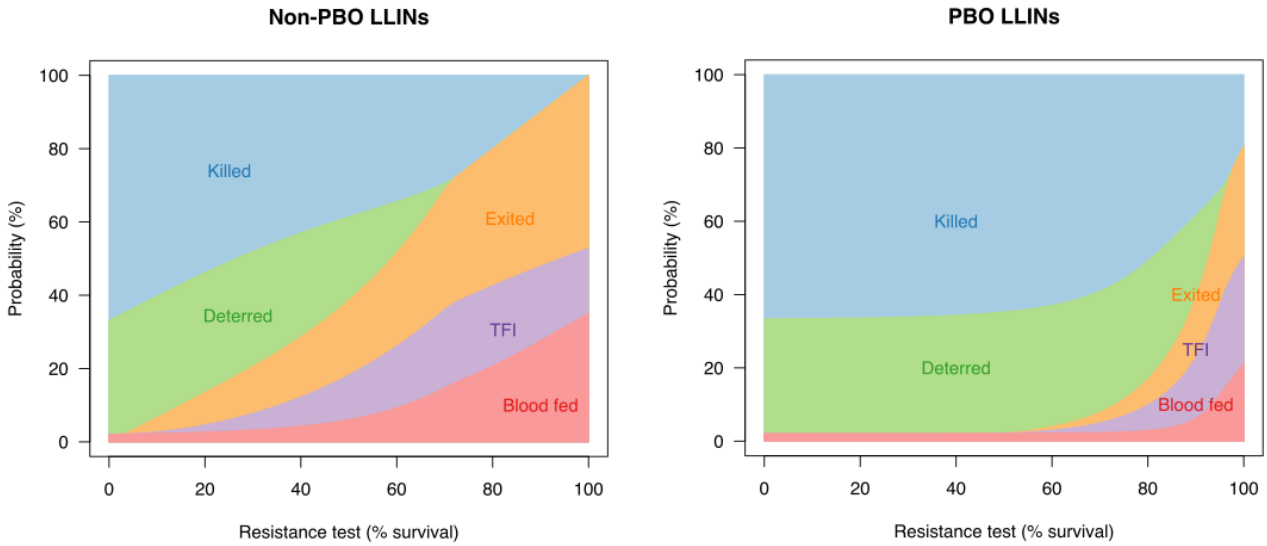

Figure 6.3: The probabilities of each entomological effect occurring for a mosquito trying to feed on a human using an unwashed LLIN at various levels of pyrethroid resistance as characterised by experimental hut trials. Panel (A) shows results for a standard pyrethroid only LLIN whilst (B) represents a PBO LLIN. Shaded regions indicate the percentage of mosquitoes being killed (blue), deterred (green), exit without blood feeding but able to start another feeding attempt immediately (yellow) exiting unfed but having temporary feeding inhibition (TFI, purple) or blood-feeding and surviving (red).

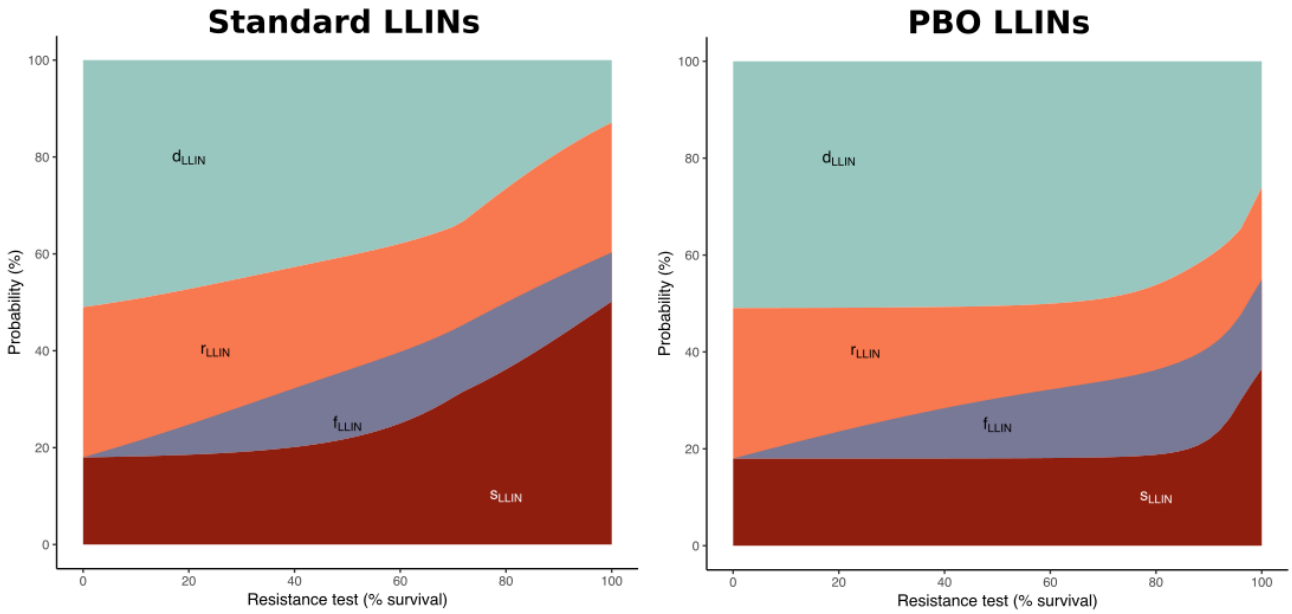

Figure 6.4: Illustration of how the model parameters change with the level of resistance. Results are similar to Figure 6.3 but here mosquitoes being deflected from the LLINs and not having TFI are combined (i.e. deterred and exited mosquitoes) and the parameters take into consideration that not all mosquitoes successfully take a blood meal when an unprotected person is sleeping in an experimental hut. The probabilities that a mosquito will die (d_{LLIN}, blue), be repelled (r_{LLIN}, orange), acquires TFI (f_{LLIN}, purple), or successfully feeds (s_{LLIN}, maroon) per feeding attempt.

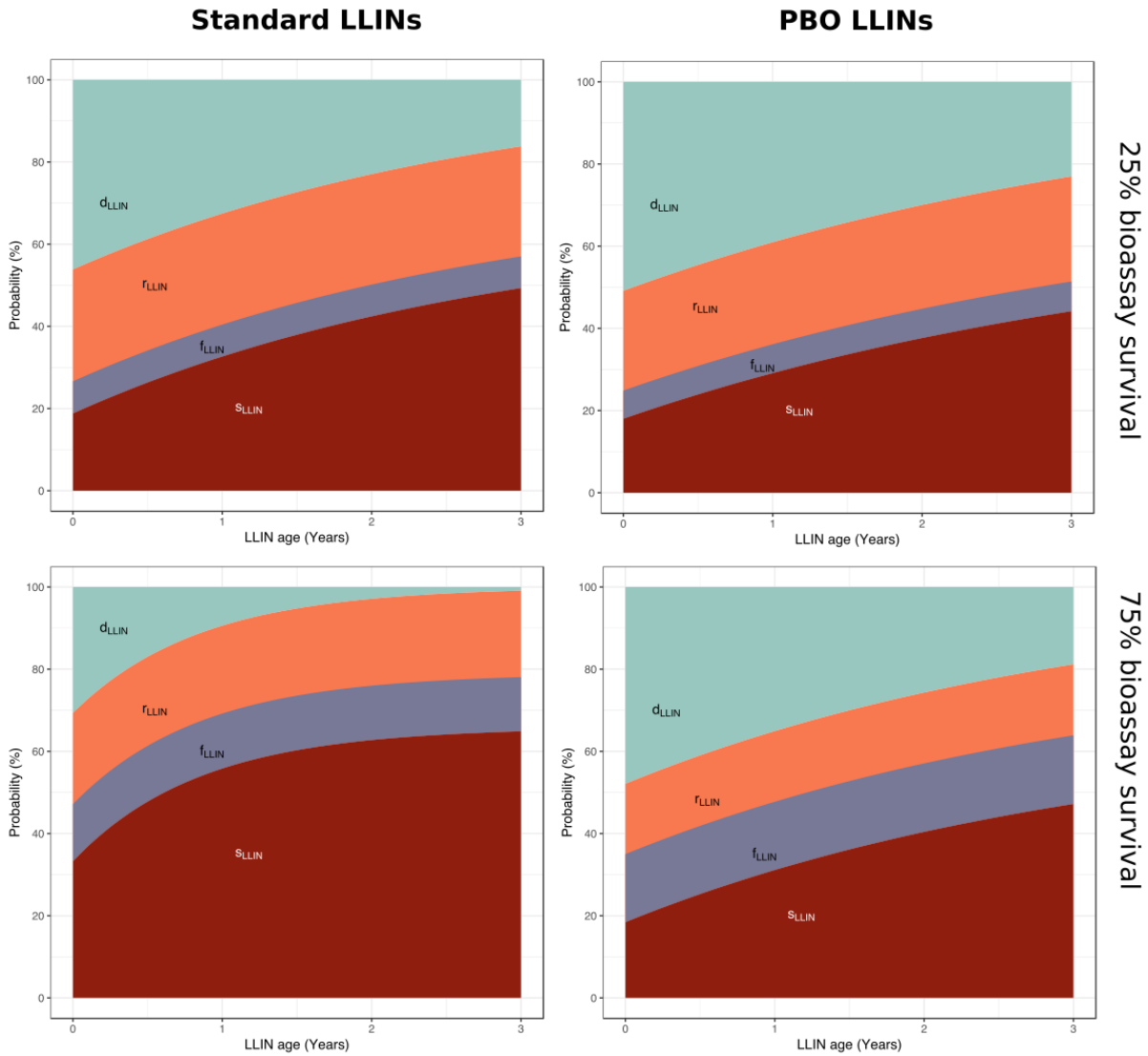

Figure 6.5: How the probabilities of each entomological effect change over the effective lifetime of a net for mosquitoes with moderate pyrethroid resistance (25% bioassay survival, top row) or high pyrethroid resistance (75% survival). The proportion of mosquitoes that receive a TFI effect is assumed not to decay over time in line with the results of Section 6.3.1. The effects are: death (d_{LLIN}, blue), repelled (r_{LLIN}, green), acquires TFI (f_{LLIN}, purple), or successfully feeds (s_{LLIN}, red).

6.3.2 Arguments for the excess blood feeding inhibition being caused by a TFI effect

In this analysis, it is assumed that the excess blood feeding inhibition from treated nets is entirely due to a TFI effect, as discussed above. Experimental huts are often designed with one-way entry systems and veranda traps so that mosquitoes entering the house can be counted, allowing researchers to compare house entry rates between different huts. The experimental hut trial data analysed here does not specify whether mosquitoes caught in the veranda trap (or not - i.e. caught within the body of the hut) are blood fed or not. As discussed in Section 1.2.1.2, it is hard to disentangle the processes that are causing blood feeding inhibition in an experimental hut trial. In this case, the excess blood feeding inhibition could therefore be explained by mosquitoes entering the house, trying to exit again upon

encountering the net, becoming stuck in the veranda trap, and then remaining unfed. However, analysing the hut trial data provides several arguments that this is not the case, and that the TFI explanation of excess blood feeding inhibition is preferable.

Firstly, if the number of mosquitoes caught in the veranda trap signifies that mosquitoes were trying to exit the hut, then we would expect more mosquitoes (of the ones remaining alive) to be caught in the trap when LLIN mortality is high. This is because mosquitoes would try to exit the hut because of the potent LLIN, or, because the mosquitoes that don't exit are more likely to die. Either way, more mosquitoes should be found alive in the veranda traps when LLIN mortality and deterrence is high. However, the proportion of alive mosquitoes caught in the veranda trap actually decreases as LLIN mortality increases (Figure 6.6A). Secondly, the proportion of alive mosquitoes caught in the veranda trap increases as the proportion of alive mosquitoes that were blood-fed increases (Figure 6.6B). Therefore, it seems more reasonable to assume that mosquitoes caught in the veranda trap are trying to exit the hut because they have fed, rather than because of an effect from the pyrethroid on the LLIN. This is consistent with our TFI assumption: mosquitoes remain in the hut unfed but cannot respond to host cues and so cannot take a blood meal.

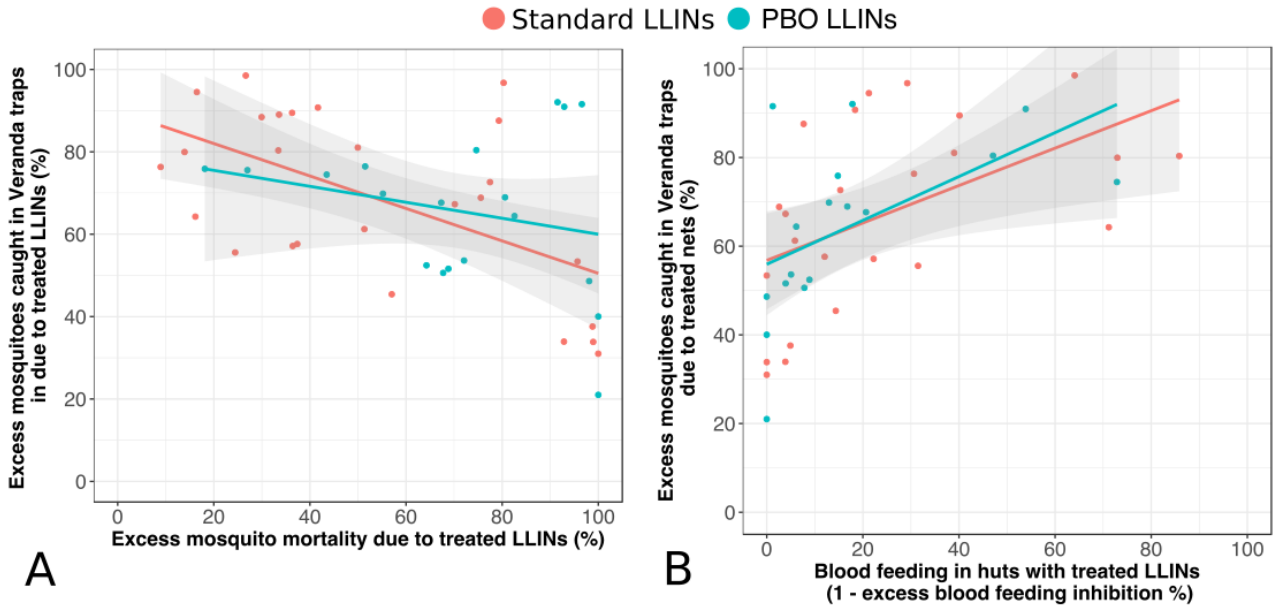

Figure 6.6: Experimental hut trial data showing how the excess proportion of alive mosquitoes caught in veranda traps (in treated nets on top of untreated nets) varies with (A) induced mosquito mortality (as assessed by comparing mortality in treated and untreated arms) and (B) Blood feeding in huts with treated LLINs (calculated as 1 - the proportion of excess blood feeding inhibition in huts with a treated net over an untreated net). Lines show predictions (and 95% confidence interval) from fitting a simple linear regression model between the two variables. These values are taken from the same dataset used for the mixed effects regression model, the sources are described in Table 6.1.

6.3.3 Epidemiological impact of the TFI effect

The Imperial model that has been expanded in this chapter was previously used to predict that increasing pyrethroid resistance is a large public health concern that will increase both the prevalence and incidence of malaria as the severity of resistance increases (Churcher *et al.*, 2016). When LLINs fail to deter or kill mosquitoes, humans will experience more infectious bites that will develop into asymptomatic infections or clinical malaria episodes. In a scenario with a 10% baseline prevalence of infection in under-fives, mosquito bioassay survival increasing from 0% to 50% will increase the under-five prevalence to 25% three years after the arrival of resistance (with LLINs at 80% coverage). This translates to approximately 600 extra cases per year per 1000 people across all age groups. Introducing the LLIN-caused TFI effect calibrated above prevents some of this increase from occurring: the prevalence in under-fives is predicted to increase to 20% instead, i.e. the increase in slide prevalence due to resistance is ~150% without TFI and ~100% with TFI (Figure 6.7A). This equates to approximately a 33% reduction in cases over the three year life-time of the LLIN (Figure 6.7B). Though the increase is substantially less than would have been the case if the TFI mosquito behaviour did not exist, the rise in malaria burden due to pyrethroid resistance is still considerable.

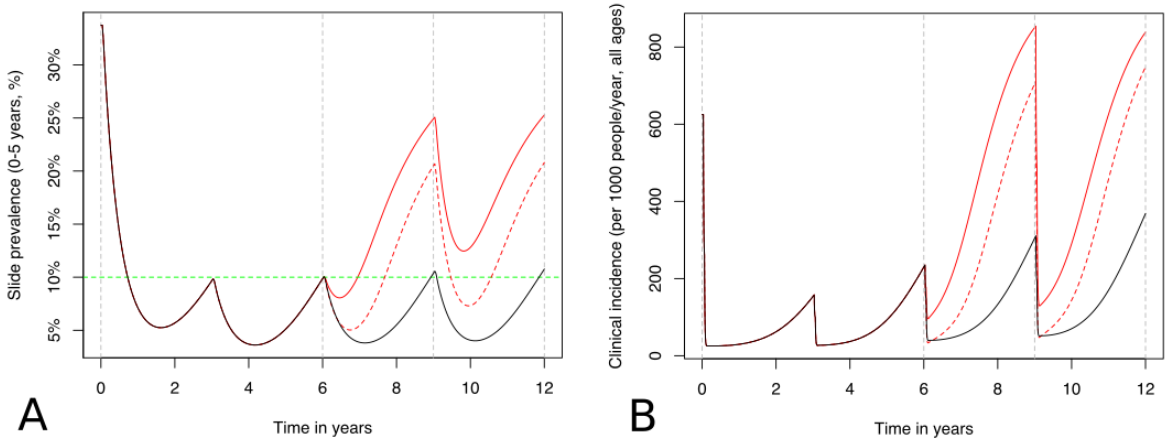

Figure 6.7: The epidemiological impact of pyrethroid resistance is mitigated by an LLIN-induced TFI effect. In this scenario standard LLINs are distributed every 3 years starting at year 0. At year 6 mosquito bioassay survival increases from 0% to 50% overnight. The black line shows the scenario where resistance never increases. The solid red line shows the predicted increase in (A) prevalence of infection in under-fives by microscopy and (B) clinical incidence per 1000 people per year in all age groups associated with the bioassay survival increase without a LLIN-induced TFI effect. The dotted red line shows the increase in each burden measured when including a LLIN-induced TFI effect in the model. Dotted green line indicates the prevalence at the time resistance arrives.

Repeating the same scenario as in Figure 6.7 for bioassay survival between 0-100% and recording the mean clinical incidence between years 6 and 7 shows that as pyrethroid resistance increases in severity, the predicted rise in malaria burden grows larger (Figure 6.8). The rise in clinical incidence happens at a lower level of resistance for standard LLINs than for PBO LLINs. The malaria burden is only predicted to increase for a population using PBO LLINs when mosquito bioassay survival reaches around 70%. In the scenario considered here the presence of a TFI effect for standard LLINs

would cause a relative reduction in the increase in the number of cases in the first year from LLIN distribution by 27% in an area where there was 90% survival in a discriminating dose bioassay. Similarly, for PBO LLINs a TFI effect would cause a relative reduction in the increase in the number of cases by 22%. There is considerable benefit to switching to PBO LLINs between 70% and 100% bioassay survival when the LLIN-induced TFI effect is present (Figure 6.8). This range was only 70% to 90% when no TFI effect is present (Churcher et al. (2016), Figure 5A). At 100% bioassay survival, clinical incidence expected from PBO LLIN use is around half that of standard LLINs (Figure 6.8, Standard LLINs). Crucially, inclusion of TFI in the model indicates that PBO LLINs maintain good effectiveness, even with very high levels of resistance, whereas previous work suggested that the benefit was only at moderate levels of resistance.

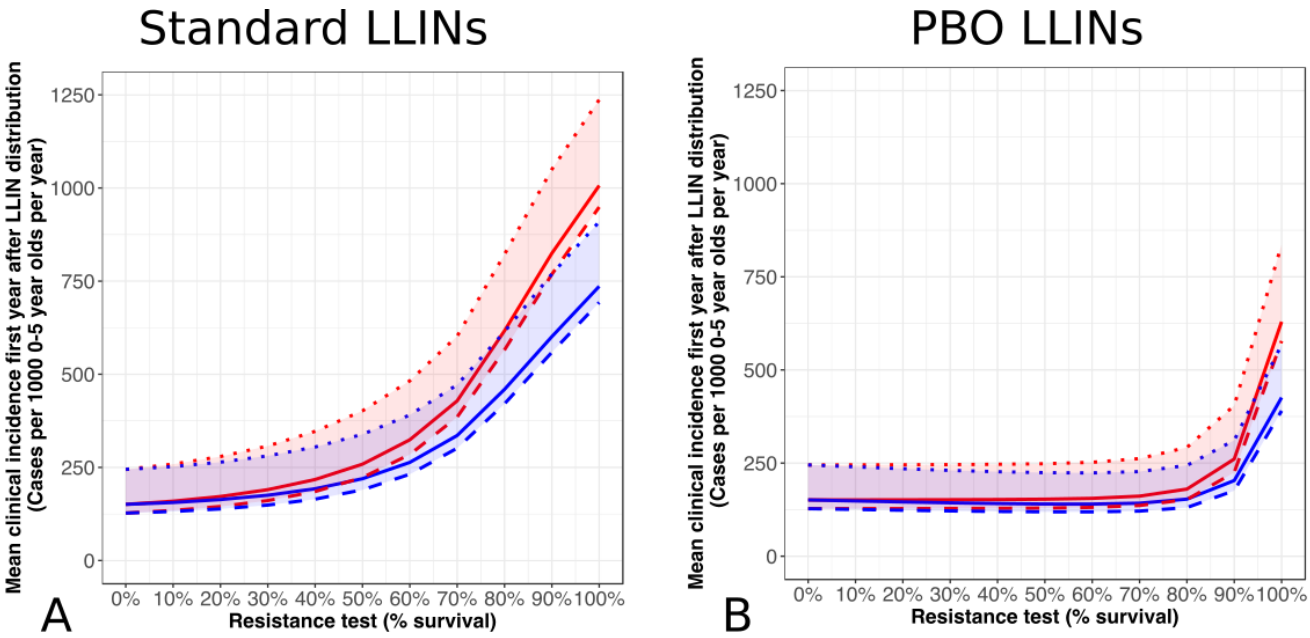

Figure 6.8: Changes in pyrethroid resistance reduces the effectiveness of newly distributed LLINs. Running the scenario in Figure 6.7 with bioassay survival ranging between 0-100% being introduced at year 6. Each line shows mean incidence in under-fives a year after 80% of the population are given LLINs (year 7). Dashed line denotes LLIN users only, dotted line denotes only people without LLINs, solid line denotes population average. Red lines are from the model without the TFI effect, blue lines are from the model with the TFI effect.

6.3.4 Comparison of model predictions to results of a randomised controlled LLIN trial

Previous work has shown that the Imperial College transmission dynamics mathematical model parameterised with experimental hut trial data can broadly recreate the epidemiological patterns observed in a recently published RCT comparing standard and PBO LLINs. Nevertheless, the model was slightly optimistic in its predictions compared to trial results, particularly at the third timepoint (1.5 years following mass distribution). Including the LLIN-induced TFI effect in the model moderately reduced the predicted prevalence of infection in the follow-up surveys of the Kagera RCT, which improved the accuracy of the model predictions (Figure 6.9). The root mean squared error of the model predictions for standard LLINs were reduced from 0.070 to 0.014 when including the TFI effect in the model. For PBO LLINs, the root mean squared error of the model predictions were reduced from 0.034 to 0.004 when including the TFI effect in the model. The maximum difference in the predicted prevalence of infection in 0 to 15 year olds with or without the TFI effect was 9.2% for standard LLINs and 7.8% for PBO LLINs. Now, for PBO LLINs the prediction interval for the prevalence over time includes all of the observed prevalence estimates from follow-up surveys when accounting for variation in the observation (Figure 6.9B). For standard LLINs the observed prevalence estimate third follow-up survey is still much lower than the model predictions when including the TFI effect. The additional impact of including the TFI effect increases over time, this is because the LLINs the mortality effect of the LLIN decays over time whereas the TFI effect does not (see Section 6.3.1). If less mosquitoes die, more will instead acquire TFI because there is still a smaller concentration of pyrethroids on the net.

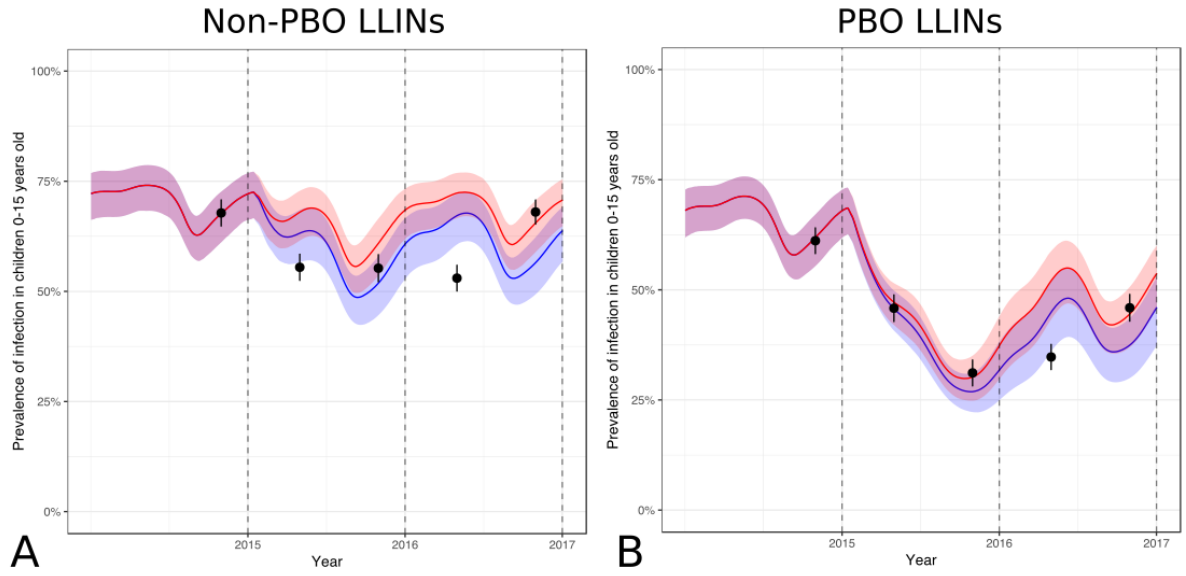

Figure 6.9: Imperial malaria transmission model predictions for the impact of standard (A) and PBO (B) LLINs in the RCT conducted by Protopopoff et al. (2018) in Kagera, Tanzania. The coloured lines show the model predictions with (blue) and without (red) a TFI effect. The coloured intervals around the lines are the result of re-running the model using 1000 samples from the fitted posteriors of other model parameters. The circular points show the observed prevalence in the surveys conducted during the RCT and their corresponding 95% confidence intervals.

6.4 Discussion

This analysis found that treated LLINs in experimental hut trials cause considerably lower mosquito blood feeding than when evaluating untreated nets. This inhibition is reduced as the severity of pyrethroid resistance increases but is still present even at very high levels of bioassay survival. Assuming that this blood feeding inhibition was entirely caused by an LLIN-induced TFI effect led to modelling predictions that standard LLINs would maintain a slightly larger epidemiological impact when pyrethroid resistance increased than would otherwise be the case. Comparing the model predictions of the randomised controlled trial undertaken in Kagera showed that the TFI effect improved the model predictions but was unable to explain all of the discrepancy between observed bioassay survival and LLIN impact.

The logistic mixed effects regression model found that PBO LLINs prevent more excess blood feeding inhibition than standard LLINs and maintain more excess feeding inhibition as resistance increases. For this reason, adding the LLIN-induced TFI effect into the Imperial College model predicted that the benefit of switching to PBO LLINs over standard LLINs is greater than previously thought (Figure 6.8). Churcher *et al.*, (2016) found previously that areas with 40-90% mosquito bioassay survival could benefit from switching to PBO nets. In this analysis, when including the TFI effect, this range is extended to 100% bioassay survival.

Therefore, PBO LLINs could have a greater role to play as a vector control method for areas with extremely high resistance such as Kagera. These results also suggest that PBO increases the likelihood of TFI in resistant mosquitoes similar to mosquito mortality.

Washing the nets 20 times, which markedly reduces its ability to kill and deter mosquitoes (Atieli *et al.*, 2010b; Sheikhi *et al.*, 2017), did not reduce the mean level of excess blood feeding that they prevented. Seemingly, the concentration of pyrethroid on the net needed to induce the TFI effect in mosquitoes can be relatively low. The mortality effect of LLINs decays more quickly when mosquitoes have pyrethroid resistance, and it is proposed that this is because the concentration of pyrethroids on the LLIN is quicker to drop below a level where it cannot kill the mosquito, reducing the epidemiological impact (Ochomo *et al.*, 2013). Further observations of mosquitoes that have acquired the TFI effect of pyrethroid contact could determine how the likelihood of TFI interacts with pyrethroid resistance and net age.

Including the TFI effect in the model reduced the predicted public health impact of pyrethroid resistance, but this should not downplay the serious threat that resistance still poses to malaria control efforts (Sougoufara *et al.*, 2017). Even with the TFI effect, there are still considerable increases in malaria transmission associated with greater levels of pyrethroid resistance. Since excess blood feeding inhibition is reduced as LLIN mortality decreases (Figure 6.2), it is possible that there will reach a point where the probability of a mosquito being susceptible to the TFI effect is low given the increasing prevalence and intensity of pyrethroid resistance. Further work is needed to assess this in mosquito populations exhibiting very high levels of resistance. Therefore, any malaria transmission currently prevented by the TFI effect on LLINs may eventually not be prevented as resistance intensity increases. Instead, these results should be seen as quantifying the epidemiological effects of sub-lethal effects of pyrethroids used in vector control. Understanding these effects is of particular importance now that it appears the endpoints of high mosquito mortality and deterrence are no longer achievable with the LLINs currently on the market.

Sub-lethal effects of pyrethroids have remained underappreciated until now because they have a small epidemiological impact when pyrethroids cause significant mortality and deterrence. The excess blood feeding prevented by LLINs is greatest when they are the most effective at killing mosquitoes, resulting in few mosquitoes actually surviving to acquire the TFI effect (Figure 6.2). Sub-lethal entomological effects only become an important

consideration when considering malaria control in settings with pyrethroid resistance vectors. If there is no resistance, sub-lethal effects add little to the overall impact of pyrethroids. Similar results have been found in the modelling of Ivermectin (IVM) by Slater et al. (2014), who found that adding in additional sub-lethal effects of IVM to the Imperial College model (namely, reduced re-feeding rate and reduced sporogony) caused negligible changes in the predicted epidemiological impact of IVM. This makes sense, as the impact of reduced re-feeding or sporogony cannot be realised; the mosquitoes die before this impact is observed. If IVM were to begin to lose its effectiveness at killing mosquitoes it would be interesting to see whether it would retain some of its public health impact, assuming that it still causes sub-lethal effects. This is not a wholly hypothetical scenario, since a family of proteins responsible for pyrethroid resistance in anopheline mosquitoes also seems to play a role in ivermectin resistance in cattle ticks (Müller *et al.*, 2008; Le Gall, Klafke and Torres, 2018).

The excess blood feeding inhibition, which in this analysis has been fully ascribed to the LLIN-induced TFI effect, could be explained through alternative processes. For instance, behavioural changes in the mosquito could lead to their avoidance of an LLIN upon entering a hut and discovering the net (Sokhna, Ndiath and Rogier, 2013; Killeen and Chitnis, 2014). This would provide an alternative explanation as to why more mosquitoes are found alive and unfed in huts with treated nets in. Use of infra-red tracking technology found that pyrethroid-susceptible mosquitoes changed their flight behaviour when approaching treated nets, but that mosquitoes stopped interacting with treated and untreated nets after a short period of time (Parker *et al.*, 2015). The mosquitoes could not be followed up to determine if they could feed or would suffer delayed mortality due to pyrethroid exposure. The hut trial data shown in Section 6.3.2 provides some indirect evidence that some of the excess blood feeding inhibition is caused by the TFI effect rather than LLIN avoidance. There is also evidence that the duration of landings for *An. quadrimaculatus* are not significantly shorter on nets treated with some (but not all) pyrethroids compared to untreated nets, suggesting that these mosquitoes do not avoid treated nets (Cooperband and Allan, 2009). However further work will be needed to validate this assumption. Untangling the processes that lead to treated nets preventing blood feeding would require monitoring mosquitoes during and after experimental trials. For instance, repeating the infra-red tracking experiment with resistant mosquitoes would determine how, if at all, resistance changes the way that mosquitoes interact with LLINs. Future tracking technologies will be able to measure more specific attributes of mosquito flight behaviour that are linked to mosquitoes following odour cues, if this is possible then it might also be feasible to determine when a mosquito stops following any

odour clues and linking this to previous LLIN contact (Spitzen and Takken, 2018). Something approaching this has already been performed for *Culex quinquefasciatus* mosquitoes, which flew more slowly and turned more frequently during flight after pyrethroid exposure (Cohnstaedt and Allan, 2011). Alternatively, mosquitoes captured alive in experimental huts could be given an opportunity to feed as soon as possible to estimate the probability of LLINs causing the TFI effect.

Including the TFI effect in the Imperial model improved the accuracy of using bioassay survival as way to predict LLIN impact in the field as the root mean squared error of the predictions from the Imperial model was greatly reduced, however there were only four observations in each arm of the trial. The prediction error was largest for the third follow-up survey for standard LLINs (Figure 6.9A). This could be because the Imperial College model needs to incorporate more sub-lethal LLIN effects, such as delayed mortality or reduced egg-laying, to give LLINs a larger or more sustained impact. Future modelling work could investigate whether combinations of sub-lethal effects could explain any discrepancy between the severity of resistance and the effectiveness of LLIN use. An alternative explanation could be that other factors influencing malaria transmission were unobserved and different during the trial. For instance, rainfall could have been unusually low around the time of the third follow-up survey (mid 2016). Preliminary analysis of satellite rainfall data estimated that the rainfall between September and December 2016 in Kagera was around 66% of the average rainfall in the same period in 2015 and 2017. This could have led to lower observed prevalence than the model prediction, since the model uses a rainfall curve averaged over many years. Nevertheless, the results presented here come from a single study and a broader approach including results from other RCTs is needed before we can determine whether including TFI substantially improves the predictive power of the model.

6.5 Conclusion

Given the observation of a LLIN-induced TFI effect in a laboratory setting it is highly likely that this effect is at least partially responsible for the considerable additional blood feeding prevented by treated nets over untreated nets. This analysis shows how such a TFI effect allows LLINs to retain some of their effectiveness that would be reduced by pyrethroid resistant vectors. While the TFI effect alone does not cause LLINs to remain effective enough to prevent serious increases in malaria burden, studying combinations of the sub-lethal effects of pyrethroids can help us to fully understand the implications of pyrethroid resistance on

malaria control effects. Characterising the sub-lethal effects of LLINs and how they interact with pyrethroid resistance in mosquitoes, will require modifying the protocols of experimental hut trials beyond monitor death and deterrence. Seeing as vector control tools utilising alternatives to pyrethroids remain some time from reaching approval and being distributed, it would be prudent to comprehensively understand how useful pyrethroids will be as a malaria control beyond the short-term.

7 General Discussion

This thesis has investigated methods of estimating and reducing the malaria burden in the context of situations encountered by MSF: poor epidemiological data, small geographical scale, and considerable pyrethroid resistance. This has required the development of new statistical models (previously these methods have only been used twice in malaria epidemiology, looking at associations between rainfall and malaria incidence in China (Guo *et al.*, 2015; Wu *et al.*, 2017)), as well as extending the Imperial College malaria transmission model. These contexts are of interest because after nearly 20 years of renewed effort to control malaria using long-lasting insecticidal nets and indoor residual spraying, the decision-making process for those running malaria control programmes is moving away from broad top-down recommendations adopted at the country level and towards individualised programmes for smaller spatial units. This is for two main reasons. Firstly, the toolbox of malaria interventions has expanded to incorporate new ways of interrupting the transmission cycle, at different times of day, or against mosquito species with specific feeding behaviours (Hemingway *et al.*, 2016; Killeen *et al.*, 2017). How effective these new interventions are will depend on local demographic, entomological and geographical factors that are all spatially heterogeneous. Mapping malaria risk shows large variability in malaria risk even within communities (Bejon *et al.*, 2014). Often, optimising packages of interventions at the province level rather than the country level leads to more cost-efficient results (Walker *et al.*, 2016; Drake *et al.*, 2017). Secondly, institutions such as the WHO are viewed as having been slow to respond to changes in the malaria landscape, such as the malaria parasite developing resistance to treatment or mosquitoes developing resistance to pyrethroids used on nets. The rigid and costly testing protocols often mean that the problem develops faster than any viable solutions to it. For instance, PBO LLINs have been under testing for nearly 10 years as a solution to pyrethroid resistance but have only recently been approved by the WHO (World Health Organization, 2015). While this was happening, some countries in Sub-Saharan Africa developed severe pyrethroid resistance in their local vector populations (Coleman *et al.*, 2017).

As a result of these two factors, national malaria campaign programmes and other institutions that decide upon malaria control strategy are starting to make their own choices using local data to try and decide upon the optimum approach to malaria control (malERA, 2017). This requires the ability to contextualise the local malaria burden geographically and temporally, as well as being able to measure changes in malaria transmission that happen after introducing interventions. In this thesis I have developed statistical approaches for both problems. Collecting adequate routine malaria surveillance data at a spatial resolution finer than the province level (provided by DHS surveys) is challenging, especially in areas with ongoing conflicts. Working in an area with an ongoing humanitarian crisis prevented MSF from being able to take cross-sectional surveys of the population (which are expensive and hazardous) or knowing up-to-date information about the local catchment populations of their hospitals. I showed how their routinely collected data from testing pregnant

women with RDTs could be used to a) compare the burden around MSF hospitals to province-wide estimates of burden (Chapter 2) and b) analyse trends in malaria burden over time, since ANC prevalence follows trends in the clinical incidence in under-fives (Chapter 3). As a direct result of this work, MSF have adopted monthly ANC prevalence as their metric for monitoring malaria at their field sites in Sub-Saharan Africa. This is an improvement on their previous use of case count data, as ANC prevalence is not directly biased by the size of the local population.

Detecting small changes in ANC prevalence is only possible if a large number of pregnant women are sampled each month. This provides enough statistical power to rule out variation in the observed prevalence due to randomness introduced by finite sample sizes. There are several reasons to think that the changes in ANC prevalence caused by new interventions might be fairly small and therefore hard to detect. New interventions might target specific pockets of residual transmission that cannot be addressed by LLINs and IRS. In Chapter 4 I explored how emanators would fare at protecting people while they are in bed when LLINs cannot be used. Emanators have a much smaller impact than LLINs, since they are thought to lack the ability to kill mosquitoes. In Chapter 5 I showed that outdoor biting during the evenings could be responsible for continued malaria transmission in places where LLIN coverage is excellent. New insecticide chemistries are being tested such as pyriproxyfen, which reduces the egg-laying capacity of mosquitoes but does not kill them (Mbare, Lindsay and Fillinger, 2014). Such effects might have small overall impact on the prevalence of infection but could play an important role in a combination of vector control tools. Finally, it is likely that pyrethroid resistance in vectors will increase further and the epidemiological impact of pyrethroid-based interventions will rely more and more upon sub-lethal effects, which can cause decreases in the prevalence of infection smaller than would be expected for fully-working LLINs. For instance, the impact of an LLIN-induced TFI effect is shown to be at most around a 10% prevalence reduction in the Kagera RCT in Chapter 6 (Section 6.3.4).

In order to be realistic, future trials of vector control tools should compare populations using the new tool in combination with LLINs against populations using only LLINs (malERA, 2017). The additional impact of new tools on top of LLINs is predicted to be fairly small (Kiware *et al.*, 2017). However, epidemiological trials are unlikely to be able to disentangle how much of the overall impact is due to each individual intervention, unless the trial has an unrealistic number of arms with different subsets of intervention combinations. The proposed solution to this is to use modelling to prioritise which vector control combinations should be tested against each other. The results presented in this thesis lead to several suggestions about how this modelling process could be expanded.

Currently, new intervention methods that fall outside of an existing intervention class require empirical proof of their epidemiological impact as well as their entomological efficacy (World Health Organization, 2018). Trials demonstrating epidemiological impact make sure that the interventions

will be effective in the field, but they also happen to be the most expensive because they involve close monitoring of a cohort for a long period of time. They are also the efficacy of the intervention in a specific site against a specific vector population which may not be representative of the area the intervention will be deployed. However, formulations of interventions in an existing class only need to prove entomological efficacy (as well as safety) (World Health Organization, 2017a). If using the results of entomological testing could allow transmission models to make trustworthy predictions of the epidemiological impact of new classes of interventions, this could lower the financial and logistical barriers preventing new interventions from reaching the field in a timely manner. This could be supported by local routinely collected data (such as prevalence of infection in pregnant women) in places where interventions are trialled.

Using entomological data to predict epidemiological impact accurately would require that experimental hut trials capture all of the entomological effects of new interventions on mosquitoes. As discussed in Section 6.4, experimental hut trials could be expanded to measure a temporary feeding inhibition effect by seeing if mosquitoes can feed in the hours following exposure. The mosquitoes caught alive and fed in a hut could also be followed up to see if they go on to lay eggs, which could demonstrate reduced egg-laying after exposure to insecticides. Mathematical models of malaria transmission would then need to demonstrate their ability to accurately predict the epidemiological impacts of interventions. All of the models mentioned in Section 1.3 are fitted to age-prevalence profiles and have not been tested or built specifically for their ability to predict the results of RCTs, past or future. If these models are to be used to choose the optimum intervention programme, then further testing of their capability to accurately predict epidemiological impacts is required. There are changes that can be made to the Imperial College model in terms of structure and parameterisation that would help make its predictions more accurate. I make several suggestions most relevant to the work in this thesis below.

In terms of calibrating the model to predict the results of a particular RCT, the model would benefit from a way of linking mosquito population density to the actual rainfall and temperature observed during the RCT. The model currently uses a curve that represents the shape of average rainfall, repeating each year. This is adequate for general predictions but not for trying to replicate the results of an intervention that actually happened. There is also room for demographic data collected during the trial to inform the values of other parameters in the model. For example, the parameters for the proportion of bites taken indoors or in bed were originally chosen based on two mosquito biting time studies available eight years ago. As I show in Chapter 4, small changes in the assumptions about how much of the population is outside during the evening can lead to big changes in the proportion of all bites taken in bed or indoors. Alongside entomological tests for pyrethroid resistance during RCTs, there could also be estimates made of the mosquito biting rates at different times of day.

Currently, the Imperial transmission model has a limited number of entomological effects, which vary with pyrethroid resistance depending upon the results of a simplistic assay. Firstly, mosquitoes currently die at a constant background rate based on the mean life expectancy of mosquitoes from the literature (Griffin *et al.*, 2010). This life expectancy could instead vary with the level of exposure to pyrethroids (mitigated by the level of pyrethroid resistance in the vector). A more complex mortality bioassay could be performed in the field that repeatedly exposes to pyrethroids the mosquitoes that are not immediately killed. This would measure delayed mortality at the trial site (a simplified version of the experiments by Viana et al. (2016)). The delayed mortality test results could translate into a new mosquito death rate in the model after the intervention has begun.

The birth rate of mosquito larvae in the model is dependent only on a seasonal carrying capacity, the mosquito feeding rate, and the mosquito death rate. The latter two parameters change with the introduction of LLINs that stop mosquitoes feeding and kill them. However, it also seems like that pyrethroid exposure could cause a separate reduction in mosquito fecundity. This would further reduce mosquito egg-laying dependent upon the level of pyrethroid exposure that mosquitoes experience. An age-dependent level of pyrethroid exposure could be calculated for a mosquito based upon how often they feed and how likely they are to be exposed to pyrethroids at each feed, given the current level of coverage for each pyrethroid-based intervention. Similar to the modelling work undertaken in Chapter 6, modelling the epidemiological impact of TFI, delayed mortality and reduced fecundity both alone and in combination could explain the continued effectiveness of LLINs in areas with pyrethroid resistant vectors. It would also be interesting to explore why LLINs may fail in some areas with pyrethroid resistance but not others, and how that failure could be understood in terms of the importance of these three sub-lethal effects varying across transmission settings.

There is also a pressing need to understand how using volatile pyrethroids in emanators might contribute to the development of pyrethroid resistance in vectors. Alternatives to pyrethroids are chosen for IRS campaigns so that they do not put selection pressure on the local vectors. This is done to preserve the effect of pyrethroids on LLINs (for which there are no alternative chemicals available to put on nets) for as long as possible. If the pyrethroids released by emanators caused a similar selection pressure, this would bring into question their use alongside LLINs as a way of preventing outdoor evening biting. Resistance to the spatial repellent effect of transfluthrin has been artificially bred into lab-reared mosquitoes, this resistance also conferred resistance to tranfluthrin toxicity from higher concentrations in bioassays (Wagman, Achee and Grieco, 2015b). It remains unclear how repeated exposure to low concentrations of pyrethroids in the air will change resistance in the vector population. If vectors that are resistant to mortality induced by LLINs retain some sensitivity to volatile pyrethroids, emanators could ease selection pressure by reducing the overall level of exposure that mosquitoes have to lethal concentrations of pyrethroids. On the other hand, emanators might add to the selection pressure since resistant vectors will be more likely to be able to take a blood meal and

lay eggs (assuming that humans are the primary feeding target for the local vector population). The recommendations set out by the WHO for managing resistance envisage rotating the chemicals used for IRS campaigns and moving away from pyrethroid LLINs as soon as alternatives become available (Organització Mundial de la Salut Global Malaria Programme, 2012). New IRS chemicals could also be tested for their spatial repellent properties at low concentrations, as well as other properties required for use on an emanator such as volatility and safety to humans. These new chemicals will have new entomological modes of action that will need to be understood and measured, similar to the sub-lethal effects of pyrethroids, before they can be added to mathematical transmission models. Further work is needed to understand how humans interact with emanators in the field and to understand their full entomological impact before more accurate predictions can be made regarding their public health value. Evaluating emanators in well recognised assays such as experimental hut trials would provide answers to some of these questions even if the primary use case of emanators would for them to be used outside in conjuncture with LLINs.

In this thesis I have been motivated to answer the type of questions that could be asked by non-governmental organisations such as MSF as they begin to take a more preventative approach to malaria control. It is important to begin to answer these questions as thoroughly as possible, since it seems unfortunately probable that the humanitarian crises that MSF respond to in DRC and surrounding countries will continue. Hopefully, the concluding thoughts regarding how to monitor vector control impact for tools with new entomological impacts, or in difficult settings, will have wider applications in malaria modelling. There is still a lot to learn about the effects of pyrethroids, and these will increasingly come into play as resistance worsens. Coming to understand these processes better will therefore be beneficial in the future when trying to achieve malaria elimination.

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Appendix

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Malaria Journal

RESEARCH

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Using ante-natal clinic prevalence data to monitor temporal changes in malaria incidence in a humanitarian setting in the Democratic Republic of Congo

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Abstract

Background: The number of clinical cases of malaria is often recorded in resource constrained or conflict settings as a proxy for disease burden. Interpreting case count data in areas of humanitarian need is challenging due to uncertainties in population size caused by security concerns, resource constraints and population movement. Malaria prevalence in women visiting ante-natal care (ANC) clinics has the potential to be an easier and more accurate metric for malaria surveillance that is unbiased by population size if malaria testing is routinely conducted irrespective of symptoms.

Methods: A suite of distributed lag non-linear models was fitted to clinical incidence time-series data in children under 5 years and ANC prevalence data from health centres run by Médecins Sans Frontières in the Democratic Republic of Congo, which implement routine intermittent screening and treatment alongside intermittent preventative treatment in pregnancy. These statistical models enable the temporal relationship between the two metrics to be disentangled.

Results: There was a strong relationship between the ANC prevalence and clinical incidence suggesting that both can be used to describe current malaria endemicity. There was no evidence that ANC prevalence could predict future clinical incidence, though a change in clinical incidence was shown to influence ANC prevalence up to 3 months into the future.

Conclusions: The results indicate that ANC prevalence may be a suitable metric for retrospective evaluations of the impact of malaria interventions and is a useful method for evaluating long-term malaria trends in resource constrained settings.

Keywords: *Plasmodium falciparum*, Malaria in pregnancy, Epidemiology

Background

Malaria remains endemic across large portions of the world, with an estimated 216 million clinical cases and 445,000 deaths globally during 2016 [1]. This burden falls disproportionately on young children in countries where the climate is amenable to endemic malaria transmission [2], predominantly sub-Saharan Africa. The increased investment in malaria treatment and prevention, along with the diverse methods available for malaria control, makes the effective measuring of temporal trends in malaria burden critically important [3]. The effectiveness of control interventions varies from site to site due to the epidemiology of infection and factors, such as the susceptibility of the local mosquito population to insecticides [4]. Local control programmes need to monitor the impact of interventions to identify the optimum package,

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justify future financial investment, and identify changes in transmission in a timely manner [5].

Africa-wide estimates of burden reduction have primarily utilized cross-sectional survey data conducted by the Demographic and Health Surveys Programme [6, 7]. These surveys are undertaken at the province level, usually every 2–3 years, where children are tested for malaria in randomly selected clusters. Province-wide estimates can hide substantial spatial heterogeneity generated by local healthcare provision or local geographical, demographic or climatic differences, therefore, populations in some areas face higher malaria burdens than the province-wide average [8, 9]. Finer scale estimates of burden can be collated passively using the number of malaria cases reported from local health centres. To generate meaningful incidence rates requires good estimates of the size of the health catchment population, which is unlikely to be available in many parts of sub-Saharan Africa. The problems are exaggerated in humanitarian settings where populations may be highly transient, or size estimates hard to generate due to security concerns or resource constrains. This is especially the case in 'open' chronic conflict settings where displaced populations often live amongst the local population and not in a defined enclosed area or are frequently on the move due to insecurity. The prevalence of the malaria parasite in refugee and internally displaced populations is often higher than in local more stable populations due to inequalities in resources and health provision [10].

A novel method for routine malaria surveillance could be the use of ante-natal care (ANC) data [11]. Such data are used in sentinel surveillance surveys for HIV, as it corresponds well with national HIV survey data of the same catchment areas [12]. For malaria, the prevalence of infection in pregnant women is strongly correlated with the prevalence of infection in children under 5 in cross-sectional survey data from across Africa [13]. During standard intermittent preventative treatment during pregnancy (IPTp) programmes, any woman that is symptomatic is tested by RDT and given artemisinin-based combination therapy (ACT), if they test positive. Any women who are not symptomatic or are test-negative are given chemoprevention in the form of sulfadoxine-pyrimethamine (SP). Since 2011, Médecins Sans Frontières (MSF) has rolled out a model of routine intermittent screening and treatment (IST) of all pregnant women combined with the IPTp-SP programme described above. This entails testing all pregnant women at every ANC appointment, women who are test-positive are given ACT and women who are test-negative are given SP (Fig. 1).

Since all women are tested regardless of symptoms, this reduces under-reporting bias due to the presence of asymptomatic infections. ANC programmes run by MSF in malaria endemic countries record the number of RDTs administered and the number of positive test results during ANC appointments at each health facility or hospital every month.

Here, methods are developed to predict the relationship between the prevalence of infection in pregnant women and the clinical incidence in children under 5 years old, using field data collected at five MSF field sites in the Democratic Republic of Congo (DRC). There is population denominator data available at these five field sites, which is uncommon for many of the sites where MSF works and more widely across sub-Saharan Africa. Nested statistical models are used to investigate the relationship between ANC prevalence and clinical incidence and determine whether this association is immediate or spread out over time. The utility of routinely collected ANC data for malaria surveillance and the evaluation of control interventions is then discussed, with special regard for settings where such denominator data are not available.

Methods

The data comprises time series from 5 different MSF health centres across the DRC for varying amounts of time between 2010 and 2016. These MSF missions vary in size and represent a mixture of hospitals, health centres and community clinics in the Great Lakes region; from North and South Kivu, close to the eastern border with Rwanda and Burundi (Baraka, Kimbi-Lulimba, Mweso and Walikale) and from the South-East province of Katanga, bordering Tanzania and Zambia (Shamwana, closed by the end of 2016). All sites are considered 'open' humanitarian settings, i.e. areas of chronic conflict mainly from the ongoing Congolese civil war, including internally displaced peoples (IDPs) and with frequent population movement due to fighting.

The ANC prevalence time series is the number of pregnant women tested for malaria using RDTs and the proportion of these that tested positive. Data is collated each month and all women that attend ANC appointments are tested for malaria regardless of whether they are symptomatic. The second time series is the monthly clinical incidence in children under 5 confirmed by RDT (i.e. symptomatic cases arriving as outpatients that tested positive by RDT). The size of the under 5 population at Mweso, Walikale and Shamwana is estimated by MSF each month using population surveys. The size of the under 5 population at Baraka and Kimbi-Lulimba, which cover larger areas, is taken from national census data conducted during the period of investigation by the DRC Department of Health.

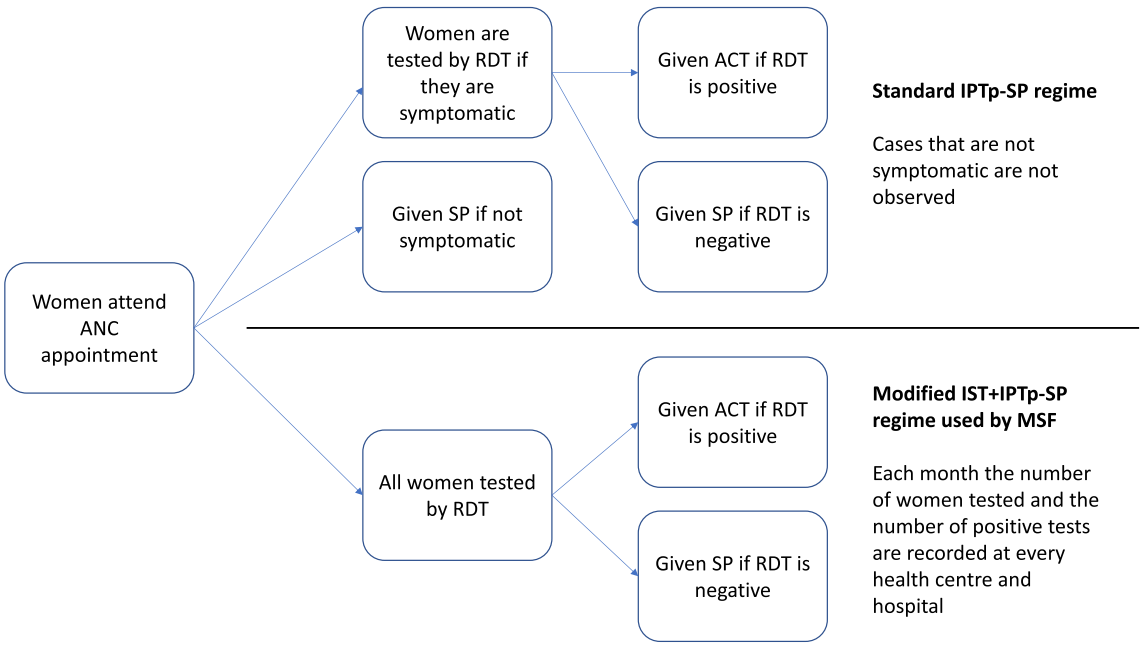

Fig. 1 Flowchart illustrating the difference between the standard intermittent preventative treatment during pregnancy using sulfadoxine-pyrimethamine (IPTp-SP) regimen and the expanded intermittent screen and treat plus IPTp-SP (IST+IPTp-SP) regime used by MSF in their ANC programmes in malaria endemic countries

An illustration of how the change in one metric may continue to influence another metric in the future (a lagged effect) is shown in Fig. 2. If one metric can affect another second metric for a long period of time, then the value of the second metric will depend on the current and historical values of the first metric.

A causal framework was utilized to characterize the relationship between ANC prevalence and clinical incidence, as well as to determine the direction of the association between the two metrics. A variable X "Granger causes" Y if including past values of X in a predictive model of Y produces better predictions of Y than just using past values of Y alone [14]. The analysis follows a two-step process. Firstly, a Granger causality test is used to determine the direction of the association (whether changes in ANC prevalence can predict future changes in clinical incidence, or vice versa) as well as the duration of any lagged effect. Secondly, this relationship is then fully characterized using more complex statistical models to determine the magnitude of the lagged effects and how the association might change with disease endemicity.

A vector auto-regression (VAR) model is used to test for Granger causality between the two metrics, determining the direction and length of potential lagged effects between two or more time series [15]. Granger causality was tested for using a Wald test suitable for stationary time series [16]. The number of past observations that should be used in the VAR model (known as the lag order) is determined by finding the lag order that optimizes some information criterion, usually the Akaike information criterion [17]. The VAR model with the optimum lag order was assessed for goodness of fit by examining the model residuals, performing a multivariate Portmanteau test to confirm that they are not correlated with each other and an autoregressive conditional heteroscedasticity test that looks for changing variance over time. The VAR models were fit using the package 'vars' in the R statistical software [16].

Distributed lag non-linear models (DLNMs) are used to fully characterize the relationship between the two metrics, these flexible models allow a "lagged effect" as well as an "endemicity effect" of one metric upon the other. The "lagged effect" means that the effect of the explanatory metric upon the response metric happens over time (with the effect size changing with respect to time), whereas the "endemicity effect" enables the relationship between the two metrics to change according to the level of disease (the effect size varies with the value of the explanatory metric) [18]. DLNMs are specified by choosing two "basis" functions, the first basis function describes the shape of the association between the two metrics at each point in time (the transmission effect basis), the second basis function controls the shape of the lagged effects in the model (the temporal lag basis,

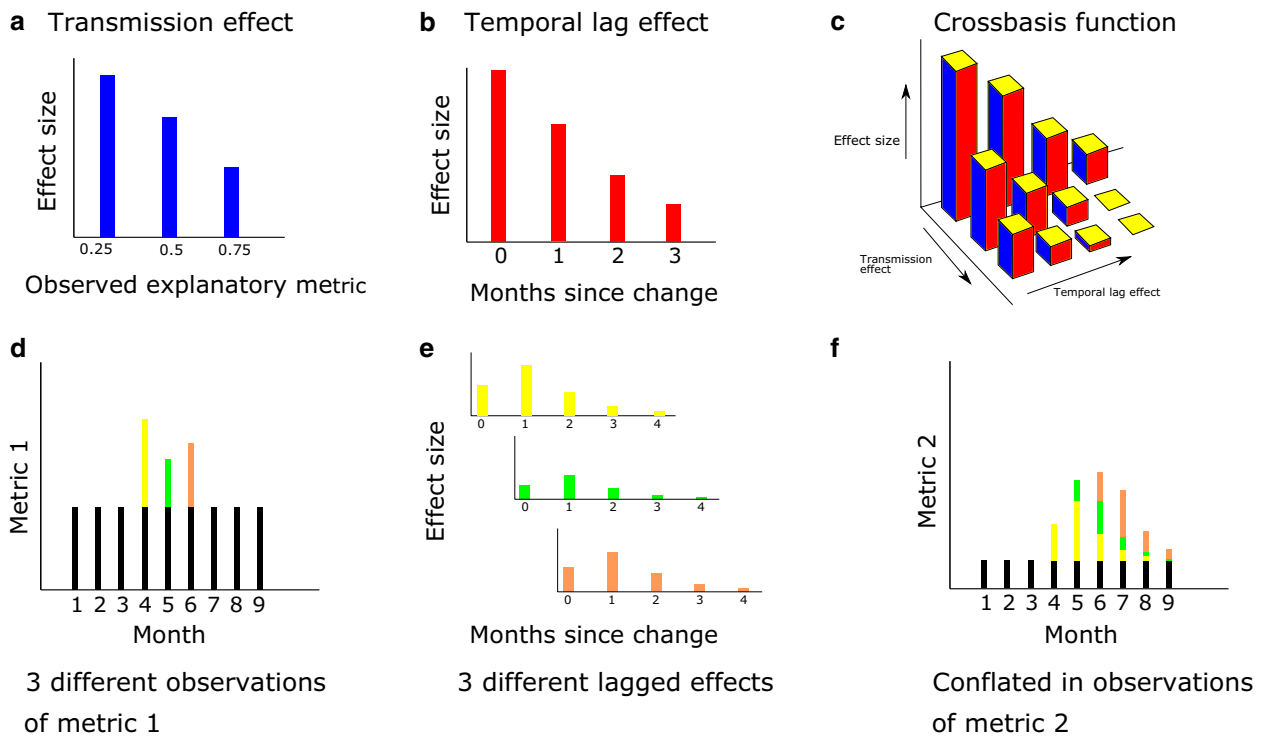

Fig. 2 **a**–**c** The concept of a crossbasis function in this context, in **a** the explanatory metric has corresponding effect on the response metric, the function that explains this relationship is the transmission effect basis. In **b** for a given value of the explanatory metric, this may have delayed effects on the response metric—in this plot for 3 months afterwards. This relationship is characterized by the temporal lag basis. In **c**, these two basis functions are combined into a bi-dimensional plot, the shape of the crossbasis function is restricted by the choice of functions in **a** and **b**. The precise shape of the crossbasis is determined during the fitting of the DLNM model. **d**–**f** How subsequent changes in one metric (Metric 1) can cause unpredictable patterns in another metric (Metric 2). **d** The different changes in Metric 1 differentiated by colour (yellow for the change in month 4, green for the change in month 5 and brown for month 6). **e** Each of these changes in Metric 1 have lagged effect that may differ with the size of the observation in Metric 1 and start at different times. These lagged effects are then observed as changes in Metric (2) over multiple months (**f**) with the lagged effects of three different changes in Metric 1 stacking up to create complex patterns in Metric 2. This is illustrated in this example where month 4 saw the greatest increase in Metric 1 whilst Metric 2 peaked in month 6

an example being Fig. 2b). These two functions are combined into a "crossbasis" function that describes the relationship between the value of an observation, how long ago it was observed and what its current effect will be on the response variable [19]. The crossbasis function can vary in shape depending on the two individual functions used to construct it. A crossbasis function can be written as $s(x_{t-l}, t-l; \eta)$, where x_{t-l} is the observation of the explanatory variable l months ago, $t-l$ is the number of months since the observation, and η are the so-called "basis parameters" which are the parameters that describe the shape of the two functions combined in the crossbasis. The crossbasis function can be included as a predictor in a generalized additive model with the following form:

$$logit(E(Y_t)) = \alpha + h_i + \sum_{l=0}^{L} s\left(x_{t-l},\ t-l; \eta\right), \quad (1)$$

where $E(Y_t)$ is the expected value of the response variable at time t (as determined by the Granger causality test outlined above), x_{t-l} is the value of the explanatory variable at time $t-l$, α is a parameter determining mean difference between the two metrics, h_i is the location-specific modifier of the mean difference between the metrics for location i, and L is the optimal lag order found when fitting the VAR model (and takes a value of 0 in models with no lagged effects). Different crossbasis functions ($s(x_{t-l}, t-l; \eta)$) made up of the two different basis functions are fit to the observed data and compared to determine the most parsimonious model. Two different functions are used to investigate how the relationship between metrics changes with endemicity, i.e. the transmission effect basis:

- Linear basis: The simplest model assumes that the endemicity effect varies linearly with the explanatory metric.

- Hill function: A function flexible enough to fit the relationship between the incidence and prevalence typically observed in non-temporal data [20].

A choice of three different basis functions are used as the temporal lag basis:

- No lagged effect.
- Linear basis: The effect of a change in the explanatory metric increases or decreases linearly with respect to time.
- Non-linear basis: A non-linear spline function that is penalized to produce a smooth curve, using penalized splines has been shown in simulations to be an effective method of reconstructing a variety of lag-exposure relationships when fitting DLNMs [21].

All combinations of endemicity effect and lagged effect basis functions are tested, giving a total of six different models. For clarity, each model is named with an acronym that represents its structure. The first two letters of the acronym represent the function used for the transmission effect basis, this can be either LE for a linear function or NE for a Hill function. The second two letters indicate the function used for the temporal lag basis, this can be LL for linear lagged effects or NL for non-linear basis spline lagged effects. If there is only one pair of letters then the model does not have lagged effects. The names of all six models are listed in Table 2.

Models were fit using the '*dlnm*' package [22] for the R statistical software and the most parsimonious model was identified using AIC value. The predictive power of each model (its ability to correctly predict into the future) was compared use a rolling origin cross-validation method. This predicted a year of unseen data at a time, with the model being fit using all previous years of data at the given location and all the data from every other location. The models can then be compared using the root mean squared error of their predictions.

Results

ANC prevalence and clinical incidence in children under 5 across the five locations are shown in Fig. 3. Visually, it is clear that the temporal trends in the metrics are broadly the same, though the association has substantial variability over time and between different locations. Baraka and Shamwana show pronounced seasonal patterns in both transmission metrics, whereas the other sites do not show obvious seasonal variation in transmission. In Fig. 3 the sites are ordered from the northernmost site to the southernmost site when moving from left to right along the top row and then the bottom row, there is a steep gradient in the degree of seasonality of malaria transmission when moving from north to south [23].

Different sites also have differing levels of ANC prevalence despite similar incidence rates in children under 5. For example, Shamwana and Kimbi-Lulimba have median observed clinical incidence rates in children under 5 of 1.714 and 1.711 respectively, but their median observed ANC prevalence is 34.6% in Shamwana and 18.5% in Kimbi-Lulimba (Table 1). A direct cross-sectional comparison of the two metrics each month is shown in Fig. 4.

The Granger causality test indicated that past clinical incidence can significantly improve predictions of future ANC prevalence compared to past values of ANC prevalence alone ($p=0.002$). Conversely, ANC prevalence was unable to predict future clinical incidence with significantly more accuracy compared to using past values of clinical incidence alone ($p=0.42$). The subsequent analysis therefore uses clinical incidence in children under 5 years as the explanatory variable and ANC prevalence as the response variable. The VAR model used for Granger causality testing also determined the length of the lagged effect (how many previous months of clinical incidence in under 5 s are predictive of the current ANC prevalence), the VAR model with the optimum AIC value had a maximum lag value of 3 months (1 month AIC $=-6.544$, 2 months AIC $=-6.556$, 3 months AIC $=-6.581$, 4 months AIC $=-6.574$). Since the difference in AIC values between the models with different lag values was not large enough to decisively prefer one model, the later DLNM model NENL was also fit using maximum lag values of 1, 2 and 4 months (see Additional file 1).

The "NENL" model provides the best fit (in terms of both AIC value and out-of-sample predictive power) indicating that changes in clinical incidence impact ANC prevalence non-linearly according to the level of endemicity, and that these effects manifest themselves (again non-linearly) immediately and over the subsequent months (Table 2). The 3D relationship (crossbasis function) is shown in Fig. 5a whilst a representation of the temporal lag basis function is depicted for various endemicity levels in Fig. 5b. The lagged effects are significant for 3 months, with the effect size being greatest in the month that the change in incidence is observed and then decreasing over time. The best fitting model that uses non-linear splines to model lagged effects (NENL) is an improvement, albeit a smaller one, upon the similar model that uses a linear function to model lagged effects (NELL). The non-linear lagged effects (NENL) estimate that incidence has a bigger effect on ANC prevalence with 1 and 2 months lag than the linear model (NELL) predicts (Fig. 5b).

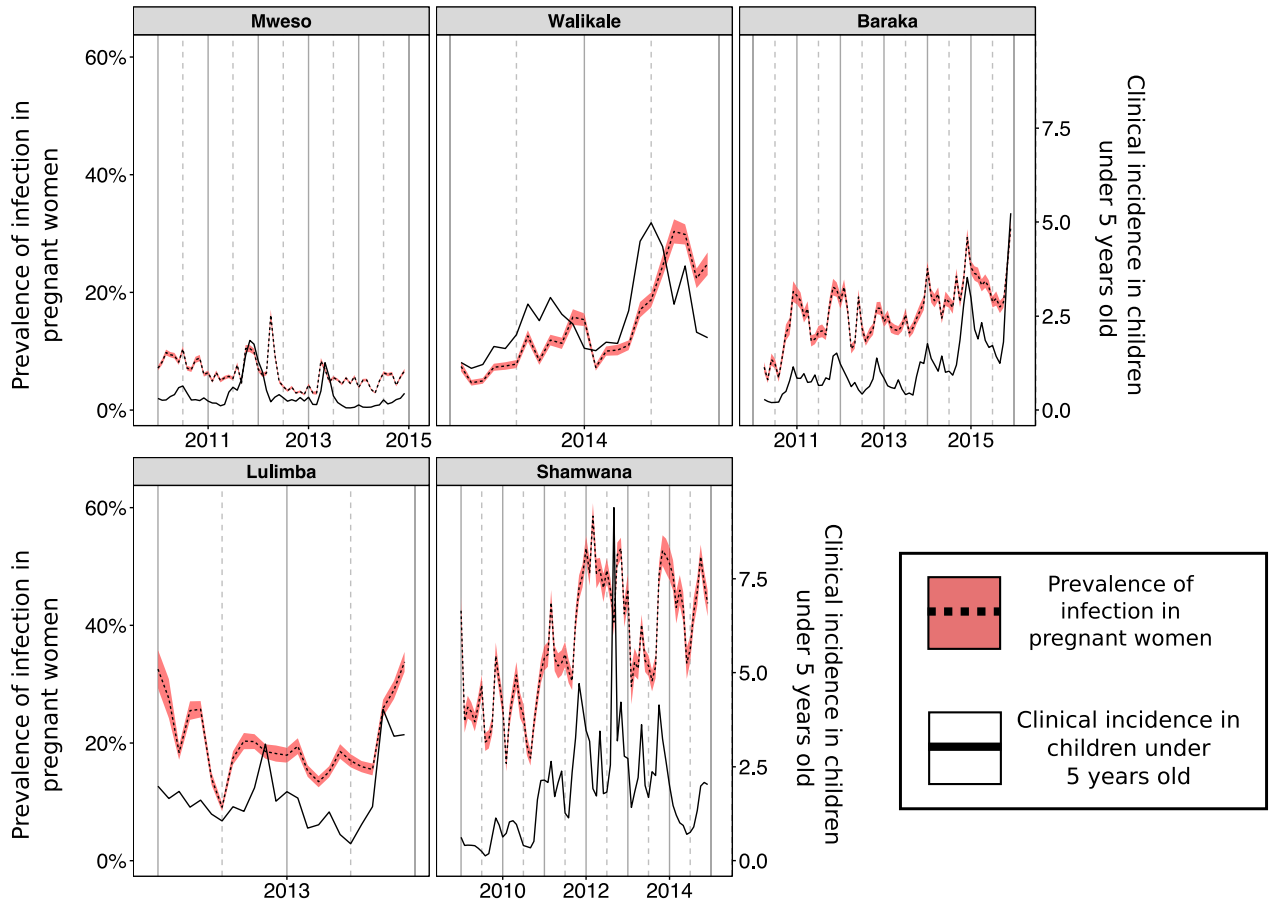

Fig. 3 Time series data from the five different settings used in the analyses. The solid black line shows the recorded clinical incidence rate in children under 5 years old each month (cases per child per year). The dotted black line shows the recorded anti-natal clinic prevalence recorded each month with the red shaded area indicating the 95% confidence intervals using the normal approximation method. Data are available for different durations in the different settings

Table 1 Summary of the time series data collected during the same month from the different DRC settings

Location	Number of data points in time-series	Median population size	Median monthly ANC visits	Median monthly ANC prevalence (%)	Median incidence in children under 5 years (minimum, maximum)
Baraka	69	71,238	636	17.3	0.929 (0.199, 5.24)
Mweso	60	65,867	1074	5.7	0.277 (0.059, 1.854)
Walikale	23	31,536	437	11.3	2.072 (1.112, 4.986)
Shamwana	72	36,000	455	34.6	1.714 (0.129, 9.397)
Kimbi-Lulimba	24	15,812	582	18.5	1.711 (0.451, 4.028)

The population size of the catchment area (used to convert case numbers into clinical incidence rates are and the number of women attending anti-natal clinics (ANC visits) are summarized using the median value. The longitudinal time series is shown graphically in Fig. 3

Allowing the relationship between clinical incidence and ANC prevalence to be non-linear substantially improves model fit (Table 2). A graphical representation of the out-of-sample predictive power of the best "NENL" model is shown in Fig. 6. Though the best-fit model is unable to predict small changes in prevalence the overall trends are well captured. How well the model captures trends in prevalence is demonstrated both when the model is fit to all available data and when using the rolling origin cross validation technique, where predictions

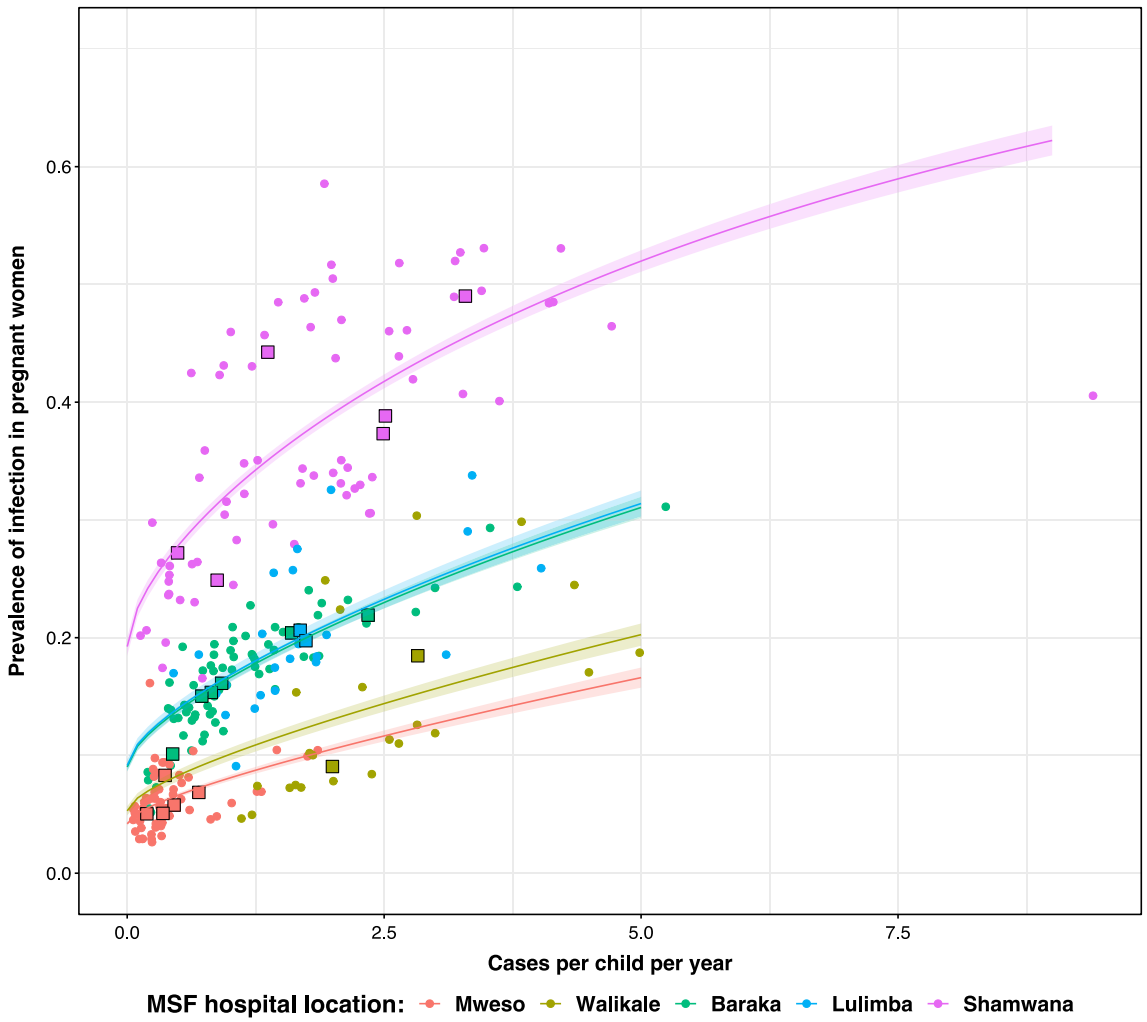

Fig. 4 Cross-sectional relationship between prevalence of infection in pregnant women attending anti-natal clinics (ANC) and clinical incidence in children under 5 years reported at the same site. The small circular points show the raw monthly values, coloured by location. The large square points show the same data aggregated by calendar year. The coloured curves show a simple non-linear relationship between the two metrics with no lagged effects (equivalent to model NE) and corresponding 95% confidence interval

Table 2 Summary of the different distributed lag non-linear models (DLNMs) characterizing the relationship between clinical incidence and ante natal clinic (ANC) parasite prevalence

Acronym	Endemicity effect	Lagged effect	Number of parameters	AIC	RMSE (rolling cross-validation)
LE	Linear	No lagged effects	6	3859.2	0.0667
LELL	Linear	Linear	7	3116.6	0.0563
LENL	Linear	Non-linear	13	3116.0	0.0564
NE	Hill function	No lagged effects	8	3499.8	0.1126
NELL	Hill function	Linear	9	2982.0	0.05434
NENL	Hill function	Non-linear	15	*2978.9*	*0.05431*

The second and third columns indicate the shape of the basis function used to characterize how the relationship is influenced by endemicity and the lagged effect. Models are compared using Akaike information criterion (AIC, lowest value in italic indicating most parsimonious model) and root mean squared error (RMSE, lowest value in italic indicating most predictive model)

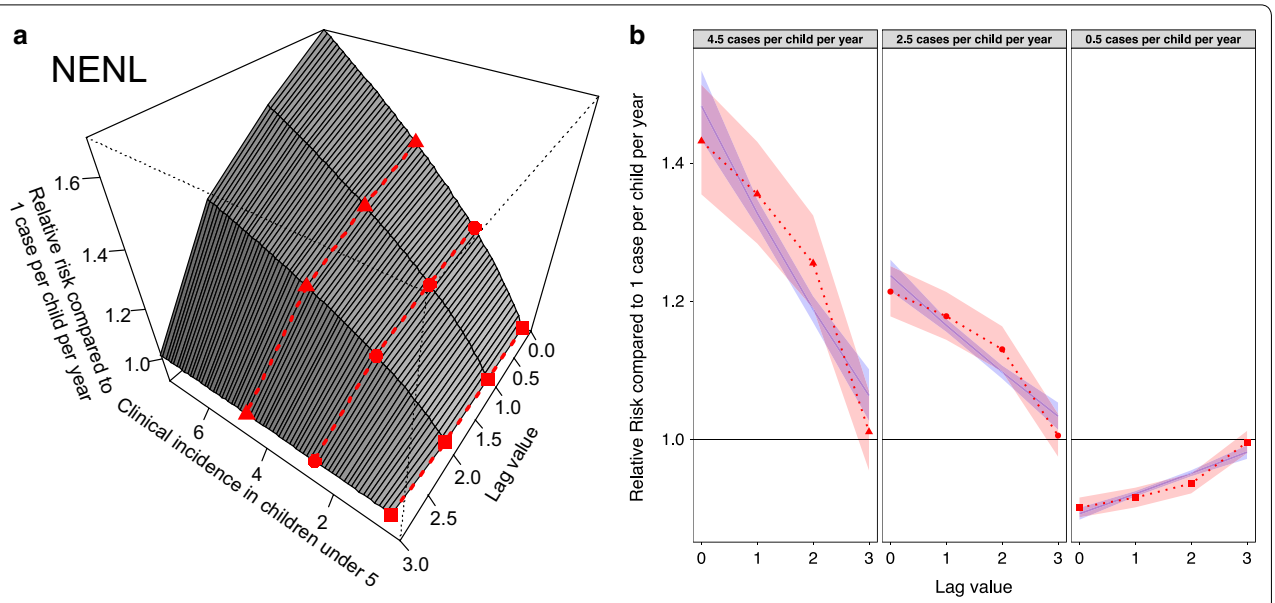

Fig. 5 The best fit "NENL" model showing how clinical incidence over the last 3 months influences current anti-natal clinic (ANC) prevalence in terms of relative risk when compared to an observation of 1 case per child per year. **a** Gives the full 3D relationship (the crossbasis function). Values greater than 1 indicate an increase in ANC prevalence whilst values less than one signify a decline. **b** Cross-sectional slices through the crossbasis function at three different clinical incidence values denoted by the shape of the points and corresponding lines on **a**. The red shaded band shows the 95% confidence interval in the fitted lagged effects whilst the blue line and associated band show the lagged effects predicted by the model NELL (allowing a comparison between the linear and non-linear lagged effects)

are made using the history of infection from the last year or more.

Discussion

Clinical incidence in children under 5 years old could predict ANC prevalence but not vice versa. This matches our current understanding of the epidemiology of malaria. Clinical incidence in children under 5 years, who have low levels of malaria immunity, is likely to closely reflect the incidence of new infections and thus be a good proxy for the current intensity of transmission. Conversely, in pregnant women an infection, and associated HRP-2 antigenaemia, can persist asymptomatically for a prolonged period of time. Since pregnant women are being tested routinely, regardless of symptoms, ANC-based prevalence is likely to be a measure of exposure accumulated in preceding months [24, 25]. This is consistent with the findings of this analysis where high clinical incidence rates in under 5 s were associated with an increased risk of a positive RDT in pregnant women for the next 3 months, as well as a recent study demonstrating that in areas of sustained, seasonal transmission a substantial proportion of women attending ANC appointments remain infected throughout the dry season [26]. The models that assumed a non-linear relationship between clinical incidence in under 5 s and ANC prevalence were superior in terms of AIC value and out-of-sample predictive power. The best-fit function produces a curve whereby increasing clinical incidence in children under 5 is approximately linearly associated with larger effects upon ANC prevalence up until around 3 cases per child per year, where it begins to plateau. This shape has been observed in multiple cross-sectional surveys comparing malaria prevalence with clinical incidence [20]. This is likely a product of heterogeneity in mosquito biting (some people are bitten substantially more than others) leading to repeatedly infected people developing asymptomatic infections (so new infections occur in people already infected meaning that there is no change in prevalence).

Due to the changes in the model fit between sites (significantly different h parameter values), the model cannot currently be used to predict ANC prevalence from incidence alone. For example, the best fitting model systematically under-predicted the level of ANC prevalence in Walikale, which has similar rates of incidence in children under 5 s as seen in Shamwana but much lower ANC prevalence (Fig. 3). Some of the differences between sites may be accounted for if there was more precise ANC data on factors known to affect the epidemiology of malaria in pregnancy such as timing of gestation [27] and parity. The sensitivity of malaria RDTs are known to vary depending on the number of children that a woman has already had, with more children meaning a likely history

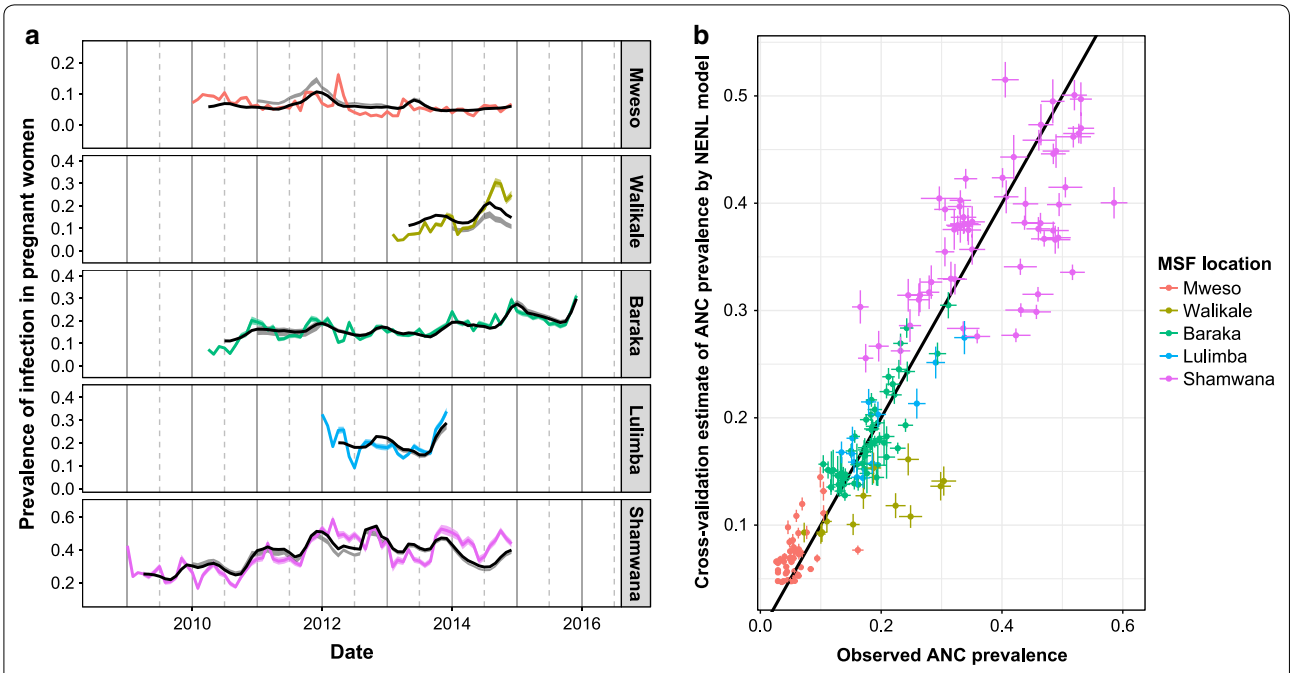

Fig. 6 The results of the out-of-sample prediction for the best fitting "NENL" model. This uses at least one previous year of data as a training dataset before trying to make out-of-sample predictions for the subsequent years. **a** The coloured lines show the observed ANC prevalence each month at each location and their corresponding 95% confidence interval. The black line shows the model predictions of the ANC prevalence when the model was fit using all data. The grey band shows a 95% confidence interval for the rolling origin cross validation technique. **b** Points show a comparison of observed ANC prevalence and the corresponding out-of-sample predictions, coloured by site. Lines around the points show the 95% confidence interval for the observations and out-of-sample prediction. The black line shows a perfect correspondence between observation and prediction

of exposure to the parasite during pregnancy and a developed placental immunity [28]. Alternatively, the variation between sites could be attributable to poor incidence estimates at some locations due to sparse health systems, insecurity, inaccurate estimates of population size, or short-term population movement into areas of higher risk (e.g. forested areas). Analysis of mobile phone data in malaria endemic countries shows large-scale population movement within and between countries [29, 30]. The infrequency of national census surveys may therefore limit the accuracy of incidence estimates derived from these surveys. However, census data was only used for two of the sites in the MSF dataset and the incidence recorded at those two sites (Baraka and Kimbi-Lulimba) was not unusual when compared to the other locations. To redress some of the uncertainty in the data, the NENL model was fit using several different maximum lag values (see Additional file 1), with the general results remaining the same for maximum lag values of 2 or 4. However there is still uncertainty in the data that the current model is unable to capture (Fig. 6). The analysis should be repeated as more data become available in order to reduce uncertainty in the model and refine predictions (Additional file 2).

These results have practical implications for the proposed use of ANC prevalence as a tool to monitor malaria. This method has established, at these 5 sites at least, that ANC prevalence seems to be a promising, simple, and cost-effective measure of recent malaria incidence. This has important applications in humanitarian settings and beyond. Good quality population size estimates are difficult, expensive to obtain, and are only available in a small number of sites where MSF operate. ANC data is much more widely available, and this work suggests it should be used to monitor recent trends in malaria endemicity over simple case count data alone. As an illustration of its importance it was unclear from hospital case counts data whether malaria transmission was increasing in sites in Eastern DRC around Baraka or not. Case counts had risen dramatically, though this may have been because of increased investment by MSF (for example the use of mobile malaria teams to diagnose and treat the wider population) or a true increase in disease transmission. The spectrum of mosquitoes resistant to pyrethroid insecticide and the possibility of the spread of drug resistant parasites means that local control interventions need to monitor secular trends in transmission regularly and tailor their programmes to maintain good levels of control. Examination of ANC data in these sites

during this period would have provided a simple, unbiased method of raising concerns over recent increases in transmission. This method also provides a way of singling out changes in incidence that should be matched by a corresponding change in ANC prevalence, but this does not happen. For example, a change in reporting capacity or surveillance may induce an increase in incidence, but this would then not be followed by an increase in ANC prevalence so those responsible for monitoring malaria can be confident that the increase in incidence was not due to increase in overall transmission.

Humanitarian organizations and other bodies are regularly trialling new methods of malaria control in specific areas to try and meet local needs. For example, MSF have used mobile malaria teams, community-based malaria management and different models of health centre support in different areas of the DRC. The evidence-base to support these interventions is lacking due to the huge expense and infeasibility of conducting large RCTs in some areas. The full effect of a sustained decrease in transmission due to an intervention may not be observable in ANC prevalence measurements until several months after it begins, therefore availability of routine ANC data from a strategy of IST alongside IPTp in area where the intervention is introduced, combined with the model outlined here, could provide a low-cost measure of triaging new interventions to see which should go on for more thorough investigation.

ANC prevalence was found not to be useful for predicting future incidence in children under 5 years old, so there is no evidence to support its use in predicting future malaria trends from this work. However, it may be that combining ANC prevalence with other data such as the amount of rainfall may allow for models with better predictive power, though this analysis is beyond the scope of this work. In the future, it would be beneficial to invert the relationship used in this work to use ANC prevalence to predict past trends in incidence, useful in many of humanitarian contexts discussed where cases or denominator populations cannot be reliably recorded.

Conclusions

This work found that time-series data of clinical incidence in children under 5 years predicts future prevalence of infection in pregnant women, but not the other way around. Increases in clinical incidence were associated with increased risk of a positive RDT in a pregnant woman for the next 3 months, with the opposite being true for decreases in incidence. This helps us to understand the role that ANC prevalence can play as a tool for retrospectively examining how malaria transmission has changed in a location over time. Though ANC prevalence derived from routinely collected clinical data may not directly reflect clinical incidence rates calculated from accurate population data, this analysis establishes that it does correspond to recent trends in malaria transmission and provides a simple to collect metric in situations where good malaria data is sparse, such as chaotic, rapidly changing humanitarian crises.

Additional files

Additional file 1: Figure S1. A table of the values of four information criteria for different lag orders, used to determine the lag order of the VAR model. **Figures S2**–**S4.** Copies of Fig. 4 whereby the NENL model is fitted to data using a lag order of 1, 2, or 4 months. **Figure S5.** A copy of Fig. 4 using the NELL model described in the analysis rather than the NENL model.

Additional file 2. This dataset contains monthly time series data for all 5 MSF locations, including ANC visits, ANC prevalence, and clinical incidence in children under 5 years old.

Abbreviations

ANC: ante natal care; AIC: Akaike information criterion; ACT: artemisinin-based combination therapy; SP: sulfadoxine-pyrimethamine; MSF: Médecins Sans Frontières; RDT: rapid diagnostic test; IST: intermittent screening and treatment; IDP: internally displaced persons; IPTp: intermittent preventative treatment during pregnancy; DRC: Democratic Republic of Congo; DLNM: distributed lag non-linear models; VAR: vector autoregressive model.

Authors' contributions

BR provided the data collected by MSF. JH, TC and BR conceived of the initial project. TC, BR, PW and AG provided feedback on early stages of the work. JH performed the statistical analysis and prepared the initial draft of the manuscript. All authors provided feedback and suggestions on the manuscript before submission. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its additional files.

Consent for publication

Not applicable.

Ethics approval and consent to participate

All data was collected by MSF and provided in an anonymized format aggregated by month.

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