UNIVERSITY OF OXFORD

NUFFIELD DEPARTMENT of MEDICINE

# Malaria transmission in Cambodia:

## Are plantations a hotspot?

**MSc in International Health and Tropical Medicine**

Trinity Term, August 2016

Candidate Number: 582470

Word count = 9,997
(excluding preface sections i. – viii., tables and figures, references, and appendix)

## i. Authorship Statement

All work detailed in this dissertation (including data cleaning, analysis and interpretation, mathematical model development, and photography) is the product of the author. Collection and provision of primary data used in the analysis was by the following organisations:

- Population Services International (PSI) provided the Cambodian plantation demographic profiles (from a plantation mapping survey conducted in 2013) and mobile malaria worker malaria incidence data. They also provided the data from a 2014 plantation worker malaria survey, officially titled "Developing the evidence base for assessing the feasibility of malaria elimination efforts among workers employed on private plantations in Cambodia." This was a research collaboration between PSI, the London School of Hygiene and Tropical Medicine (LSHTM), the Institut Pasteur du Cambodge, and Partners for Development, with funding provided by the Bill and Melinda Gates Foundation.
- The Cambodian National Center for Parasitology, Entomology and Malaria Control (CNM) provided the village level malaria incidence and population data.
- Cambodian rainfall data were provided by the Cambodian Ministry of Water Resources and Meteorology.

## ii. Research Ethics Approval

Ethics approval for the plantation malaria survey was obtained from the National Ethics Committee for Health Research of Cambodia in February 2014 in addition to PSI's internal institutional review board, and the LSHTM's institutional review board. Individual written consent was obtained from each participant in the survey. Participant blood tests and surveys were linked according to a unique identification number.

A waiver of additional ethics approval was granted for use of this data and all other analysis within this dissertation by the Oxford MSc in International Health and the Oxford Tropical Research Ethics Committee (OxTREC).

## iii. Acknowledgements

This project would not have been possible without the collaboration of the Mathematical and Economic Modelling group at the Mahidol-Oxford Tropical Medicine Research Unit (MORU) in Bangkok, Thailand, and PSI in Phnom Penh, Cambodia.

At MORU, Lisa White and Wirichada Pan-Ngum facilitated an essential 2-week introductory mathematical and economics modelling course. My project supervisor, Lisa White, provided invaluable support and guidance with the development of the malaria transmission model.

Richard Maude and Eric Ohuma supported the epidemiology and statistics component of the dissertation. Pengby Ngor obtained the rainfall, provincial malaria incidence, and village population data. Divaree Franssen afforded a seamless experience in Southeast Asia through her efficient logistical and administrative support.

Proochista Ariana also provided immeasurable support and advice throughout the project. My academic advisor, Laura Merson, kindly reviewed a final draft of this dissertation, providing valuable feedback.

I would also like to thank Abigail Pratt and Panharith Nou of PSK who provided on-the-ground support in Cambodia, including a tour of PSK MMW-supported plantations in Kratié province, and access to their data.

## iv. Abstract

**Background:** Malaria is a leading cause of morbidity in Cambodia with an estimated 55,000 cases in 2014. Malaria prevalence is highest in forested rural areas, where a large proportion of the population live and work. Plantations are hypothesised to be "hotspots" for malaria transmission due to their proximity to forests and the vectors therein.

**Methods:** An analysis of malaria incidence data collected on plantations and in surrounding local villages in north-eastern Cambodia, as well as epidemiological analysis of risk factors for malaria prevalence on plantations. A deterministic compartmental model was developed to simulate malaria transmission dynamics on plantations.

**Findings and conclusions:** Malaria incidence in both villages and plantations follows a seasonal pattern with a peak in cases toward the end of the wet season in October to November. There was no statistical difference in incidence between settings. Mondulkiri province has a second peak at the start of the wet season which may be attributed to the type of work conducted on the plantations at that time or population migration. Permanent non-vector permeable housing in the wet season (OR 0.30; 95% CI: 0.11–0.86, $p < 0.05$) and use of bed nets in the dry season (OR 0.15; 95% CI: 0.03–0.62, $p < 0.001$) were the only factors found to have a significantly reduced odds of malaria prevalence. The plantation malaria model demonstrates that importation of cases by migrant workers is currently not a factor in malaria transmission on plantations. Plantation workers with immunity and asymptomatic malaria act as a reservoir enhancing malaria transmission and need to be targeted in elimination efforts. The malaria model also shows that as the plantations approach elimination, the transmission pattern will shift from a stable endemic to unstable epidemic pattern due to declining immunity which is likely to result in more clinical and severe malaria.

## v. List of Abbreviations

| | |
|---|---|
| CI | Confidence interval |
| CNM | Cambodia National Center for Parasitology, Entomology and Malaria Control |
| ITN | Insecticide-treated bed nets |
| LLIN | Long-lasting insecticide treated nets |
| LSHTM | London School of Hygiene and Tropical Medicine |
| MDA | Mass drug administration |
| MMW | Mobile malaria worker |
| MORU | Mahidol-Oxford Tropical Medicine Research Unit |
| OR | Odds ratio |
| PCR | Polymerase chain reaction |
| PSI | Population Services International |
| PSK | Population Services Khmer (Cambodia) |
| RDT | Rapid diagnostic test |
| VMW | Village malaria worker |

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# 1. Introduction

In this dissertation I examine the hypothesis that plantations form "hotspots" for malaria transmission in rural areas of Cambodia. I compare the incidence of malaria on plantations to the surrounding villages to assess whether data supports this claim, and examine plantation-specific risk factors that may increase transmission.

To better understand why plantations may form "hotspots", it is necessary to examine the plantation life cycle, human dynamics, and interaction of the workers with the plantation ecosystem. I focused on the rubber plantation, which is a dominant agricultural industry in Cambodia and Southeast Asia. Through field visits to plantations in Kratié province with Population Services International/Khmer (PSI/PSK), and a literature review, I identified several factors which may be contributory to transmission of malaria on plantations. The migratory workforce, style of housing, and working environment changes depending on the stage of the plantation. The relationship between this plantation life cycle and the risk of malaria to the workers has not been fully described.

I use available data to construct a deterministic mathematical model to predict malaria transmission on plantations and the effects of migrant worker movement. There are currently no published mathematical models that describe how the plantation life cycle, with the migration of workers, affects the transmission of malaria on Cambodian plantations. A model that takes these factors into account would be helpful to simulate targeted delivery of novel as well as more intensive interventions to diminish the impact of plantations on malaria transmission. The results of this study can be used to propose measures which could potentially curb transmission. By investigating the detrimental effects that plantations have on the control of malaria, this research will aid malaria elimination programmes within countries of the Mekong region.

## 2. Background

Cambodia saw a dramatic decrease in *Plasmodium falciparum* malaria cases between 2004 and 2013.[1] The most striking decline in cases by 81% between 2009 to 2013 coincided with the scaling up of village malaria worker (VMW) and insecticide-treated bed net (ITN) programmes, as well as increasing deforestation.[1] However, malaria is still a leading cause of morbidity in Cambodia with an estimated 55,000 cases in 2014.[2] *P. falciparum* accounted for around 64% of these cases, with *P. vivax* accounting for the remainder.

Despite the marked decline in *P. falciparum* malaria incidence and mortality over the past decade, artemisinin resistance is on the rise. Since 2009, the World Health Organisation in partnership with the Cambodian and Thailand ministries of health have been working to contain drug resistance through malaria prevention, early diagnosis and treatment, and increased surveillance.[3] It has been demonstrated that local elimination of *P. falciparum* may be necessary in order to prevent artemisinin resistance,[4] and Cambodia has set itself the goal of eliminating malaria by 2025.[3] This ambitious target makes identifying the risk factors for local transmission in Cambodia all the more pertinent.[5]

### 2.1 Malaria transmission in Cambodia

Malaria prevalence is highest in rural forested regions (15–40%) compared to urban and unforested regions (0–3%).[6] This is significant as an estimated 77.5% of Cambodia's 15.2 million population live in rural areas, and 3.7 million people (45.3% of the employed populace) work in the agriculture industry in these areas.[7] Malaria is transmitted by species of *Anopheles* mosquito that breed in the forests which cover

48% of the land area in Cambodia.[8] Around 1.6 million people live and work in high transmission areas in close proximity to forests.[6]

## 2.2 Rubber plantations in Cambodia

Rubber trees, *Hevea brasiliensis*, were first introduced to Southeast Asia in the latter half of the 19th century and the first seedlings were planted in Cambodia in 1910.[9] The climactic conditions in the region—warm temperature and well-balanced rainfall—are ideal for rubber tree growth.[10] Mature trees are tapped for rubber latex, a resin which when vulcanised has high tensile strength and abrasion resistance and is used in the production of variety of products; from car tyres, to footwear, to toys. Once the yield of latex starts to decline, the trees are harvested as a cheap source of biofuel.[9]

Due to the high global demand and price of latex, combined with the ease of cultivation and maintenance, rubber farming has become one of the dominant agricultural industries in Cambodia and much of the Mekong region.[10] The plantations have become a major source of economic growth by providing jobs on state-owned and private-industrial plantations, as well as poverty alleviation for household or "small-holder" farms.[9] Due to the profitability of rubber farming, there has been significant crop and forest conversion to semi-permanent agroforestry.[10] Indeed, most rubber growing areas in Cambodia have been converted from forests.[9] A total of roughly 70,000 hectares of rubber plantations (2007) are mainly located in the central and north-eastern provinces bordering Vietnam and Laos as well as Battambang and Pailin provinces bordering Thailand (Figure 1).[9,11]

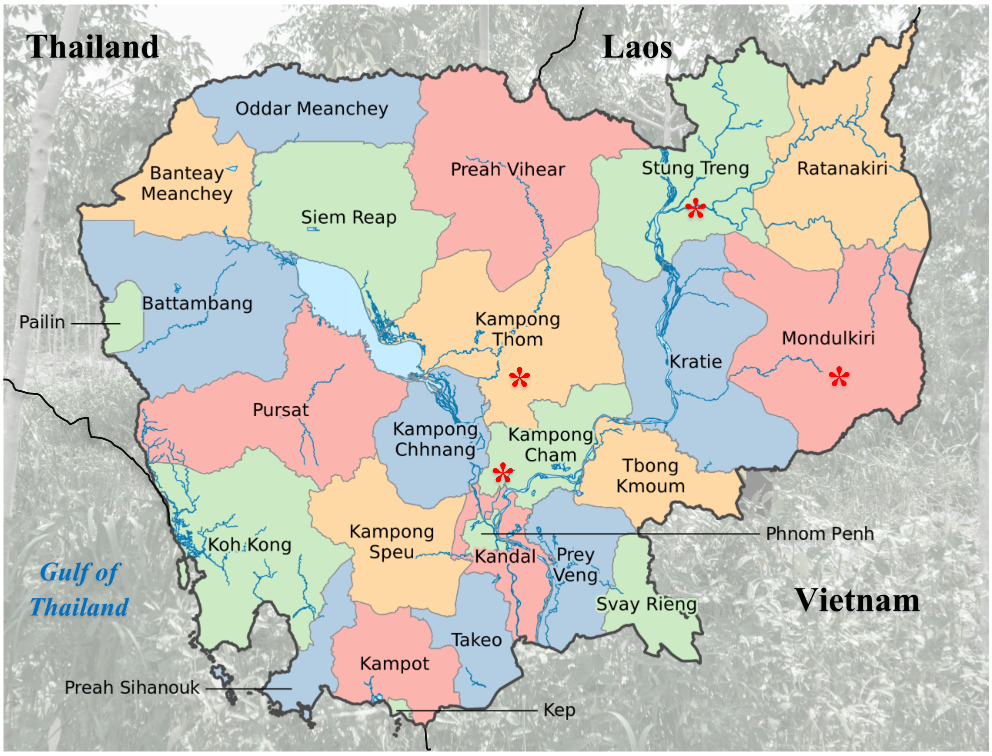



**Figure 1.** Provinces of Cambodia (Adapted[12]). Provinces with asterisks (*) were included in this study.

## 2.3 Rubber plantation life cycle

Rubber plantations in Cambodia go through a series of stages from forest clearing to tapping of the rubber tree resin or latex (Figure 2).[10] The first stage is the clearing of indigenous forest (Figure 2A–B), which takes place towards the end of the dry season or during the early wet season. Planting takes place predominantly in the wet season when there is sufficient rainfall to sustain newly planted seedlings (Figure 2C).[13] It takes between 5 to 10 years for rubber trees to reach full maturity, during which time the main year-round daytime activity on the plantation is tending to young saplings by clearing surrounding undergrowth, insecticide and herbicide spraying, and pruning (Figure 2D–E).[10] Once the trunks have reached a suitable circumference, they are ready for tapping of rubber latex.


**Figure 2A–H.** Rubber plantation life cycle: (A, B) indigenous forest, (C) clearing of forest and planting seedlings, (D) young rubber trees (less than 6 years old), (E) workers tending to young rubber trees by clearing weeds and stripping lower branches, (F), worker tapping mature rubber tree, (G) mature rubber trees with rubber latex collecting in pots , and (H) worker collecting pooled rubber latex.

Mature rubber trees are tapped year round every 2 to 3 days depending on their maturity and yield.[13] The tapping process starts early in the morning, often as early as 2am, as the coolness increases exudation of latex. Tapping rubber latex is accomplished by cutting the trunk in a spiral pattern, which allows the white resin to flow into small pots over the following hours (Figure 2F–G). The resin is then collected later in the morning or afternoon when it has stopped flowing (Figure 2H). The trees have a lifespan of approximately 25 to 30 years before latex production declines significantly and the trees need to be removed and new seedlings planted.[10]

### 2.4 Ecological dynamics on plantations

The plantation evolution affects the local ecology. Rubber plantations are often located within or near to forested areas, and plantation workers often hunt, fish, or travel through the forest.[14] As plantations expand in size, forests on the boundaries of the estates are cleared and turned into arable land. With this expansion the plantation epicentre and areas of human habitation move further away from the forested areas.

The proximity of plantations to forest remains a major risk factor for malaria: living and working close to these vector breeding sites increases the risk of transmission.[2,15-16] Plantations that are less than 1km away from forests have a higher prevalence of malaria.[14] Workers on smaller plantations are generally closer to the forest surrounding the plantation and at higher risk of infection.[14] The August to September peak in seasonal malaria transmission during the wet season coincides with the planting of crops on plantations. In 2014, an estimated 2.7 million households planted crops in the wet season in Cambodia, compared to 0.8 million in the dry season.[7]

The complexity of the vector–environment interactions, both in and outside of the forest, makes malaria transmission difficult to predict. Deforestation might result in lower densities of primary malaria vectors, but may conversely lead to higher densities of secondary vectors in deforested areas.[11] Even in extremely deforested areas malaria vectors persist, suggesting that even small fragments of forest are sufficient to allow mosquito survival during the dry season.[11] *Anopheles dirus,* a vector of malaria, has also been shown to adapt to deforested areas where natural forests are replaced with orchards, tea, coffee, and rubber plantations.[17] Higher accessibility of the remaining forest due to construction of roads may also result in increased man-mosquito contact.[11]

## 2.5 Human dynamics on plantations

The evolution of a rubber plantation is a dynamic process with different groves of trees at different stages of maturation, from newly planted seedlings and juvenile saplings, to mature trees ready for tapping. Older and larger plantations are likely to have more mature groves being harvested, while newly established and early plantations will have young saplings and juvenile groves. This evolution effects the human population dynamics on the plantation: older and more mature plantations are likely to have a larger workforce (Figure 3), predominantly consisting of permanent workers, with work focussed on tending to mature rubber tree groves and tapping rubber.

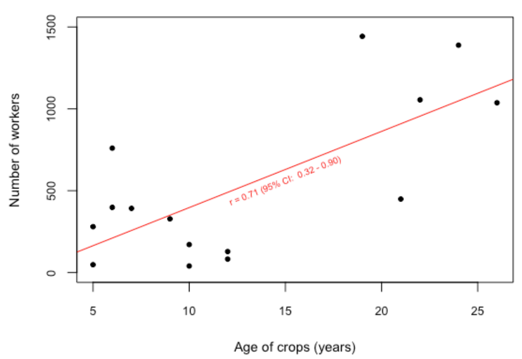



**Figure 3.** Correlation between plantation age and total numbers of workers on Cambodian rubber plantations in 2012. From plantation survey data provided by Population Services International (PSI).

Early plantations by contrast have a smaller workforce with a greater proportion of temporary or migrant workers. Work on these plantations focuses on clearing forests, planting of trees, and tending to immature saplings.[14] As the plantation grows, so too does the size of the workforce and population at risk of malaria.

## 2.6 Mobile migrant workers

Mobile migrant or seasonal workers account for 26.5% of the Cambodian population (3.5 million people) and are at a particularly high risk of malaria transmission.[14] They work for varying periods of time on the plantations, ranging from weeks to months. During the wet or rainy season (from June to early November), when malaria transmission is greatest, there is an influx of migrant or temporary workers on the plantations to help with the clearing of forests and planting of new groves.[19] These workers may come from areas with a lower prevalence of malaria and have little or no immunity to the parasite, placing them at higher risk of severe malaria.


**Figure 4A–B.** Mobile migrant workers on a rubber plantation in Cambodia.

In an addition to unsatisfactory housing ( Figure 4A–B), mobile migrant workers often do not have access to ITN or repellents, increasing their exposure to bites and malaria.[5] There are several obstacles to this group accessing preventive measures, diagnostic testing and treatment. These include poor availability of services, lack of knowledge of local providers, and cost barriers. If the workers do seek treatment, it is often of unknown origin and efficacy from informal providers.[14]

Uncontrolled cross-border population movements also affect malaria transmission, and may fuel the spread of artemisinin resistance. The mobile plantation workforce may act as a mobile reservoir. A study looking at a farming community on the Cambodia-Vietnam border found that 46.3% of the household heads regularly spent a night across the border in Vietnam for economic opportunities such as selling vegetables.[5] Another study looking at the behaviour of agriculture workers on a Cambodian plantation found that half the workforce travelled outside their commune in the past 6 months, and 34.7% travelled outside the commune in the prior month during the wet season.[14] Two percent of those who had travelled in the wet season were parasitaemic, highlighting the increased risk in the mobile migrant population. Due to their mobility, malaria plantation workers may be important drivers of anti-malarial drug resistance in the Mekong region. Mobile and migrant workers have been identified as a target population for malaria control as part of a long-term elimination strategy by the Cambodia National Centre for Parasitology Entomology and Malaria Control (CNM).[20]

## 2.7 Plantation housing

The type of planation housing varies depending on the cycle of the season and location of the house. An ethnographic study of a rural Cambodian Jarai community describes how families combine sleeping in their village homes, with sleeping at their homes at the forest farms or plantations.[5] The village houses are wooden-stilted longhouses (Figure 5A) inhabited by the nuclear or extended family mostly during the dry season, when work on the fields is completed, or during celebrations. During the rainy season, which is the most labour-intensive period, Jarai farmers will more often sleep at the plantations in stilted bamboo houses intended for the nuclear family (Figure 5B–C), or outdoors in hammocks (Figure 5D).[5]

House construction affects exposure to malaria vectors: houses built on stilts have a lower vector density than houses at ground level, reducing malaria transmission.[21] Poorly constructed houses with vector-permeable walls and floors, have a 30% greater risk for harbouring *Anopheles species* than houses built with permanent and less permeable materials such as wood or metal.[22,23] Migrant or temporary workers may be provided with poorly constructed barrack-style accommodation (Figure 5E) or live in makeshift tents with plastic walls (Figure 5F) which are permeable to *Anopheles* mosquitos. This places migrant workers at especial risk of malaria infection.


**Figure 5A–F.** Plantation housing: (A) stilted house with tin roof and non-vector-permeable wooden walls/floor, (B) stilted house with tin roof and vector-permeable bamboo walls/floor, (C) stilted house with permeable roof and walls/floor made of bamboo and leaves, (D) hammock, (E) barracks housing with tin roof and permeable walls made of leaves, and (F) makeshift tent with plastic roof and walls.

## 2.8 Malaria prevention

Several standard approaches to malaria control including ITN, indoor residual spraying, vector control, and mass drug administration (MDA) have been thoroughly studied and implemented in "static" populations. However, it is not known whether these measures are appropriate for mobile migrant plantation-worker populations. The migrant worker population may act as a mobile reservoir for malaria, making elimination difficult.

The primary malaria vectors in Cambodia, *Anopheles dirus* and *Anopheles minimus,* are exophagic (feeds outdoors) and exophilic (lives outdoors) early biters.[24-26] This limits the impact of ITN and indoor residual spraying, which only protect people when they are sleeping inside the houses.[27] Plantation workers who tap rubber in the night time or early hours of the morning are at particular risk of exposure and often do not use protective sprays or clothing.[14]

ITN and insecticide-treated hammocks may only provide partial protection against malaria, with one study showing that 71% of infectious bites occurred after 10pm, when people were expected to be sleeping under a net.[11] In another study, only a third of plantation workers self-reported that they regularly used ITN or insecticide-treated hammocks nets in the dry season, versus 55% in the wet season.[14] This may be due to a lack of  availability of affordable ITN, cost associated with dipping or replacing nets annually, or lack of knowledge of the efficacy of bed nets.


**Figure 6A–B.** (A) bed net, and (B) local primary health care centre in Kratié province of Cambodia.

## 2.9 Village and plantation mobile malaria workers

Since 2004, CNM has implemented a system of VMWs across areas of high malaria prevalence within Cambodia.[20] These community health workers provide malaria rapid diagnostic testing (RDT), treatment of uncomplicated malaria, and referral of severe malaria to hospitals, to people in rural areas who often do not have access to health care facilities. The marked decline in *P. falciparum* cases since 2009 has been attributed to the scaling-up of the VMW programme.[1]

**Figure 7A–D.** Mobile malaria workers: (A) setting up a malaria screening clinic in a rubber plantation and (B–D) performing rapid diagnostic tests and treating plantation workers and their families.

Since August 2013, PSI has begun rolling out a similar system of mobile malaria workers (MMWs) within plantation in 7 provinces: Kampong Cham, Kampong Thom, Mondulkiri, Stung Treng, Tboung Khmum, Kratié, and Rattanakiri. This programme now covers over 120 plantations, providing malaria education, RDT and treatment, as well as a package of other health services, to plantation workers and their families (Figure 7). PSI also distributes long-lasting insecticide treated nets (LLIN) and collects malaria incidence data via the MMWs. The efficacy of the plantation MMW programme has not previously been investigated.

# 3. Research Questions

## 3.1 Hypothesis

Plantations form “hotspots” of malaria transmission in Cambodia due to their proximity to forests and rudimentary housing, as well as worker migration, and the nature of the work. These factors place plantation workers at higher risk of contracting malaria due to increased exposure to malaria vectors. The plantation population is as reservoir for malaria, maintaining the seasonal transmission cycles in rural areas.

## 3.2 Aim

To explore the factors which effect malaria transmission on plantations and develop a mathematical model which will aid in reducing the detrimental impact of plantations on malaria transmission in Cambodia and the greater Mekong region.

## 3.3 Research objectives

**1)** Compare the pattern and magnitude of malaria cases among Cambodian plantation workers versus the surrounding populace: are plantations truly “hotspots” for transmission?

**2)** Characterise the relationship between the plantation life cycle and the risk of malaria to workers: what factors increase malaria transmission and hamper control on plantations?

**3)** A mathematical malaria transmission model to predict malaria incidence and prevalence based on plantation life cycle: how does population migration affect transmission? What interventions could be used to control transmission on plantations?

# 4. Methodology

This chapter presents the study population and design, and the methodology related to each of the three research objectives.

## 4.1 Study population

The study focussed on permanent and migrant (less than 6 months' residence in the study plantations) in 4 north-eastern provinces of Cambodia (Figure 1): Kampong Cham, Kampong Thom, Mondulkiri and Stung Treng.

## 4.2 Study design

Two different methodologies were used to answer the research objectives. Statistical techniques were used in the analysis and interpretation of surveillance and epidemiological data to answer the first two research objectives. The third research objective relied on mathematical modelling techniques. The data utilised in the analysis was from several sources summarised in Figure 8.

**Figure 8. Data sources.**

**Population Services International (PSI)**

- Cambodian plantation demographic profiles (plantation mapping survey, 2013)
- Mobile malaria worker malaria incidence data
- Data from a 2014 plantation worker malaria survey, officially titled "Developing the evidence base for assessing the feasibility of malaria elimination efforts among workers employed on private plantations in Cambodia." This was a research collaboration between PSI, LSHTM, Institut Pasteur du Cambodge, and Partners for Development, with funding provided by the Bill and Melinda Gates Foundation.

**The Cambodian National Center for Parasitology, Entomology and Malaria Control (CNM)**

- Village level malaria incidence and population data

**Cambodian Ministry of Water Resources and Meteorology:**

- Cambodian monthly provincial rainfall data

## 4.3 Research objective 1: Comparison of the pattern and magnitude of malaria cases among Cambodian plantation workers versus the surrounding populace

**Malaria incidence data analysis.** All data were imported into R (version 3.3.0 for Mac), cleaned and analysed. Binomial proportion confidence intervals were used for all population proportions and incidences. The pattern and magnitude of malaria incidence on plantations versus the surrounding village populations were compared in 4 of the provinces (Kampong Cham, Kampong Thom, Mondulkiri, and Stung Treng). From here onwards, malaria will only refer to *P. falciparum* malaria. Village population data from 2008 and VMW malaria RDT incidence data (tests conducted and results by month) from January 2010 to June 2015 were obtained from CNM for the 4 provinces. Plantation population data from 2013 and plantation MMW malaria RDT incidence data (tests conducted and results by plantation and month) from August 2013 to March 2016 were obtained from PSI for the 7 provinces in which it operates. Tboung Khmum, Kratié, and Rattanakiri provinces were excluded as there were no corresponding VMW data. Plantation and village population sizes for the 4 provinces included in this dissertation are summarised in Table 1.

Table 1. **Total village and plantation population size.**

| Province | Villages' population 2008 | Plantations' worker population (wet season survey 2013) | | | Plantations' worker population (dry season survey 2013) | | |
|---|---|---|---|---|---|---|---|
| | | Temporary | Permanent | Total | Temporary | Permanent | Total |
| **Kampong Cham** | 128,337 | 620 (36.6%) | 1,076 (63.4%) | 1,696 | 1,148 (49.9%) | 1,154 (50.1%) | 2,302 |
| **Kampong Thom** | 168,117 | 865 (25.0%) | 2,596 (75.0%) | 3,461 | 670 (29.5%) | 1,602 (70.5%) | 2,272 |
| **Mondulkiri** | 56,206 | 1,290 (56.7%) | 987 (43.3%) | 2,277 | 497 (39.3%) | 768 (60.7%) | 1,265 |
| **Stung Treng** | 81,998 | 623 (44.7%) | 772 (55.3%) | 1,395 | 628 (48.9%) | 655 (51.1%) | 1,283 |

Plantation worker population sizes in the wet and dry season were adjusted to include the family members living with the workers on the plantations. The numbers of accompanying family members, obtained from the plantation worker survey, varied by province (Table 2).

Table 2. **Accompanying family members living with each worker.**

| Province | Mean number of family members per worker | 95% CI |
|---|---|---|
| Kampong Cham | 2.07 | 1.97 – 2.17 |
| Kampong Thom | 1.43 | 1.34 – 1.52 |
| Mondulkiri | 0.98 | 0.89 – 1.08 |
| Stung Treng | 1.40 | 1.29 – 1.52 |

CI = confidence interval

Monthly incidence of malaria in villages and plantations was calculated by dividing the number of RDT positive cases by the respective village or plantation population. Plantation and village malaria RDT incidence data were aggregated by province, and also combined to create a monthly incidence for all 4 provinces. Kampong Cham and Kampong Thom province malaria incidence data were not analysed as the MMW programme was only gradually introduced to plantations in these provinces from December 2013 onwards.

**Rainfall data analysis.** The most recent monthly provincial rainfall data available were for the period from January 2000 to December 2009 from the Cambodian Ministry of Water Resources and Meteorology. No rainfall data were available from 2010 onwards; instead, average monthly rainfall was calculated from the prior 10-year period for each province. No rainfall data were available for Mondulkiri province, so an average of the monthly rainfall for the 3 surrounding provinces (Stung Treng, Rattanakiri and Kratié) was used as a proxy. Malaria incidence in plantations and surrounding villages was plotted graphically against average monthly rainfall for Mondulkiri, Stung Treng, as well as an average for the 4 provinces.

**Seasonality data analysis.** Seasonality was analysed by trend analysis (seasonal and irregular), using moving averages.

## 4.4 Research objective 2: Characterisation of the relationship between the plantation life cycle and the risk of malaria to workers

**Plantation worker malaria study**. Data from the plantation worker malaria study[14] (Figure 8) was utilised to answer the second research objective. This survey was conducted in 2014 to compare characteristics and malaria prevalence of temporary and permanent workers on 40 plantations in 4 provinces of Cambodia (Mondulkiri, Stung Treng, Kampong Cham, and Kampong Thom). Temporary workers were defined as people who had been working for a fee for less than 6 months in one or more plantations, whereas permanent workers were those who had only worked on a single plantation in the last 6 months. Family members of workers were also recruited to the study.

**Plantation selection.** The plantations included in the study were selected by a combination of purposive and convenience sampling. The plantations were all relatively removed from health care facilities, located near forested areas, and had managers who were willing to participate in the PSI MMW programme.[14]

**Sample size and participant recruitment.[14]** Data collection for this study was conducted at 2 time-points: during the dry season (June 2014) and wet season (October 2014). Required sample size for the dry season was 1,320 temporary and 1,320 permanent workers, with 33 of each type from every plantation. In addition, family members of temporary (n=7) and permanent (n=7) workers were sampled from each plantation. Preliminary data revealed that temporary or permanent worker status did not contribute to malaria prevalence, and so during the wet season, participant recruitment was based on plantation size: 40 participants (15 temporary, 17 permanent, and 8 family members) were to be sampled on small plantation (<7,500 hectares), and 48 participants (15 temporary, 33 permanent, and 8 family members) on large plantations (>7,500 hectares). The margin of error, confidence level, expected prevalence, and population size for calculating these required sample sizes are unfortunately not elucidated in the study protocol.[14] Stratified (temporary and permanent workers) random sampling was used to select workers from each plantation using an up to date employee list. It is not explained in the study protocol how randomisation was performed.

**Data collection.**[14] Four study teams of approximately 20 investigators, comprising healthcare workers from the Institut Pasteur du Cambodge, interviewers led by the LSHTM, and co-ordinators from PSI, spent around 1 month visiting plantations. A quantitative survey was administered to assess workers' demographic profile, living conditions, risk behaviour, knowledge of malaria, and treatment seeking behaviour. A blood sample was taken from all participants (including family members) for an on-site malaria RDT and for laboratory Polymerase chain reaction (PCR) testing by the Institut Pasteur du Cambodge in Phnom Penh within 2 weeks. Samples were tested for both *P. vivax* and *P. falciparum* using the protocol described by Canier et al.[28] This dissertation only reports the PCR seroprevalence results for *P. falciparum* (Table 5). Interview data were double entered using EpiData, each dataset was validated, and inconsistencies were checked against the questionnaire. Laboratory data were merged with the interview data in STATA (version 12).

**Malaria prevalence data analysis.** For this dissertation, STATA files were imported into R, cleaned and analysed. 2-sample tests for equality of proportions were performed to test the difference between wet and dry season provincial prevalence. The risk of malaria was compared using odds ratios (OR) for several demographic variables and risk factors stratified by season. Fisher's test was used for testing of associations.

A multivariable logistic regression analysis of binary (season, sex, migrant vs permanent, housing style, bed net use, and night work), categorical (education level), and continuous (age) independent variables was also conducted. The outcome variable was malaria PCR result. Due to small sample sizes, the malaria prevalence data in the wet and dry season data were combined to form a binary variable for season. The logistic regression analysis provides a value for the strength of the association between risk factors and malaria adjusting for potential confounding variables.

## 4.5 Research objective 3: A mathematical malaria transmission model to predict malaria incidence and prevalence based on plantation life cycle

The aim of the mathematical modelling component of the project was to predict seasonal *P. falciparum* malaria incidence patterns in plantations and examine the effect of population dynamics (migration) during the plantation life cycle. The model could be used to determine which interventions are most effective in the control of malaria transmission on plantations. To this end, a mechanistic, deterministic compartmental model composed of ordinary non-linear differential equations was developed to represent malaria transmission dynamics. The first step of the modelling process is the conceptualisation of the model, whereby observations are related to relevant biological systems and are translated into a mathematical form.

**Model structure.** The plantation population is divided into 8 compartments (Table 3) stratified by either partial immunity ($L_2$, $A_2$, $G_2$, R) or no immunity ($L_1$, $A_1$, $G_1$, S). An additional migrant worker compartment (M) was added to examine the effects of immigration of temporary workers from different malaria prevalence settings (low, medium, or high) on plantation malaria transmission dynamics. Migrant workers enter and leave the plantations during a single month each year, May and December respectively. A flowchart of the model structure is represented in Figure 9 and the ordinary differential equations which describe the system are in Figure 10.

Infections in susceptible non-immune individuals (S) start in a pre-erythrocytic liver stage ($L_1$) before merozoites are released and the individual enters the asexual blood stage ($A_1$). Individuals in this compartment are predominantly symptomatic, receive treatment and recover, entering the R compartment, which represent recovered individuals with prior exposure and partial immunity. Individuals retain partial immunity for a period of time which protects them from clinical or severe forms of malaria, but not infection itself. The immunity will be prolonged on further exposure to malaria. Those who are asymptomatic or fail to get treatment progress to the sexual gametocyte stage ($G_1$) where they may recover naturally over a longer period or be treated. Upon re-infection (with the same force of infection as individuals in the susceptible compartment), individuals in the R compartment progress through similar stages $L_2$, $A_2$, and $G_2$. However, individuals in groups $A_2$ and $G_2$ have partial immunity and are less likely to have clinical malaria, hence less likely to receive treatment. During the gametocyte stages ($G_1$ and $G_2$), the individuals are infectious to mosquitos, which impacts the future force of infection and transmission rate for humans. Individuals in the $G_2$ remain infectious to mosquitos for a longer period. A lag was introduced into the force of infection equation to account for the time taken for sporozoite maturation in the mosquito. Vectors were not included in the model.

The force of infection ($\lambda_t$) is similar to the one used by Silal et al.[29] and is calculated as $\lambda_t = \beta \text{ x } seasonality$, where:

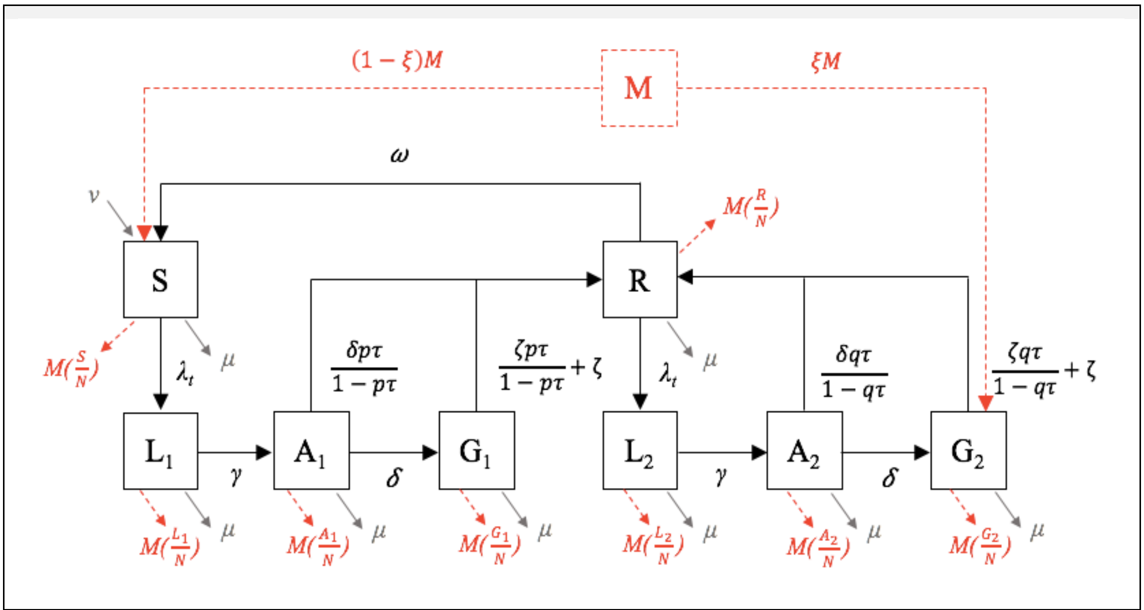
$$Seasonality = 1 + amp.\cos(\frac{2\pi(t-\phi)]}{52})$$

$$\text{Transmission rate } (\beta) = \left(1-\theta_{cov}.\theta_{eff}\right).\alpha.\left(\frac{G_1[t-lag]+G_2[t-lag]}{N}\right)$$

**Table 3.** Malaria transmission model compartments.

| | |
|---|---|
| **S** | Susceptible compartment (no immunity) |
| **$L_1$** | Liver stage compartment (no immunity) |
| **$A_1$** | Asexual blood stage compartment (no immunity) |
| **$G_1$** | Sexual (gametocyte) blood stage compartment (no immunity) |
| **R** | Recovered or treated compartment (partial immunity) |
| **$L_2$** | Liver stage compartment (partial immunity) |
| **$A_2$** | Asexual blood stage compartment (partial immunity) |
| **$G_2$** | Sexual (gametocyte) blood stage compartment (partial immunity) |
| **N** | Population size ($N = S + L_1 + A_1 + G_1 + R + L_2 + A_2 + G_2$) |
| **M** | Migrant workers |

**Figure 9.** Flowchart demonstrating the baseline structure of the compartmental malaria transmission model for plantations with time dependent variables and parameters (Table 4). Seasonal movement of migrant workers is in red.




The second step of the modelling process is to parameterise the model and quantify factors that are thought to be relevant. The model parameters were derived from the literature, malaria survey data, and estimated from the model fitting process (Table 4).

**Figure 10.** Ordinary differential equations describing the baseline malaria transmission model. Seasonal movement of migrant workers is in red.

$$\frac{dS}{dt} = \nu N + \omega R - (\mu + \lambda_t)S - \left(\frac{S}{N}\right)M_{t=12} + (1-\xi)M_{t=5}$$

$$\frac{dL_1}{dt} = \lambda_t S - (\mu + \gamma)L_1 - \left(\frac{L_1}{N}\right)M_{t=12}$$

$$\frac{dA_1}{dt} = \gamma L_1 - \left(\mu + \frac{\delta p\tau}{1-p\tau} + \delta\right)A_1 - \left(\frac{A_1}{N}\right)M_{t=12}$$

$$= \gamma L_1 - \left(\mu + \frac{\delta}{1-p\tau}\right)A_1 - \left(\frac{A_1}{N}\right)M_{t=12}$$

$$\frac{dG_1}{dt} = \delta A_1 - \left(\mu + \frac{\zeta p\tau}{1-p\tau} + \zeta\right)G_1 - \left(\frac{G_1}{N}\right)M_{t=12}$$

$$= \delta A_1 - \left(\mu + \frac{\zeta}{1-p\tau}\right)G_1 - \left(\frac{G_1}{N}\right)M_{t=12}$$

$$\frac{dR}{dt} = \left(\frac{\delta p\tau}{1-p\tau}\right)A_1 + \left(\frac{\zeta}{1-p\tau}\right)G_1 + \left(\frac{\delta q\tau}{1-q\tau}\right)A_2 + \left(\frac{\zeta}{1-q\tau}\right)G_2 - (\mu + \lambda_t + \omega)R - \left(\frac{R}{N}\right)M_{t=12}$$

$$\frac{dL_2}{dt} = \lambda_t R - (\mu + \gamma)L_2 - \left(\frac{L_2}{N}\right)M_{t=12}$$

$$\frac{dA_2}{dt} = \gamma L_2 - \left(\mu + \frac{\delta q\tau}{1-q\tau} + \delta\right)A_2 - \left(\frac{A_2}{N}\right)M_{t=12}$$

$$= \gamma L_2 - \left(\mu + \frac{\delta}{1-q\tau}\right)A_2 - \left(\frac{A_2}{N}\right)M_{t=12}$$

$$\frac{dG_2}{dt} = \delta A_2 - \left(\mu + \frac{\zeta q\tau}{1-q\tau} + \zeta\right)G_2 - \left(\frac{G_2}{N}\right)M_{t=12} + \xi M_{t=5}$$

$$= \delta A_2 - \left(\mu + \frac{\zeta}{1-q\tau}\right)G_2 - \left(\frac{G_2}{N}\right)M_{t=12} + \xi M_{t=5}$$

**Initial conditions.** The total worker population size is 10,000. A proportion of the population are migrant workers in the M compartment. This proportion is calculated as the mean of the wet and dry season temporary worker populations (Table 1). The remainder are in the permanent plantation worker compartments. A proportion of these workers ($\xi$) are in the $G_2$ compartment, with the remainder $(1-\xi)$ in the S compartment.

**Table 4.** Malaria transmission model parameters.

| Parameter | | Value | Source |
|---|---|---|---|
| **1/ν** | Birth rate | Life expectancy in Cambodia (ν) = 68.2 years | [30] |
| **1/μ** | Mortality rate | Mortality rate = Birth rate (1/ν = 1/μ) | [30] |
| **1/γ** | Rate of movement from liver to asexual blood stage (pre-patent period) | Period between liver stage and blood stage<br>γ = 11 days (range 7 – 28, mean and median 11) | [31] |
| **1/δ** | Rate of gametocyte maturation (time until appearance of gametocytes in the blood) | δ = 10 days (range 7 – 15) | [32-34] |
| **p** | Proportion of asexual blood stage patients who are clinical / symptomatic (no immunity)* | 0.93 (95% CI: 0.87 – 0.96)† in malaria patients with 3 or more days of fever.[31] | [31] |
| **q** | Proportion of asexual blood stage patients who are clinical / symptomatic (immunity) | 0.5 | Estimated from model fitting |
| **τ** | Proportion of clinical (symptomatic) infected people that are tested and receive treatment | 0.4[36]<br>A similar value was obtained by multiplying the proportion of people with fever tested by RDT (0.45)[37] by the sensitivity of the SD Bioline Ag-Pf/pan malaria RDT‡:88.1% (95% CI: 75.0 – 94.8)[38], 88.2% (95% CI, 83.2 – 92.0%)[39], 90.2% (95% CI: 87.4 – 92.6)[40] | [36] |
| **1/ζ** | Rate of natural recovery from gametocyte stage of malaria (untreated infection duration) | ζ = 120 days (range 0 – 319)<br>Other estimate in the literature: 286 days (range 59 – 714)[41] | [31] |
| **1/ω** | Rate of loss of partial immunity (barring re-exposure) | ω = 1 year (average duration of immunity)<br>(low transmission areas: 1 year, high transmission areas: 5 years, range 0.25 – 50 years) | [42, 43] |
| ***amp*** | Relative amplitude of seasonal forcing | 0.9 | Estimated from model fitting |
| $\phi$ | Peak month in seasonal forcing | 8 (August) | [6] |
| **α** | Daily number of mosquito bites per person | 0.79 | Estimated from model fitting |
| $\theta_{cov}$ | Proportion of population covered by vector control measures (ITN) | Dry season: 0.34 (95% CI: 0.33 – 0.36)<br>Wet season: 0.63 (95% CI: 0.60 – 0.65)<br>All seasons: 0.45 (95% CI: 0.43 – 0.46) | Derived from plantation survey data |
| $\theta_{eff}$ | Efficacy of vector control measures (ITN) | 0§ | Derived from plantation survey data |
| ***lag*** | Lag from mosquito biting infected human to time that mosquito can transmit malaria to humans | 14 days (duration of *P. falciparum* sporozoites development in mosquito) | [44, 45] |
| $\xi$ | Average dry season malaria prevalence of area of origin of migrant workers | Low transmission area = 0, medium transmission area = 0.05, high transmission area = 0.1 | |

* Symptomatic malaria refers to people who have fever for 3 or more days, which is the average amount of time before attending a healthcare professional.[35]

† Sporozoite- and blood- induced infection in patients with no previous history of infection and course of infection not modified with treatment.

‡ This assumes that all the people diagnosed with malaria complete an effective course of treatment.

§ No significant protection offered by ITN during peak transmission periods ($p > 0.1$) or for combined wet and dry seasons ($p > 0.05$), with significant effect only offered during the low transmission period in the dry season ($p < 0.001$).

**Model assumptions.** Modelling complex problems involves making assumptions and approximation in order to simplify the problems to a point where they can be analysed, and inferences can be drawn (i.e. to test the hypothesis about the association between migration and malaria incidence).

This model makes several assumptions in order to focus on the effects of plantation human dynamics, and the role of immunity in malaria transmission. There are many assumptions that are commonly used: the model assumes that the population is homogenous (i.e. every individual is identical), that the birth rate is equal to the mortality rate (the population is in a dynamic equilibrium), and that deaths from malaria do not substantially effect transmission dynamics and are hence not included. Although immigration and emigration occurs year round, the model has simplified population movement into bi-annual events.

The seasonal pattern of transmission related to mosquito population dynamics has been integrated into the model through a seasonality cosine function, however the mosquito population is not included in the model. The model assumes that the mosquito population is at equilibrium in respect to human population changes.[29]

The model also assumes that every mosquito bite from infectious mosquitos results in malaria infection and that every infected individual eventually receive treatment or recovers naturally. The model also makes several assumptions about immunity which are discussed in the following section.

**Immunity.** The mechanism of protective immunity to malaria in humans is complicated and not completely understood. Adaptive immunity to malaria results in lower levels of circulating parasites, lower morbidity (less symptomatic disease) and less severe disease (lower mortality).[46] It is thought that repeated malaria infections (exposure) is needed to maintain protective immunity (similar to the concept of vaccine boosting), however the immunity is short-lived.[42]

The dissertation hypothesises that plantations in Cambodia are “hotspots” for transmission, implying that immunity is frequent. In this model, partial immunity develops in everyone after only a single infection with malaria, is short-lasting (1 year), there is no super-infection nor cumulative immunity (i.e. multiple bites from infected mosquitos do not boost immunity), and immunity does not protect individuals from infection with malaria.[43] The model assumes that the partial immunity rendered through exposure to *P. falciparum* does not have transmission blocking effect, does not reduce the duration of the liver stage, and has no effect on gametocytogenesis.[43] However, partial immunity does protect the individual from clinical or severe malaria with the repercussion that re-infection may be asymptomatic and may go untreated. These partially immune patients with chronic malaria infection remain infective to mosquitoes for longer period of time. This asymptomatic carriage and transmission is explored.

**Model computation and analysis.** The mathematical model and parameters were translated into R. A delayed differential equation function was used to solve equations where the derivative depends on past values of the state variables. The model output equations (prevalence, reported incidence, and the proportion of cases with immunity) are summarised in Figure 11. Reported incidence refers to the number of people with symptomatic malaria who sought treatment per 1000 population. The monthly incidence is multiplied by 0.25 as only a quarter of people sought treatment from providers on plantations (95% CI: 0.23–0.29) according to the plantation worker survey. The remainder preferred to self-treat, use alternative medicine, or accessed public and private health services.

**Figure 11.** Equations describing the model output variables.

**Prevalence** $= \frac{\text{population with blood stage infection}}{\text{total population}}$

$= \frac{(A_1+G_1+A_2+G_2)}{N}$

**Reported incidence** $= \text{sum of all symptomatic patients who seek treatment}$

$= \left(\frac{\delta p\tau}{1-p\tau}\right) A_1 + \zeta G_1 + \left(\frac{\delta q\tau}{1-q\tau}\right) A_2$

**Proportion of cases with immunity** $= \frac{\text{blood stage cases with no immunity}}{\text{blood stage cases with partial immunity}}$

$= \frac{A_1+G_1}{A_2+G_2}$

The R code for the plantation model has been included as an appendix insert and is also available online at github.com/Simonidies/plantation_model/archive/master.zip.

**Model calibration.** Bifurcation diagrams, with seasonality omitted, were used to check the malaria model structure for biological plausibility. Incidence, prevalence, and proportion of cases with immunity in a stable equilibrium after 10 years were plotted against varying α (number of mosquito bites per day) and τ (proportion of symptomatic infected people that are tested and treated).

**Data fitting and validation.** Model fitting involves checking how representative the model output is of the raw data. The model was fitted against the monthly MMW plantation malaria incidence data, and wet and dry season seroprevalence survey data from Mondulkiri and Stung Treng provinces. The relative amplitude of seasonal forcing, daily number of infective mosquito bites per person, and proportion of asexual blood stage patients with partial immunity who are symptomatic were derived from the fitting process. No interventions were included in the model fitting process. The model was run from for 3 years to reach a steady state before being fitted to plantation level data from August 2013 to March 2016. Ideally the model should be validated against later plantation incidence and prevalence data, but only 32 months of data were available for this dissertation.

**Interventions.** The impact of scaling up of MMW programmes (increasing access to and uptake of malaria testing and treatment) on plantation malaria transmission was modelled. The years to malaria elimination was compared for a range of $\tau$ values (proportion of symptomatic infected people that are tested and treated). In addition, the efficacy of a PCR diagnosis and treatment campaign was explored. A threshold incidence and prevalence value approaching zero was used to define elimination as it is mathematically impossible to achieve absolute zero cases in a deterministic model.

# 5. Results

This chapter will present the results related to each of the three research objectives.

## 5.1 Research objective 1: Comparison of the pattern and magnitude of malaria cases among Cambodian plantation workers versus the surrounding populace

**Malaria incidence.** The pattern and magnitude of malaria cases among Cambodian plantation workers and the surrounding village populations are plotted against the average monthly rainfall for Mondulkiri (Figure 12) and Stung Treng (Figure 13) provinces. A combined inter-provincial incidence per 1000 population is also plotted for all the provinces (Kampong Thom, Kampong Cham, Mondulkiri, and Stung Treng) in Figure 14.

A low incidence of malaria is observed in villages and plantations for all provinces. The overlapping confidence intervals indicate that there is no significant difference in incidence between settings. The confidence intervals for the plantation incidence is much wider than that of the village incidence. Both villages and plantations have a seasonal peak in incidence toward the end of the wet season around October – November. In Mondulkiri province (Figure 12), there appears to be an additional or dual peak of malaria cases at the start of the wet season in June.

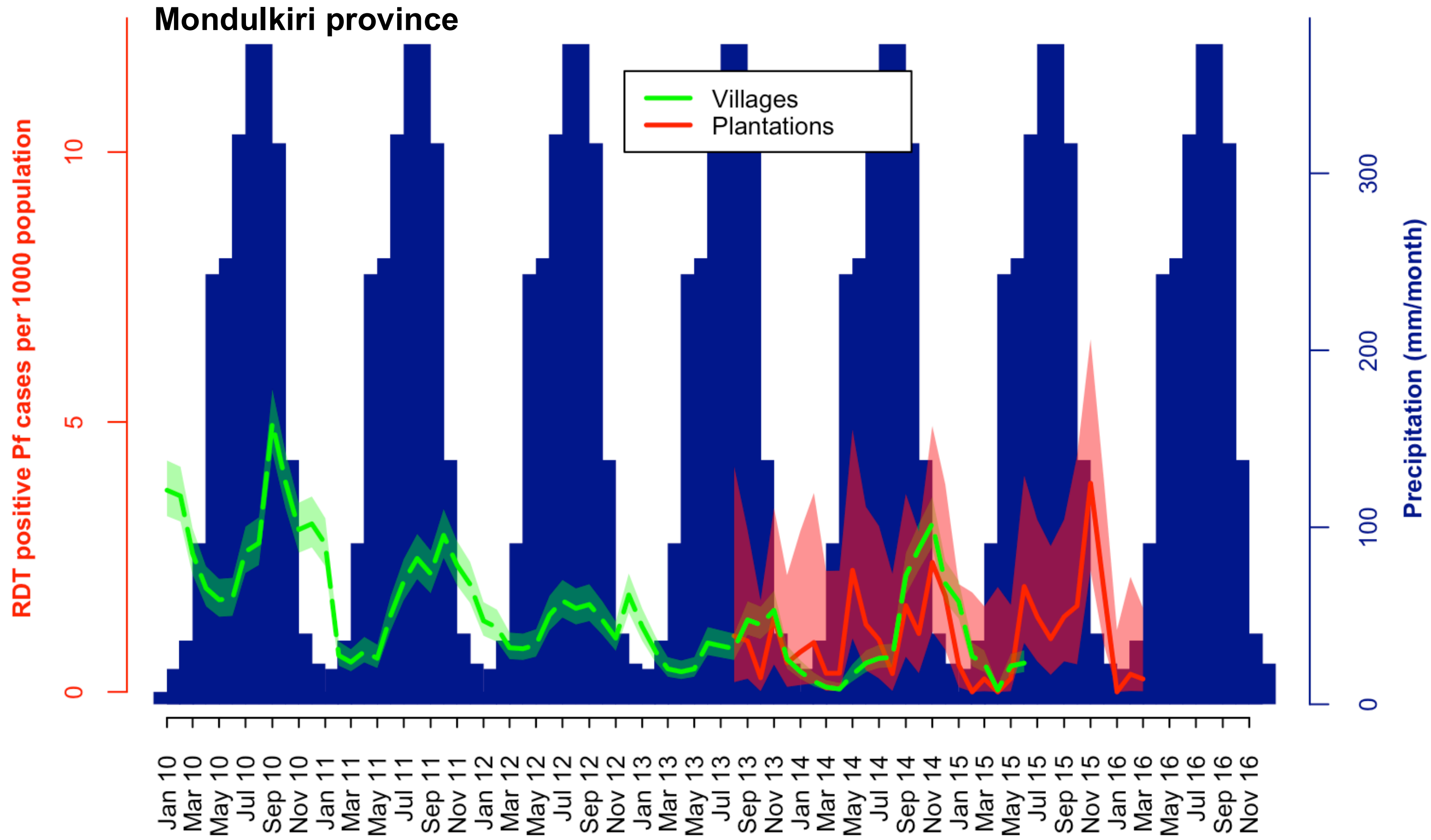



**Figure 12.** Seasonal trend and incidence of malaria on plantations versus surrounding villages in **Mondulkiri province**. The graph depicts the incidence of malaria from RDTs (left axis) performed in plantations by MMWs (August 2013 to March 2016) and surrounding villages by VMWs (January 2010 to June 2015) against the average monthly rainfall over a 10 year period from 2000-2009 (right axis). The green and red shaded areas represent the 95% confidence intervals for the VMW and plantation MMW incidence data respectively.

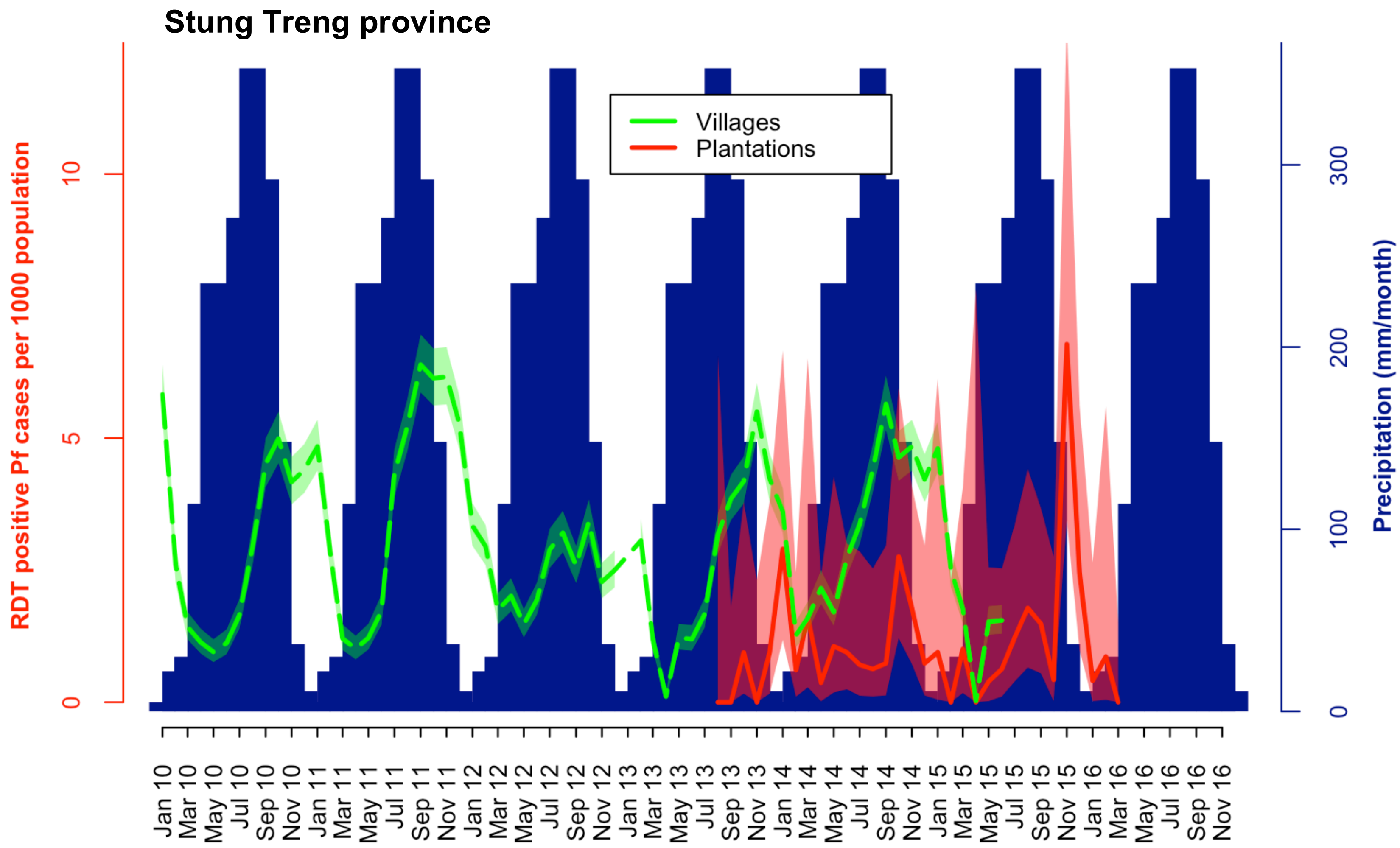



**Figure 13.** Seasonal trend and incidence of malaria on plantations versus surrounding villages in **Stung Treng province**. The graph depicts the incidence of malaria from RDTs (left axis) performed in plantations by MMWs (August 2013 to March 2016) and surrounding villages by VMWs (January 2010 to June 2015) against the average monthly rainfall over a 10 year period from 2000-2009 (right axis). The green and red shaded areas represent the 95% confidence intervals for the VMW and plantation MMW incidence data respectively.

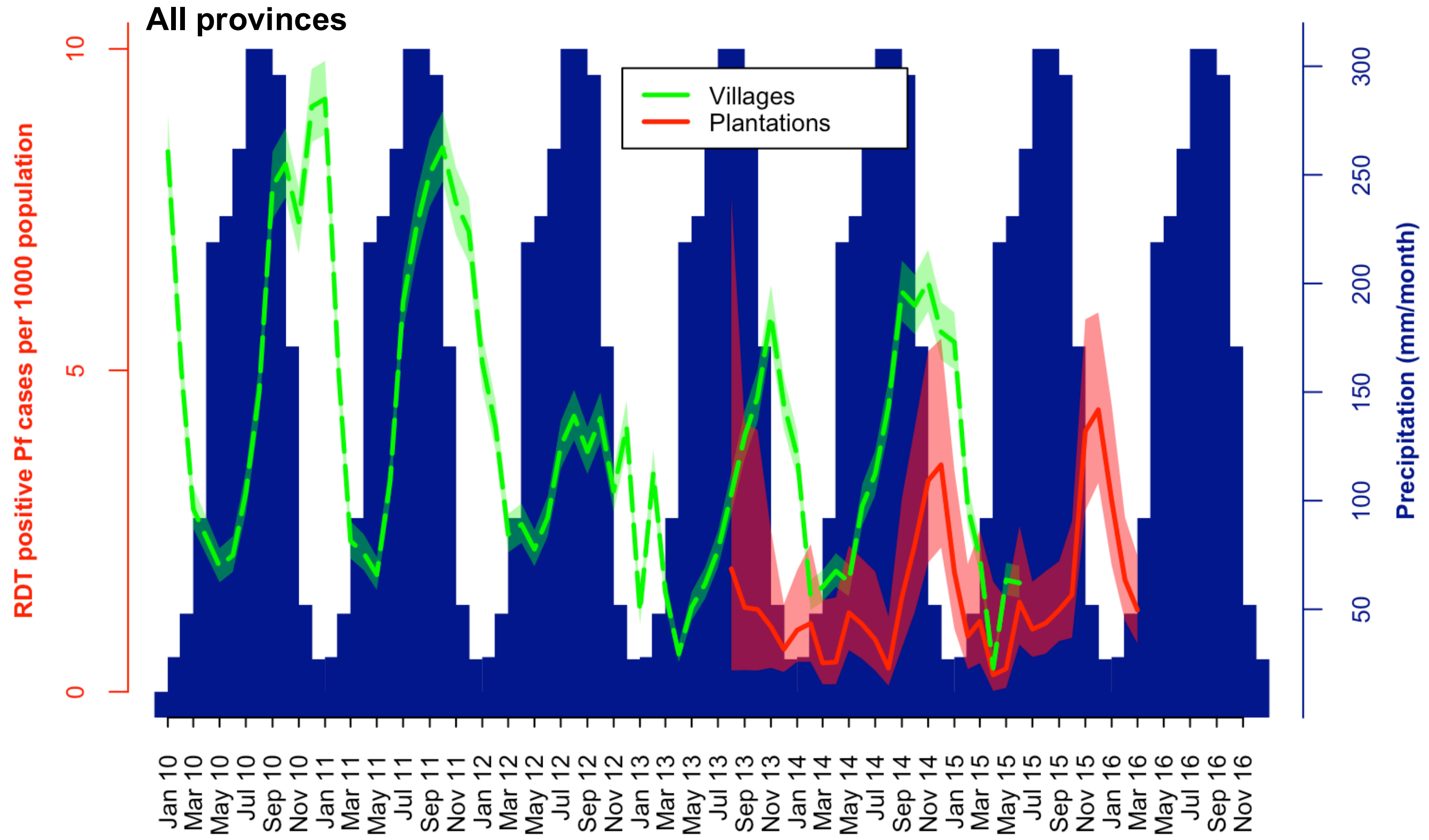



**Figure 14.** Seasonal trend and incidence of malaria on plantations versus surrounding villages in **all provinces** with sufficient available data (Kampong Thom, Kampong Cham, Mondulkiri and Stung Treng). The graph depicts the incidence of malaria from RDTs (left axis) performed in plantations by MMWs (August 2013 to March 2016) and surrounding villages by VMWs (January 2010 to June 2015) against the average monthly rainfall over a 10 year period from 2000-2009 (right axis). The green and red shaded areas represent the 95% confidence intervals for the VMW and plantation MMW incidence data respectively.

**Malaria seasonality.** To further explore the seasonality of malaria incidence on plantations versus the surrounding villages, the data were decomposed into seasonal, trend and irregular components using moving averages (Figure 15). This helps to tease out additive or multiplicative seasonal components, and remove noise.

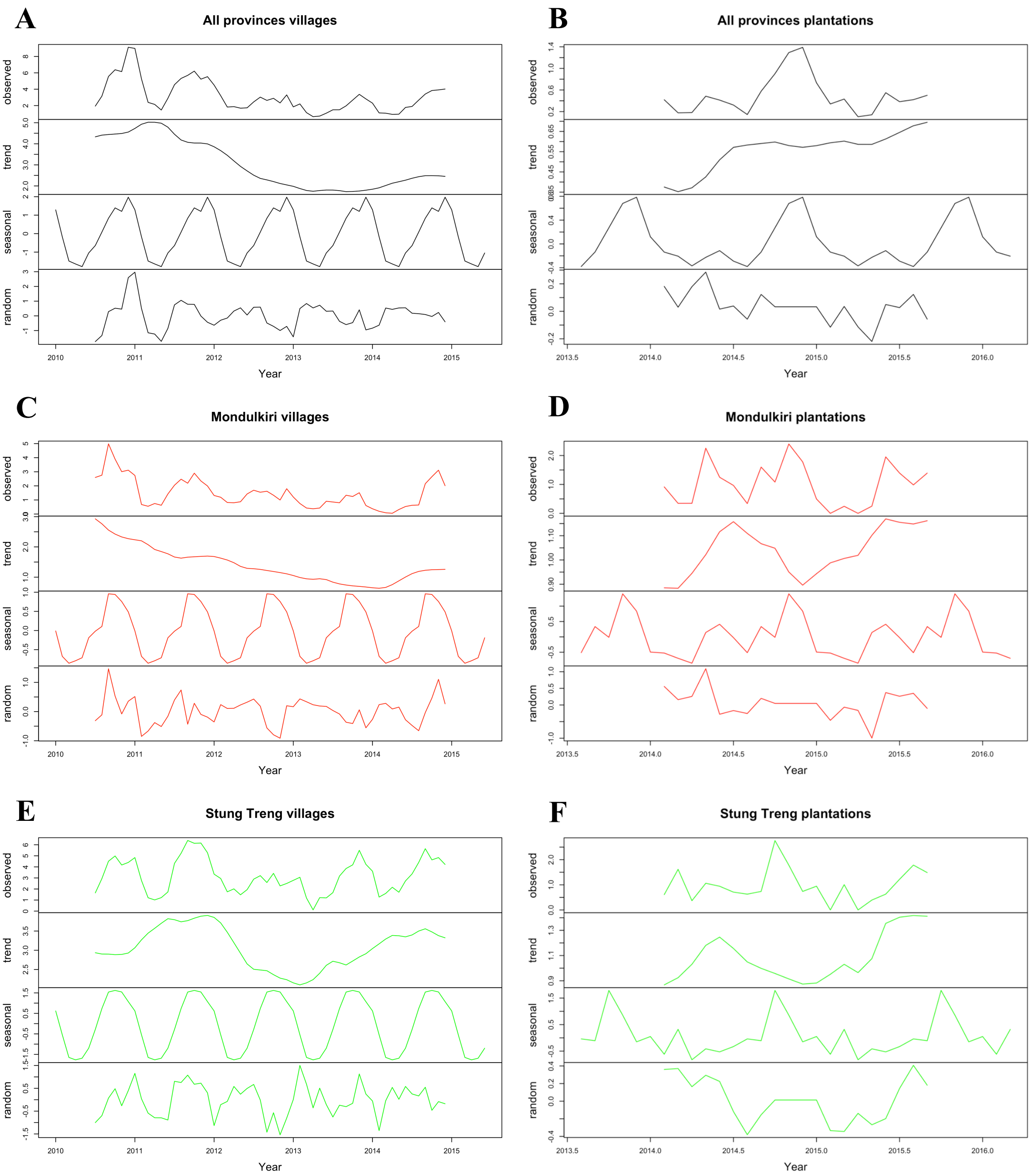



**Figure 15A–F.** Decomposition of the **observed** incidence into **seasonal**, **trend**, and irregular (**random** error or noise) components. A–B exhibit incidence in all provinces with available data (Mondulkiri, Stung Treng, Kampong Cham and Kampong Thom), C–D Mondulkiri province, and E–F Stung Treng province. Figures on the left (A, C, E) are for malaria RDT results from VMWs, and figures on the right (B, D, F) are for malaria RDT results from plantation MMWs.

The decomposed data confirms the seasonal peak in October – November in villages and plantations in all provinces, and also shows a possible dual peak in Mondulkiri province. The moving average trend component indicates that there is a downward trend in cases in Mondulkiri, but not in Stung Treng villages. The trend is also present in the combined provinces village plot. There is insufficient data to comment on the trend on plantations.

## 5.2 Research objective 2: Characterisation of the relationship between the plantation life cycle and the risk of malaria to workers

**Malaria prevalence on plantations.** Provincial wet and dry season malaria prevalence from the plantation malaria seroprevalence survey is summarised in Table 5. Baseline characteristics of the population under study are summarised in Table 6. Prevalence of malaria is low in all provinces (<3%) in both seasons. Mondulkiri is the only province with a significant difference in malaria prevalence between seasons (absolute difference 2.84, 95% CI: 1.18–4.49, $p$ <0.001). It is likely that the high $p$ values are due to insufficient sample sizes (low power) to accurately represent the very small difference between wet and dry season prevalence signals.

Table 5. **Plantation *P. falciparum* malaria seroprevalence survey by province.**

| | Wet season survey | | Dry season survey | | Wet versus dry season | |
|---|---|---|---|---|---|---|
| **Province** | ***Pf* positive PCRs (N)** | **% Prevalence (95% CI)** | ***Pf* positive PCRs (N)** | **% Prevalence (95% CI)** | **Difference (95% CI)** | ***P* value** |
| Kampong Cham | 3 (537) | 0.56 (0.19 – 1.63) | 15 (1060) | 1.42 (0.86 – 2.32) | -0.86 (-1.81 – 0.09) | 0.126 |
| Kampong Thom | 3 (510) | 0.59 (0.20 – 1.72) | 9 (952) | 0.95 (0.50 – 1.79) | -0.36 (-1.26 – 0.55) | 0.471 |
| Mondulkiri | 11 (388) | 2.84 (1.59 – 5.00) | 0 (570) | 0.00 NA | **2.84 (1.18 – 4.49)** | **<0.001** |
| Stung Treng | 6 (358) | 1.68 (0.77 – 3.61) | 3 (561) | 0.53 (0.18 – 1.56) | 1.15 (-0.32 – 2.6) | 0.087 |
| All Provinces | 23 (1793) | 1.28 (0.86 – 1.92) | 27 (3143) | 0.86 (0.59 – 1.25) | 0.42 (-0.19 – 1.04) | 0.153 |

*Pf* = *P falciparum,* PCR = polymerase chain reaction test, N = total number of tests performed, CI = confidence interval

**Malaria risk factors.** A univariate analysis of risk factors associated with *P. falciparum* malaria on Cambodian plantations, stratified by season, is presented in a contingency Table 7. The prevalence of malaria for each factor and OR for each variable is summarised.

**Table 6.** Baseline characteristics of plantation worker malaria survey stratified by season in 2013 for all provinces.

| Variable | Dry season N (%) | Wet season N (%) |
|---|---|---|
| **Sex** | | |
| Female | 1171 (44.0) | 597 (39.0) |
| Male | 1493 (56.0) | 938 (61.1) |
| **Age group** | | |
| 15 – 29 | 1310 (49.2) | 757 (49.4) |
| 30 – 44 | 930 (34.9) | 519 (33.8) |
| 45 + | 424 (15.9) | 258 (16.8) |
| **Education** | | |
| No education | 489 (18.4) | 285 (18.6) |
| Primary only | 1542 (57.9) | 910 (59.3) |
| Secondary or higher | 632 (23.7) | 340 (22.2) |
| **Worker or family** | | |
| Worker | 2699 (85.9) | 1536 (85.7) |
| Family member of worker | 444 (14.1) | 257 (14.3) |
| **Migrant** | | |
| No (≥6 months on plantation) | 1955 (63.0) | 1285 (71.8) |
| Yes (<6 months on plantation) | 1148 (37.0) | 506 (28.3) |
| **ITN vs no ITN** | | |
| No (Did not use ITN previous night) | 1748 (65.6) | 574 (37.4) |
| Yes (Used ITN previous night) | 915 (34.4) | 961 (62.6) |
| **ITN vs no net** | | |
| No (Did not use a bed- or hammock-net previous night) | 204 (18.2) | 59 (5.8) |
| Yes (Used ITN previous night) | 915 (81.8) | 961 (94.2) |
| **Hammock- or bed- net** | | |
| No (Did not use a bed- or hammock-net previous night) | 204 (7.7) | 59 (3.8) |
| Yes (Used a bed- or hammock-net previous night, not indicated if insecticide or non-insecticide treated net) | 2459 (92.3) | 1476 (96.2) |
| **Bed net** | | |
| No (Did not use bed net previous night) | 344 (12.9) | 125 (8.1) |
| Yes (Used a bed net previous night, not indicated if insecticide or non-insecticide treated net) | 2319 (87.1) | 1410 (91.9) |
| **Housing** | | |
| Tent or temporary shelter | 262 (8.4) | 210 (11.7) |
| Permanent house or barracks or house | 2842 (91.6) | 1581 (88.3) |
| **House roof** | | |
| Metal, wood, tile, or cement | 2688 (91.6) | 1513 (89.7) |
| Plastic, leaves, or bamboo | 245 (8.4) | 174 (10.3) |
| **House walls** | | |
| Metal, wood, or brick | 2536 (86.5) | 1434 (85.0) |
| Plastic, leaves, or bamboo | 397 (13.5) | 253 (15.0) |
| **Works at night from 9pm to 5am** | | |
| No | 3012 (97.8) | 1721 (96.1) |
| Yes | 68 (2.2) | 70 (3.9) |
| **Type of work at night** (Night not defined) | | |
| No work at night | 1810 (69.0) | 1180 (76.9) |
| Clearing, planting, or tending (young plantation) | 10 (0.4) | 9 (0.6) |
| Tapping, harvesting, or cutting (mature plantation) | 685 (26.1) | 292 (19.0) |
| Sedentary: Tractor, security, supervisor, or office work | 119 (4.5) | 53 (3.5) |
| **Work during the day** (Day not defined) | | |
| Clearing, planting, or tending (young plantation) | 1406 (53.3) | 952 (62.4) |
| Tapping, harvesting, or cutting (mature plantation) | 815 (30.9) | 393 (25.8) |
| Sedentary: Tractor, security, supervisor, or office work | 418 (15.8) | 180 (11.8) |
| **Tapping rubber** | | |
| During the day | 815 (54.3) | 393 (57.4) |
| At night | 685 (45.7) | 292 (42.6) |

**Table 7.** Univariate analysis of risk factors associated with *P. falciparum* malaria on Cambodian plantations stratified by season.

| | | Dry season | | | Wet season | | |
|---|---|---|---|---|---|---|---|
| Variable | Factors | *Pf* PCR Positive (%; 95% CI) | OR (95% CI) | *p* | *Pf* PCR Positive (%; 95% CI) | OR (95% CI) | *p* |
| **Sex** | Female | 8 (0.68; 0.32 – 1.40) | Reference | | 8 (1.34; 0.62 – 2.73) | Reference | |
| | Male | 17 (1.14; 0.69 – 1.86) | 1.67 (0.68 – 4.50) | 0.226 | 13 (1.39; 0.77 – 2.42) | 1.03 (0.39 – 2.90) | 0.940 |
| **Age group** | 15 – 29 | 17 (1.30; 0.78 – 2.12) | Reference | | 12 (1.59; 0.86 – 2.83) | Reference | |
| | 30 – 44 | 3 (0.32; 0.08 – 1.02) | 0.25 (0.05 – 0.85) | 0.016 | 5 (0.96; 0.36 – 2.37) | 0.60 (0.17 – 1.86) | 0.341 |
| | 45 + | 5 (1.18; 0.43 – 2.89) | 0.91 (0.26 – 2.58) | 0.850 | 4 (1.55; 0.50 – 4.19) | 0.98 (0.23 – 3.26) | 0.969 |
| **Education** | None | 2 (0.41; 0.07 – 1.64) | Reference | | 4 (1.40; 0.45 – 3.80) | Reference | |
| | Primary only | 12 (0.78; 0.42 – 1.40) | 1.91 (0.42 – 17.62) | 0.390 | 12 (1.32; 0.72 – 2.36) | 0.94 (0.28 – 4.03) | 0.913 |
| | Secondary or higher | 11 (1.74; 0.92 – 3.19) | 4.31 (0.93 – 40.19) | **0.039*** | 5 (1.47; 0.54 – 3.60) | 1.05 (0.22 – 5.34) | 0.944 |
| **Worker or family** | Worker | 25 (0.93; 0.61 – 1.39) | Reference | | 21 (1.37; 0.87 – 2.12) | Reference | |
| | Family member of worker | 2 (0.45; 0.08 – 1.80) | 0.48 (0.06 – 1.95) | 0.314 | 2 (0.78; 0.13 – 3.09) | 0.57 (0.06 – 2.34) | 0.437 |
| **Migrant** | No (≥6 months on plantation) | 19 (0.97; 0.60 – 1.54) | Reference | | 16 (1.25; 0.74 – 2.06) | Reference | |
| | Yes (<6 months on plantation) | 8 (0.70; 0.32 – 1.43) | 0.72 (0.27 – 1.72) | 0.426 | 7 (1.38; 0.61 – 2.96) | 1.11 (0.38 – 2.88) | 0.815 |
| **ITN vs no ITN** | No (Did not use ITN last night) | 21 (1.20; 0.76 – 1.86) | Reference | | 8 (1.39; 0.65 – 2.84) | Reference | |
| | Yes (Used ITN last night) | 4 (0.44; 0.14 – 1.20) | 0.36 (0.09 – 1.07) | 0.052 | 13 (1.35; 0.75 – 2.37) | 0.97 (0.37 – 2.72) | 0.947 |
| **ITN vs no net** | No (Did not use a bed- or hammock-net last night) | 6 (2.94; 1.20 – 6.59) | Reference | | 0 (0; NA) | Insufficient cases | |
| | Yes (Used ITN last night) | 4 (0.44; 0.14 – 1.20) | **0.15 (0.03 – 0.62)** | **<0.001*** ** | 13 (1.35; 0.75 – 2.37) | | |
| **Hammock or bed net** | No (Did not use a bed- or hammock-net last night) | 6 (2.94; 1.20 – 6.59) | Reference | | 0 (0; NA) | Insufficient cases | |
| | Yes (Used a bed- or hammock-net last night, not indicated if insecticide or non-insecticide treated) | 19 (0.77; 0.48 – 1.23) | **0.26 (0.10 – 0.80)** | **0.002**** | 21 (1.42; 0.91 – 2.21) | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Bed net** | No (Did not use a bed net last night) | 6 (1.74; 0.71 – 3.95) | Reference | | 2 (1.6; 0.28 – 6.24) | Reference | |
| | Yes (Used a bed net last night, not indicated if insecticide or non-insecticide treated net) | 19 (0.82; 0.51 – 1.30) | 0.47 (0.18 – 1.43) | 0.097 | 19 (1.35; 0.84 – 2.14) | 0.84 (0.20 – 7.52) | 0.816 |
| **Housing** | Tent or temporary shelter | 1 (0.38; 0.02 – 2.44) | Reference | | 7 (3.33; 1.47 – 7.03) | Reference | |
| | Permanent house or barracks or house | 26 (0.91; 0.61 – 1.36) | 2.41 (0.39 – 99.14) | 0.374 | 16 (1.01; 0.60 – 1.68) | **0.30 (0.11 – 0.86)** | **0.005**** |
| **House roof** | Metal, wood, tile, or cement | 26 (0.97; 0.65 – 1.44) | Reference | | 15 (0.99; 0.58 – 1.67) | Reference | |
| | Plastic, leaves, or bamboo | 1 (0.41; 0.02 – 2.61) | 0.42 (0.01 – 2.58) | 0.380 | 6 (3.45; 1.41 – 7.70) | **3.56 (1.12 – 9.88)** | **0.006**** |
| **House walls** | Metal, wood, or brick | 25 (0.99; 0.65 – 1.47) | Reference | | 14 (0.98; 0.56 – 1.68) | Reference | |
| | Plastic, leaves, or bamboo | 2 (0.50; 0.09 – 2.01) | 0.51 (0.06 – 2.05) | 0.350 | 7 (2.77; 1.22 – 5.86) | **2.88 (0.97 – 7.73)** | **0.018*** |
| **Works at night from 9pm to 5am** | No | 25 (0.83; 0.55 – 1.24) | Reference | | 21 (1.22; 0.78 – 1.89) | Reference | |
| | Yes | 2 (2.94; 0.51 – 11.16) | 3.62 (0.41 – 15.02) | 0.065 | 2 (2.86; 0.50 – 10.86) | 2.38 (0.27 – 10.06) | 0.233 |
| **Type of work at night** (Night not defined) | No work at night | 15 (0.83; 0.48 – 1.40) | Reference | | 16 (1.36; 0.80 – 2.24) | Reference | |
| | Clearing, planting, or tending (young plantation) | 0 (0; NA) | 0 (0 – 58.91) | 0.773 | 0 (0; NA) | 0 (0 – 40.26) | 0.725 |
| | Tapping, harvesting, or cutting (mature plantation) | 8 (1.17; 0.54 – 2.38) | 1.41 (0.52 – 3.57) | 0.429 | 5 (1.71; 0.63 – 4.18) | 1.27 (0.36 – 3.66) | 0.646 |
| | Sedentary: Tractor, security, supervisor, or office work | 2 (1.68; 0.29 – 6.54) | 2.04 (0.22 – 8.96) | 0.335 | 0 (0; NA) | 0 (0 – 5.88) | 0.394 |
| **Work during the day** (Day not defined) | Clearing, planting, or tending (young plantation) | 10 (0.71; 0.36 – 1.35) | Reference | | 14 (1.47; 0.84 – 2.52) | Reference | |
| | Tapping, harvesting, or cutting (mature plantation) | 8 (0.98; 0.46 – 2.01) | 1.38 (0.47 – 3.91) | 0.493 | 5 (1.27; 0.47 – 3.12) | 0.86 (0.24 – 2.56) | 0.779 |
| | Sedentary: Tractor, security, supervisor, or office work | 7 (1.67; 0.74 – 3.57) | 2.38 (0.76 – 6.97) | 0.072 | 2 (1.11; 0.19 – 4.38) | 0.75 (0.08 – 3.32) | 0.708 |
| **Tapping rubber** | During the day | 8 (0.98; 0.46 – 2.01) | Reference | | 5 (1.27; 0.47 – 3.12) | Reference | |
| | At night | 8 (1.17; 0.54 – 2.38) | 1.19 (0.39 – 3.66) | 0.727 | 5 (1.71; 0.63 – 4.18) | 1.35 (0.31 – 5.93) | 0.635 |

N = total number, *Pf = Plasmodium falciparum,* PCR = polymerase chain reaction, OR = odds ratio, CI = confidence interval, *p* = significance

*** *p* <0.05, ** *p* <0.01, *** *p* <0.001**

Only 3 variables, all related to housing style, had significant ORs during the wet season. The odds of malaria are significantly higher for those who dwell in temporary housing (tents and shelters) with walls and ceilings that are porous (leaves, bamboo, plastic), than those who reside in permanent housing with non-porous walls and ceilings (metal, wood, tile, or cement). During the dry season, only ITN or use of any form of net (hammock or bed, both insecticide and non-insecticide treated), had significantly lower odds of malaria than use of no net.

**Multivariable logistic regression analysis.** A multivariable logistic regression analysis was performed to assess whether any of the variables were significant with the addition of confounding variables (Table 8). Only the 30–44 year age group shows significantly lower odds than the 15–29 year age group in the analysis (OR 0.42, *p* 0.03). However, the OR had a wide 95% confidence interval (0.18–0.88).

**Table 8.** Multivariable logistic regression analysis of risk factors associated with malaria transmission on Cambodian plantations.

| Variable | N (%) | OR (95% CI) | *p* |
|---|---|---|---|
| **Sex** | | | |
| Female | 1768 (42%) | Reference | |
| Male | 2431 (58%) | 1.12 (0.59 – 2.17) | 0.731 |
| **Age** | | | |
| 15 – 29 | 2067 (49%) | Reference | |
| 30 – 44 | 1449 (35%) | 0.42 (0.18 – 0.88) | **0.030*** |
| 45+ | 682 (16%) | 1.06 (0.46 – 2.22) | 0.884 |
| **Education** | | | |
| None | 774 (18%) | Reference | |
| Primary only | 2452 (58%) | 1.14 (0.48 – 3.12) | 0.783 |
| Secondary or higher | 972 (23%) | 1.91 (0.75 – 5.55) | 0.199 |
| **Season** | | | |
| Dry | 3143 (64%) | Reference | |
| Wet | 1793 (36%) | 1.37 (0.75 – 2.49) | 0.299 |
| **Migrant** | | | |
| No | 3240 (66%) | Reference | |
| Yes | 1654 (34%) | 0.84 (0.43 – 1.57) | 0.595 |
| **Housing** | | | |
| Temporary | 472 (10%) | Reference | |
| Permanent | 4423 (90%) | 0.54 (0.24 – 1.35) | 0.149 |
| **Uses bed net?** | | | |
| No | 469 (11%) | Reference | |
| Yes | 3729 (89%) | 0.66 (0.31 – 1.58) | 0.317 |
| **Works at night? (9pm – 5am)** | | | |
| No | 4733 (97%) | Reference | |
| Yes | 138 (3%) | 2.80 (0.82 – 7.22) | 0.057 |

SE = standard error, OR = odds ratio, CI = confidence interval, *p* = significance

**** p <0.05***

## 5.3 Research objective 3: A mathematical malaria transmission model to predict malaria incidence and prevalence based on plantation life cycle

**Model calibration.** The malaria transmission rate is highly dependent on the biting rate ($\alpha$) and the proportion of symptomatic patients treated ($\tau$). The first bifurcation plots (Figure 16A) confirm that when no infected people are tested or treated ($\tau = 0$), the reported incidence at 10 years would be 0. As $\alpha$ increases, prevalence over time tends towards 100%.

Figure 16B demonstrates that as $\tau$ increases, the prevalence of malaria at 10 years tends towards zero. There is a threshold $\tau$ value at which control is achieved at 10 years, and this is dependent on $\alpha$. When $\alpha$ is increased from 1 to 3 (Figure 16C), the threshold $\tau$ value also increases. As $\tau$ increases, the reported incidence of cases also increases. A higher peak in incidence is observed when there is a higher biting or transmission rate (Figure 16B versus Figure 16C) as more people are infected.

**Malaria transmission pattern and immunity.** The proportion of cases with partial immunity also rises as $\alpha$ increases, or as $\tau$ decreases (Figure 16D–E). With increasing incidence, more people are exposed to malaria and develop immunity. In the immunity bifurcation plots, there is a range of $\alpha$ values (Figure 16D: $0.2<\alpha<0.5$, Figure 16E: $0.5<\alpha<2.3$) below which there is elimination after 10 years and above which there are stable transmission dynamics (Figure 16D–E). The range represents an unstable transmission dynamic, and is dependent on $\tau$: As $\tau$ is increased from 0.5 (Figure 16D) to 0.75 (Figure 16E), it is possible to achieve malaria control at a higher biting rate ($\alpha \approx 0.2$ versus $\alpha \approx 0.5$).

When the biting rate is fixed at 3 (Figure 16F), there is a range of τ values (0.78<τ<0.9) above which elimination is achieved, and below which there is a stable transmission dynamic. This implies that as the population heads towards malaria elimination, the proportion of symptomatic and severe to sub-clinical malaria cases will actually increase before control is achieved.

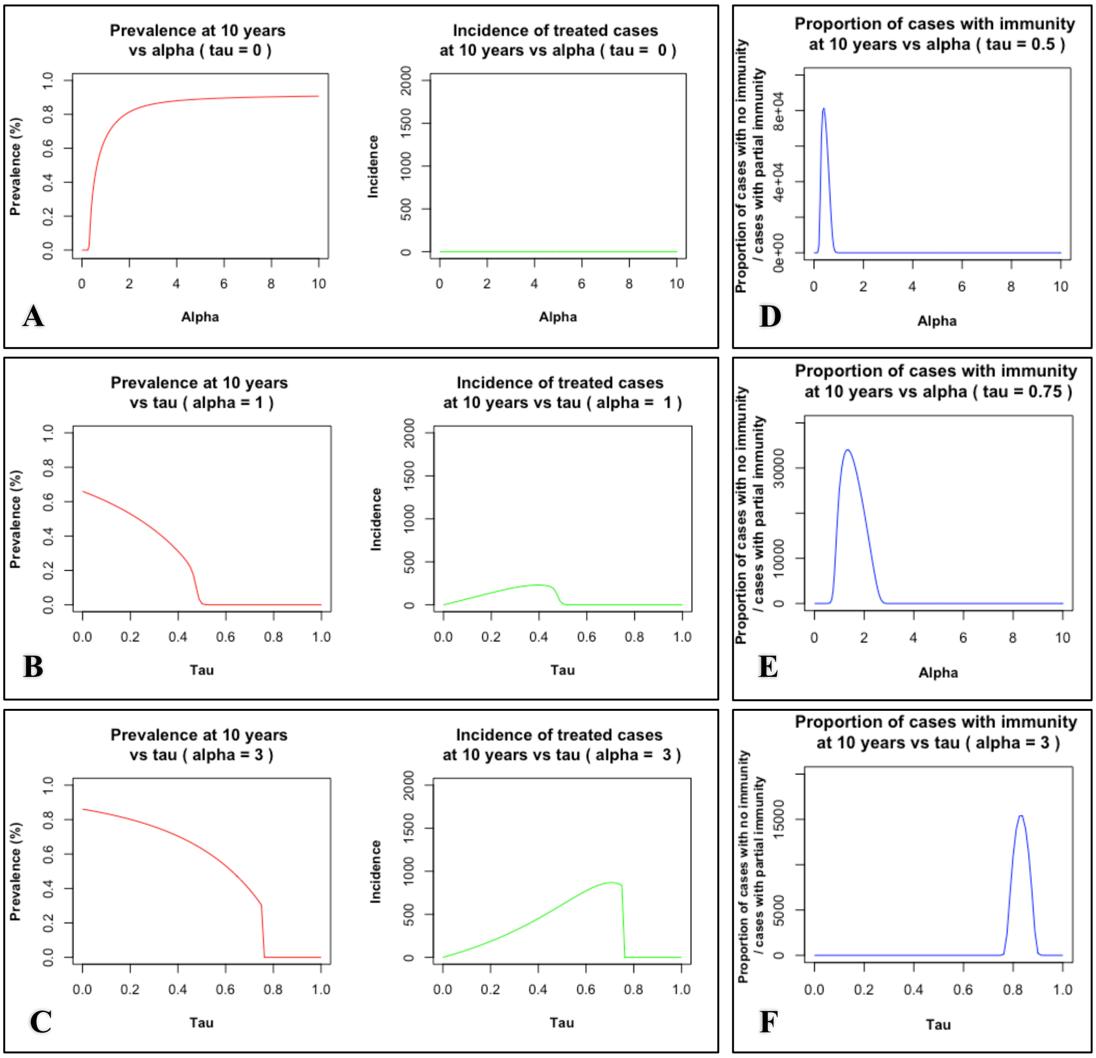
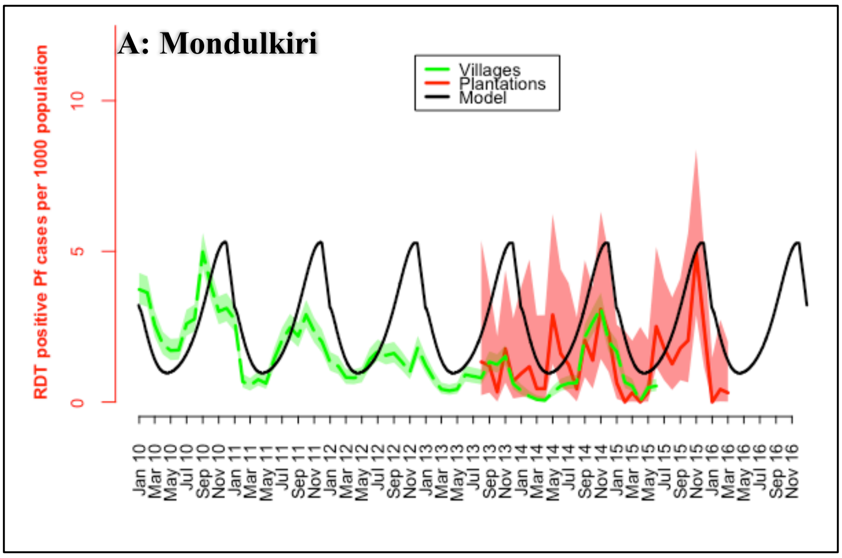



**Figure 16A–F.** Bifurcation diagrams demonstrating prevalence and incidence of malaria at a steady-state after running the model for 10 years with varying α and τ parameters: (A) Variable α values, τ = 0, (B) α = 1, variable τ values (threshold τ ≈ 0.5), and (C) α = 3, variable τ values (threshold τ ≈ 0.78).

Bifurcation diagrams demonstrating proportion of people with immunity after running the model for 10 years with varying α and τ parameters: (D) Variable α values, τ = 0.5, (E) Variable α values, τ = 0.75, and (F) α = 3, variable τ values.

**Model fitting**. The baseline model was fitted to plantation malaria incidence (Figure 17) and prevalence (Figure 18) data for Mondulkiri and Stung Treng provinces using parameters from Table 4. For the initial fitting process, the prevalence in the migrant worker population was assumed to be 0 (low transmission setting). The model was run for 10 years, taking about 3 years to reach a steady-state. Although fitted against the plantation data, the model appears to predict the village malaria incidence pattern.



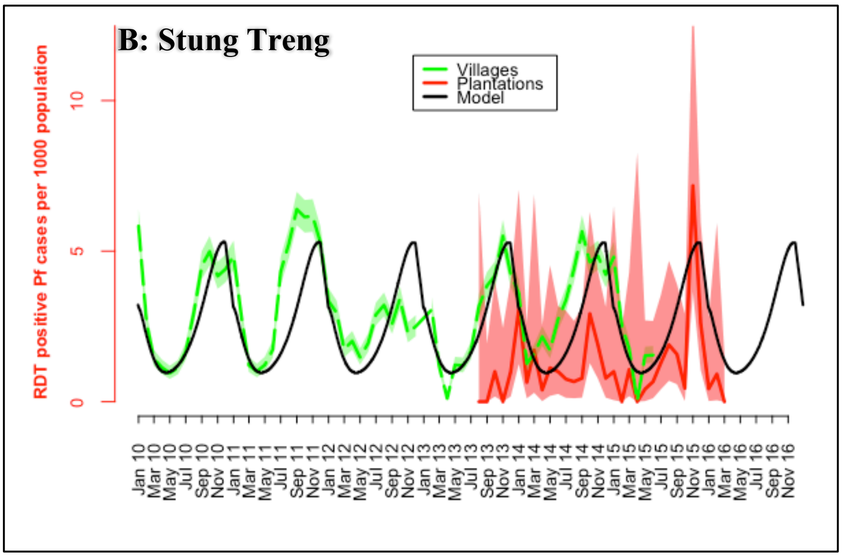




**Figure 17A–B. Fitting model to plantation incidence data.** (A) Mondulkiri province, and (B) Stung Treng province. Shaded red and green areas represent 95% binomial confidence intervals for plantations and villages respectively.

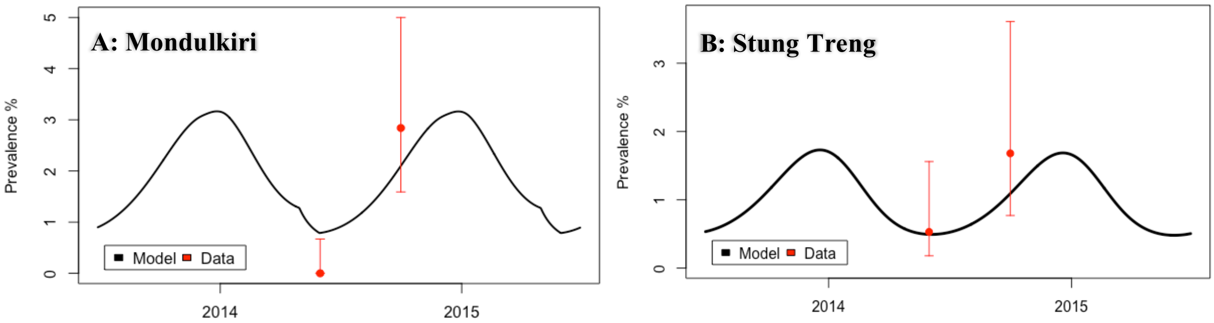



**Figure 18A–B. Fitting model to plantation prevalence data.** (A) Mondulkiri province, and (B) Stung Treng province. Error bars represent 95% CI.

Phase plots (Figure 19) compare incidence and prevalence values between peak (wet season) and trough (dry season) in a steady-state. The circular pattern demonstrates a stable equilibrium with a seasonal cycling pattern.

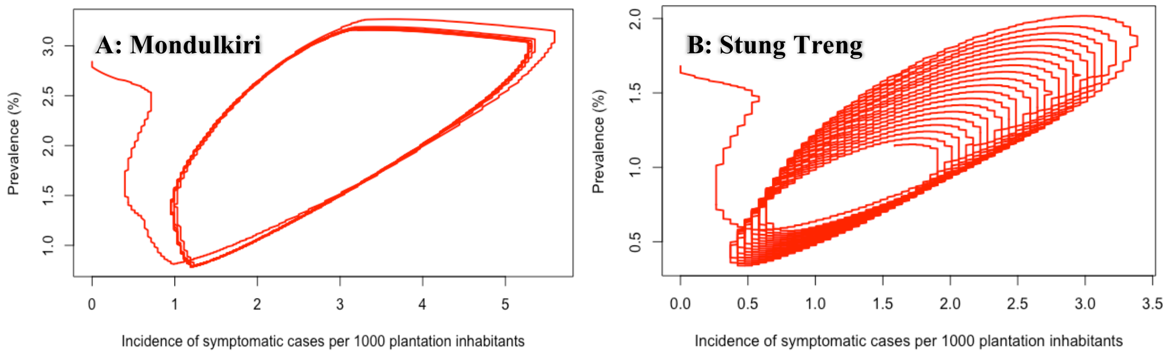



**Figure 19A–B. Phase plot of malaria incidence versus prevalence.** (A) Mondulkiri province, and (B) Stung Treng province. Error bars represent 95% CI.

The relative amplitude of seasonal forcing (*amp* = 0.9), daily number of infective mosquito bites per person ($\alpha = 0.79$), and proportion of asexual blood stage patients with partial immunity who are clinical/symptomatic ($q = 0.5$) parameters were derived from the fitting process. The high amplitude of seasonal forcing indicates a large difference in incidence between wet and dry seasons. The proportion of asexual blood stage patients with partial immunity who are symptomatic ($q = 0.5$) is much lower than for those without immunity (0.93; 95% CI: 0.87–0.96, in malaria patients with 3 or more days of fever)[31]. The proportion of symptomatic cases treated ($\tau$) is usually estimated from the data, but in this case has been obtained from the literature (0.4).[36] A similar value was obtained by multiplying the proportion of people with fever tested by RDT (0.45)[37] by the sensitivity of the SD Bioline Ag-Pf/pan malaria RDT (approximately 0.9).[38–40] There is usually little data for the number of infections that have remained untreated, and hence it is often difficult to derive $\tau$.[29]

**Modelling worker migration.** Two methods were attempted to import immigrants and export migrants from the model. The first method simulated continuous temporary worker migration throughout the year with a seasonal cosine function resulting in a peak in immigration in June and peak in emigration in December. The second method and one presented here assumes that all the workers arrive in May and leave in December. The fitted model assumes that the workers all come from a low prevalence setting ($\xi$ = 0). If the prevalence in the migrant population is increased to 5% (medium transmission area), the incidence (Figure 20) and prevalence of malaria on the plantation both increase substantially, which does not fit the data. This suggests that the migrant population does not act as a malaria reservoir, and does not increase transmission on plantations.

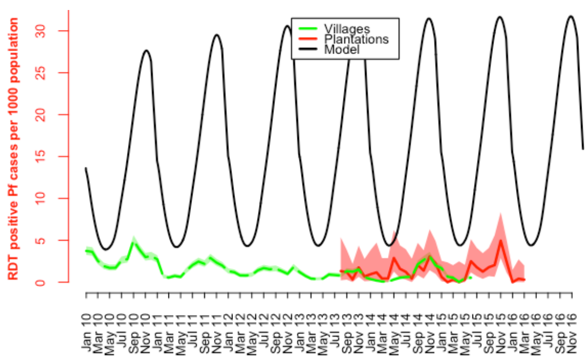



**Figure 20. Model of medium prevalence of malaria in migrant population ($\xi$ = 0.05) arriving on plantations in Mondulkiri province.**

An attempt was also made to replicate the dual peak of transmission seen in the plantation incidence data from Mondulkiri province (Figure 21). This could only be achieved if the migrants were coming from a high transmission setting ($\xi = 0.1$), and enter compartment $A_2$ (asexual blood stage with immunity) rather than G2. The biting rate ($\alpha$) also needs to be halved to 0.4 to maintain the steady-state. This suggests that the dual peak in the plantation incidence data may be an artificial finding due to noisy data and not a reflection of reality.

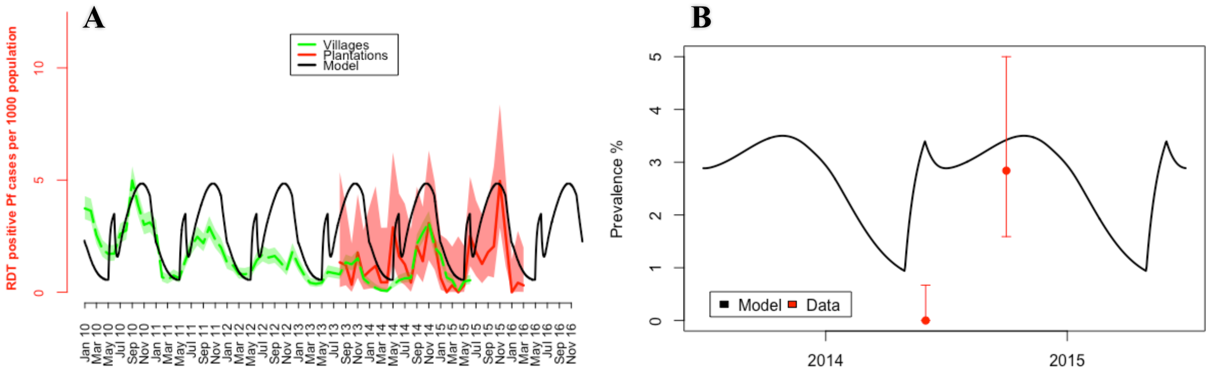



**Figure 21A–B. Dual peak model.** (A) Incidence, and (B) prevalence fitted to Mondulkiri data. The prevalence in the migrant population ($\xi$) = 0.1, $\alpha$ = 0.4, with migrants entering S and $A_2$ compartments. Red and green shaded areas (A), and error bars (B) represent 95% CI.

**Interventions.** A conservative assumption is made that malaria is not going to burn itself out and additional or ramped-up interventions are needed to achieve elimination. Two interventions were included in the model and the time to elimination in years was the output. Both models were run for 50 years to see if elimination was achieved with the set parameters. The first intervention looked at scaling up of access and uptake of malaria RDT and treatment (Figure 22A). The proportion of symptomatic patients who are correctly diagnosed and receive treatment ($\tau$, range 0–1) is plotted against time in years, producing an exponential curve. No elimination is achieved in 50 years for tau $<0.42$. If $\tau > 0.58$ is reached, then the time to elimination is estimated at $<5$ years.

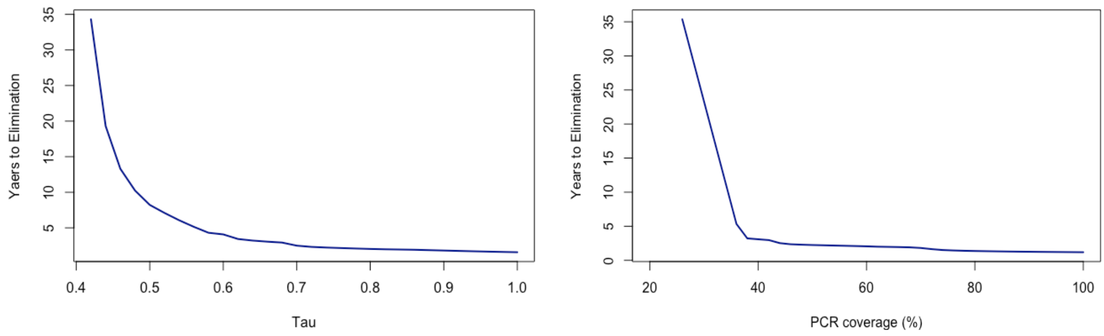



**Figure 22A–B. Years to malaria elimination with scaled-up or additional interventions in Mondulkiri province.** (A) Scaled-up RDT and treatment. The proportion of symptomatic patients who receive treatment (τ) is plotted against years to elimination. (B) A PCR screening and treatment campaign. The proportion of the population covered by the PCR campaign is plotted against years to elimination.

The second intervention examined the effect of a mass PCR screening campaign in the dry season in which a proportion of the population is screened by PCR and treated (Figure 22B). The sensitivity of testing is assumed to be 100%,[28] but with an optimistic 10% loss to follow up from testing to completing treatment (i.e. the efficacy of PCR screening and treatment = 90%). The campaign only affects the population in the blood stage compartments, who are treated and move to the recovered with immunity (R) compartment. The population coverage of the PCR campaign (range 0–100%) is plotted against time in years, producing an exponential curve. No elimination is achieved in 50 years for PCR coverage <24%. If the population coverage is >38%, and there is only 10% loss to follow up, the time to elimination is <5 years. These scenarios are only plausible in a setting where there is no immigration (importation) of individuals from areas with malaria ($\xi = 0$).

# 6. Discussion

This chapter will the discuss the results, and compare and contrast the findings with the literature.

## 6.1 Research objective 1: Comparison of the pattern and magnitude of malaria cases among Cambodian plantation workers versus the surrounding populace

The results demonstrate that plantations in north-eastern Cambodia do not have significantly higher transmission than surrounding areas, and are therefore unlikely to be "hotspots" for transmission. The prevalence of malaria on the plantations during both wet and dry season was found to be much lower than previously reported.[18] This could be a result of successful malaria control measures (including scaling up of VMW programme and ITN distribution) in the past decade.[1] Underreporting is a likely explanation for the lower incidence than anticipated. Only 25% of plantation inhabitants seek treatment from the MMWs, with the remainder using private providers such as pharmacies, self-treating, using alternative medicine, or not seeking treatment.

Snow et al.[48] estimate that in a hypoendemic setting (defined as malaria prevalence of less than 10%) the median incidence of clinical cases per year will be 43/1,000 population (interquartile range 6–117). The incidence and prevalence of malaria from the local data are in keeping with what one would expect in a hypoendemic setting in south east Asia. In Mondulkiri and Stung Treng provinces, the wet and dry season seroprevalence was 2.84% and 1.68% respectively. This is significantly lower than the prevalence demonstrated by Cook et al.[18] in Rattanakiri province (8.1 and 3.4% respectively). The mean annual incidence of cases reported by MMWs in Mondulkiri and Stung Treng provinces was 15.8 and 13.9 cases per 1,000 population respectively, which correlates well with the study by Snow et al.[48]

It also appears that the pattern and magnitude of new cases is similar on plantations and villages in the same localities. Both sets of data have an overlapping seasonal peak during the rainy season. The VMW malaria data has a broader peak which spans from June to January, in keeping with the study by Maude et al.[1] Both sets of incidence data have their maximal peak around October to November, at the end of the rainy season.

**Why do villages have similar transmission patterns to plantations in rural areas?** Both plantations and villages in rural areas are in close proximity to forests which are the breeding sites of mosquitos, placing the village inhabitants at increased risk.[18,49] Workers may commute between their village homes, forests, and plantations. Forest activity has been shown to be a strong risk factor for malaria transmission.[49] Villagers also engage in forest farming, hunting, fishing, and logging which increases their exposure to outdoor feeding vectors.[50] They often sleep in the forest overnight, sometimes without ITN or in housing completely or partly open to the outside.[50] The presence of domestic animals, which attract and provide a food source for the zoophilic vectors, is another explanation for the increased transmission in rural villages.[11]

**Dual seasonal peak of cases in Mondulkiri province.** The VMW incidence data is more robust and has a larger sample size than the MMW data. This results in variable and noisy monthly incidence figures in the plantations, which could account for the first peak at the start of the wet season. Further data in the coming years will clarify this. However, the first peak could also be due to increased exposure to vectors through clearing and planting activities near forests during the early wet season. Another hypothesis is that these peaks are a result of population migration, with migrant workers importing malaria to the plantations. An alternative explanation is that the migrant workers come from areas of low prevalence, and thus have less immunity to malaria. Arriving at the plantations at the start of the peak transmission period and working in close proximity to forests places this susceptible group at a higher risk of symptomatic or severe malaria.[19] In addition, the migrant workers often do not have knowledge of, or access to, malaria testing and treatment.[51] The effect of worker migration on plantation transmission is explored further in the mathematical modelling component of the dissertation.

## 6.2 Research objective 2: Characterisation of the relationship between the plantation life cycle and the risk of malaria to workers

The second component of the project explored risk factors for malaria transmission on plantations. Factors, such as permanent or temporary worker status, type of work (working in close proximity to forests), and working at night had no significant effect on the odds of malaria. Only two factors, housing style and use of nets, showed a significant association with malaria prevalence. However, both associations had wide confidence intervals. All the significant variables have large discrepancies in sample size between comparison groups. This leads one to question whether the odds are truly different, or merely being driven by the differences in sample size. Given the low event rate, the robustness of the results should be interpreted with caution.

This dissertation demonstrates no efficacy in the use of insecticide treated bed and hammock nets in the wet season for worker populations, although there is some evidence of protection during the dry season. Bed net usage was proportionally much higher in the wet than the dry season Table 6. During the field visits and from discussion with PSI, it appears that the ITN are not replaced or retreated annually and many of the nets seen on the field visits had many holes or had been repurposed (e.g. as fishing nets). A cluster-randomised trial looking at the use of ITN for the prevention of malaria in Rattanakiri province found that use of ITN was associated with a 9% reduction in prevalence, however was not statistically significant.[52] In an entomological survey conducted in Western Cambodia by Sochantha et al.,[53] long lasting insecticide treated hammocks were shown to provide significant protection from bites of *Anopheles minimus* and *dirus*. The study did not report whether this reduced biting rate results in a lower incidence of malaria. Another cluster-randomised trial in Vietnam however did demonstrate a significant reduction in malaria in malaria incidence.[54]

The use of ITN in south east Asia has not been as effective in controlling malaria as compared to its prominent role in Africa. This is due to the exophagic and exophilic behaviour of the *Anopheles minimus* and *dirus* species. Mosquito biting predominantly takes place outside in the early hours, reducing the efficacy of indoor residual spraying and bed nets and used while sleeping at night, particularly in permanent houses with non-permeable walls built on stilts.[53] In contrast, temporary shelters and tents have vector-permeable walls and are open to the elements exposing the occupants to the vectors. There is significant evidence which supports the association between housing characteristics (non vector-permeable walls, window screens, and stilts) and malaria transmission.[21-23,55]

**Multivariable logistic regression limitations.** There were several limitations to the multivariable logistic regression analysis with this dataset, and the results should be interpreted with caution. Firstly, there are marked differences in sample size between variable factors. Ideally, one needs sufficient participants with each possible set of explanatory variables. I combined the wet and dry season data to increase the sample sizes and added a binary variable "season" to compensate for the small sample sizes.

Secondly, risk factors which are not significant in the univariate analysis, or are below a significance threshold, should be excluded from the regression analysis. Known confounding variables are typically still included. The univariate analysis in this case had very few significant factors, but the multivariable regression analysis is still a worthwhile exercise to examine whether the risk factors are significant with the addition of potential confounders.

Thirdly, collinearity between variables (such as housing style and type of roof or wall) may cause over-adjustment. Collinear explanatory variables were removed from this analysis.

## 6.3 Research objective 3: A mathematical malaria transmission model to predict malaria incidence and prevalence based on plantation life cycle

When interpreting the results of the malaria model it is worth keeping in mind the aphorism "All models are wrong; but some are useful."[56] The aim of the mathematical model was to describe how population migration affects malaria transmission on plantations and to evaluate the effectiveness of elimination-focused interventions which could be used to control malaria in this setting. While the model is useful in answering these questions, it only simplifies or approximates reality.

To answer the questions, a deterministic mathematical malaria transmission model was developed to predict the incidence and prevalence of malaria on plantations in north-eastern Cambodia. The model also predicts malaria incidence in areas surrounding the plantations, which is not surprising as the first research objective demonstrated that the seasonal pattern and magnitude of malaria on plantations is similar to surrounding villages.

**Malaria transmission pattern and immunity.** Malaria transmission dynamics may be unstable (low transmission rate, low proportion of population with immunity) or stable (medium to high transmission rate, high proportion of population with immunity). The model demonstrates a controlled low-endemic stable state of malaria transmission.[57] Stable endemic populations have a high proportion of people with immunity, resulting in less cases of severe malaria, but also higher asymptomatic carriage and transmission. Before malaria elimination (disease-free state) is achieved in a population with a stable transmission dynamic, there will be an upsurge in clinical and severe cases (unstable transmission state).[43] As transmission declines from an endemic stable state to an unstable dynamic, the number of people with immunity drops. This results in higher proportion of clinical new malaria cases which can be detected and treated. Individuals with clinical malaria are infectious for a shorter time period than those with an asymptomatic infection.[43] However, new cases are also more likely to be severe due to dwindling immunity in the population.[43]

**Migration and malaria transmission.** Imported infections do not appear to play an important role in transmission dynamics in the north-eastern provinces of Cambodia. The model suggests that migrant workers arriving on plantations in north-eastern Cambodia have a low prevalence of malaria. As such, they do not act as a "hot population" fuelling the spread of malaria. If the migrants (who make up a substantial proportion of the population) had a higher prevalence of malaria (>5%), the transmission dynamics on the plantations would be drastically altered, with increased incidence. This does not appear to be the case.

If migrants were continually importing malaria into the province and fuelling the infectious reservoir, then it is unlikely that local elimination would be possible without foreign source reduction (reducing malaria prevalence in the province of origin).[29] If this were the case, national and not focal malaria control programmes would be advisable.

In the Cambodian rural setting, vector control is not a possibility due to extensive mosquito breeding sites in forests within close proximity to human habitation. In the post-elimination setting, since the vectors remain, imported infections may re-ignite transmission in previously controlled areas leading to a resurgence of malaria.[29]

**Interventions.** According to the model, to achieve elimination by 2025, the proportion of symptomatic patients who receive and complete an effective course of treatment (currently estimated to be around 40% in VMW areas)[36] needs to increase to over 50%. This could be achieved through an increase in access to and uptake of MMWs on plantations. The scaling up of VMW programmes in Cambodia over the past 7 years has had a significant impact on malaria control, with an 81% decline in cases from 2009 to 2013.[1]

The model also demonstrates that dry season PCR screening and treatment of positive cases could drastically reduce the time to elimination. Malaria PCR screening is highly sensitive (approaching 100% sensitivity)[28] even in asymptomatic patients with immunity. PCR is able to detect asymptomatic cases of malaria at low-density parasitaemia (<100 parasites/L). By contrast, RDTs have poor performance at low parasitaemia in individuals, with a sensitivity of around 55%.[58] These undetected asymptomatic cases act as a malaria reservoir, fuelling malaria transmission, and may be considerable even in a low transmission setting.[59] A PCR screening and treatment campaign covering over 35% of the population would help to reduce this reservoir, could lead to malaria elimination within 10 years. This assumes that the loss to follow up is less than 10%, that the period from diagnosis to treatment is rapid (less than 1 week), and that there is no immigration of workers importing malaria into the province.

# 7. Conclusions

This chapter highlights the key findings related to the research objectives, discusses the strengths and limitations of the methodology, and concludes with policy and research recommendations.

## 7.1 Key findings

Although plantations do not appear to be "hotspots", areas of human habitation in rural areas which are in close proximity to forests experience considerable malaria transmission. The magnitude and pattern of transmission in villages and plantations appears to be similar with a peak in cases toward the end of the wet season. The malaria seroprevalence among plantation workers and their families was far lower than expected, but incidence was higher than reported in the literature.

Only plantation housing style appears to have a significant effect on malaria prevalence in the wet season, while bed nets show some efficacy in the dry season. The type of work, working at night, and migrant status do not appear to have a significant effect on prevalence.

As Cambodia heads toward malaria elimination, the transmission pattern will shift from a stable endemic to unstable epidemic pattern. The model demonstrates that as malaria cases become more sporadic, immunity is lost and people without immunity are at higher risk of severe malaria. The lower proportion of people with immunity to malaria means that people infected with malaria are more likely to be symptomatic, which also means they are more likely to seek treatment. It is likely that the migrant workers are at increased risk of malaria and adverse outcomes due to lower immunity and poor knowledge and uptake of health care services.

In order for Cambodia to progress towards malaria elimination by 2025, a concerted effort will be required, with scaled-up diagnosis and treatment as well as additional interventions. Evidence-based, cost-effective strategies need to be investigated and priorities developed. There is a need for robust data to decide where and how to implement these interventions effectively.

## 7.2 Limitations of methodology and analysis

The malaria surveillance data from the plantations had wide confidence intervals and were very irregular. Malaria surveillance data is subject to collection bias due to improperly trained data collectors and under-reporting.[1]

The epidemiological data from the plantation survey is also of sub-optimal quality. There were significant problems with research methodology and internal validity. Sample sizes were too small to demonstrate significant causal relationships. The judgment and convenience method of sampling may result in a study sample that is not truly representative of the plantation population. I would recommend future studies use random stratified sampling of both migrant and permanent workers and their families, in both wet and dry seasons. In terms of data analysis, multivariate regression techniques were not suitable for this dataset as multiple interactions and collinearity resulted in a lack of power.

The primary limitations of the modelling component were lack of sufficient incidence and prevalence data for model fitting and validation, and data which were not recent (rainfall and population size).

## 7.3 Policy and research recommendations

The results presented should be used to inform, improve upon, and develop approaches for providing Cambodian plantation workers and rural dwellers with the most effective and efficient malaria diagnostic and treatment services feasible. The low uptake and efficacy of bed nets necessitates a different approach to malaria control in south east Asia to that used in Africa.

Plantation workers are often mobile, migrant, and hard to reach, making provision of healthcare services difficult. Due to high cross-border and inter-provincial mobility of the population and importation of malaria cases, MDA may not be the most effective approach as efficacy would rely on a quick and systematic implementation through all regions to prevent re-importation of cases. A general policy of screening and treating migrants upon entry into the plantation would not be effective as the migrant workers appear to have a low prevalence of malaria. This policy may even be detrimental to the migrants, resulting in fear, stigma, segregation, or loss of employment. However, if migrants were known to come from an area of high prevalence, early PCR screening and treatment of this group may be efficacious.

The mathematical model demonstrates that the key to controlling malaria transmission in highly mobile populations is increased access to malaria screening and treatment for symptomatic cases to shorten the infective period. In addition, efforts should be made to detect and treat asymptomatic cases which act as a malaria reservoir. Novel strategies are needed to eliminate this reservoir. Rapid and sensitive PCR testing is an option for screening and treatment of inhabitants in low-endemicity areas.[60] It may not be feasible or affordable at a population level, but should form part of malaria surveillance in all rural areas. This could help to identify areas for mass drug administration, or for intensive PCR screening and treatment campaigns.

PSI and other civil society groups have a role in increasing knowledge about malaria, and increasing access to and uptake of MMW and VMW services in order that malaria cases are treated early. Another intervention which may be effective is upgrading worker housing to permanent, non-vector-permeable walled structures. Protective clothing and repellents may have value in tackling residual malaria transmission and should be explored further.[11]

Finally, the waning immunity as the transmission dynamic shifts from stable to unstable will result in an upswing of clinical and severe malaria cases. Interventions that reduce transmission need to be combined with improved access to health services for migrants and referral facilities for severe cases to prevent a paradoxical increase in disease morbidity and mortality.[43]

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