Université catholique de Louvain
Secteur des sciences de la santé

Institut de Recherche Santé et Société
Promoteurs : Niko Speybroeck
Annette Erhart
Alejandro Llanos-Cuentas



Angel Rosas-Aguirre is a medical doctor with substantial work experience managing malaria control projects in South America. Because of the importance of the evidence-based decision making, his professional career has always been continuously interwoven with teaching and research activities. His current research aims to understand malaria transmission dynamics in Peruvian endemic areas and to generate evidence for improving case detection and treatment strategies for the malaria control and elimination.

Malaria remains an important public health problem in Peru. Two parasitological and serological surveys were conducted to characterize malaria transmission in endemic areas of the Pacific northern coast (2010) and the Amazon region (2012) in Peru. Results showed high heterogeneity in malaria exposure at micro-geographical level and over time, and that most malaria infections were asymptomatic and sub-microscopic. Since focal screening and treatment (FSAT) has been introduced to overcome the limitations of routine passive case detection for detecting asymptomatic infections, a modelling approach was developed to assess the potential impact of this strategy on the *P. falciparum* transmission in the Amazon. Although model predictions suggest that FSAT enabled to reach elimination levels, the progression rates to those levels varied substantially according to different operational criteria used for the intervention. The asymptomatic reservoir calls for an immediate shift in the malaria policy in Peru from detecting and controlling clinical malaria to targeting and eliminating all malaria infections.

Le paludisme reste un problème important de santé publique au Pérou. Deux enquêtes parasitologiques et sérologiques ont été effectuées pour caractériser la transmission palustre dans les zones endémiques de la côte Pacifique Nord (2010) et la région de l'Amazonie Péruvienne (2012). Les résultats ont montré une forte hétérogénéité de l'exposition au paludisme, au niveau micro-géographique ainsi que dans le temps, et que la plupart des infections palustres étaient asymptomatiques et sub-microscopiques. Le dépistage focal et traitement (DFT) ayant été introduit récemment afin de surmonter les limites de la détection passive afin de détecter les infections asymptomatiques, une approche de modélisation a été développée pour évaluer l'impact potentiel de cette stratégie sur la transmission de P. falciparum *en Amazonie. Bien que les prédictions du modèle suggèrent que la DFT permette d'atteindre les niveaux d'élimination, les taux de progression vers ces niveaux varient sensiblement selon les différents critères opérationnels utilisés pour l'intervention. L'existence de ce réservoir asymptomatique nécessite une réponse immédiate de la politique de control du paludisme au Pérou, afin de passer de la détection et de contrôle des cas cliniques au ciblage et l'élimination de toutes les infections palustres.*

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Angel Rosas-Aguirre

Understanding malaria transmission dynamics in Peru

Understanding transmission dynamics for malaria control and elimination in Peru

Angel Rosas-Aguirre

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Thèse présentée en vue de l'obtention du grade
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Co-Promotor :	**Prof. Alejandro Llanos** Instituto de Medicina Tropical Alexander von Humboldt Universidad Peruana Cayetano Heredia, Lima, Peru
Co-Promotor :	**Prof. Anna Rosanas** Department of Biomedical Sciences Institute of Tropical Medicine, Anvers, Belgique
Jury Member:	**PhD. Emmanuel Abatih** Department of Biomedical Sciences Institute of Tropical Medicine, Anvers, Belgique
External Jury Member:	**Prof. Teum Bousema** Department of Immunology and Infection London School of Hygiene and Tropical Medicine, London, UK.

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LIST OF ABBREVIATIONS

ABER	Annual blood examination rate
ACD	Active case detection
ACT	Artemisinin-based combination therapy
API	Annual parasite index
APIf	Annual parasite index for *P. falciparum*
APIv	Annual parasite index for *P. vivax*
AS	Artesunate
BCC	Behaviour change communication
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CQ	Chloroquine
DDT	Dichloro-diphenyl-trichloroethane
DGCD	Directorate General for Development Cooperation of the Belgian Government
DOT	Directly observed therapy
EIR	Entomological inoculation rate
ELISA	Enzyme-linked-immune-sorbent assay
ENSO	El Niño Southern Oscillation phenomenon
FSAT	Focal screening and treatment
GFATM	Global Fund to Fight AIDS, Tuberculosis and Malaria
GMAP	Global Malaria Action Plan
GMEP	Global Malaria Eradication Program
GDP	Gross domestic product
GNI	Gross national income
GPS	Global Positioning System
HBR	Human biting rate
HTS	High transmission season
HH	Household
IPHS	Interamerican Public Health Service
Ig	Immunoglobulin
IRB	Institutional Review Board
IRS	Indoor residual spraying
ITM AvH	Institute of Tropical Medicine Alexander von Humboldt
ITN	Insecticide treated net
LAMP	Loop-mediated isothermal amplification
LLINs	Long-lasting insecticide-treated nets
LM	Light microscopy

LTS	Low transmission season
M&E	Monitoring and evaluation
MDA	Mass drug administration
MDG	Millennium Development Goal
MIGIA	Malaria Immunology and Genetic project
MQ	Mefloquine
MSAT	Mass screening and treatment
MTI	Malaria transmission intensity
NIAD-NIH	National Institute of Allergy and Infectious Diseases – National Institutes of Health.
NCHS	US National Center for Health Statistics
NMCP	National Malaria Control Programme
NMES	National Malaria Eradication Service
NYU	New York University
OD	Optical densities
OR	Odds ratio
PCD	Passive case detection
PCR	Polymerase chain reaction
PfAMA-1	*Plasmodium falciparum* apical membrane antigen-1
PfGLURP	*Plasmodium falciparum* glutamate-rich protein
PMI	US President's Malaria Initiative
PP	Percent positivity
PQ	Primaquine
PR	Parasite rate
PvAMA-1	*Plasmodium vivax* apical membrane antigen-1
PvMSP119	*Plasmodium vivax* merozoite surface protein119
RBM	Roll Back Malaria
RDTs	Rapid diagnostic tests
Ro	Basic reproductive ratio
SCR	Seroconversion rate
SP	Sulphadoxine-pyrimethamine
SPR	Slide positivity rate
SR	Sporozoite rate
SRR	Seroreversion rate
SRHDS	Sub-Regional Health Direction of Sullana
USAID	US Agency for International Development
USD	US dollars
WBBP	World Bank's Booster Program
WBC	White blood cells
WHA	World Health Assembly
WHO	World Health Organization

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SUMMARY

Despite the decline in its incidence in the past decade, malaria remains an important public health problem in Peru. A challenge faced by the National Malaria Control Programme (NMCP) is the ability to efficiently target and eliminate the remaining parasite reservoir in endemic areas, where malaria transmission is low, seasonal and increasingly focal.

Two cross-sectional surveys that combined parasitological (PCR, microscopy) and serological metrics (seroprevalence, seroconversion rates) were conducted to characterize malaria transmission dynamics in low endemic areas of the Pacific northern coast and the Amazon region in Peru. Besides the serological evidence for high heterogeneity in malaria exposure, both to *Plasmodium falciparum* and *Plasmodium vivax,* at micro-geographical level and over time, PCR results confirmed that most malaria infections in the study areas were asymptomatic and sub-microscopic, and therefore unlikely to be detected by the routine passive case detection (PCD). Since the NMCP has recently introduced focal screening and treatment (FSAT) to overcome the limitations of PCD for detecting asymptomatic carriers, a modelling approach was developed to assess the potential impact of this strategy on the *P. falciparum* transmission in the Amazon region and to provide insights for the best criteria for its implementation. Although model predictions suggest that combined interventions enabled to reach elimination levels, the progression rates to those levels varied substantially according to different operational criteria (timing, duration, diagnostic tool) used for the intervention.

The uncovered asymptomatic and sub-patent reservoir calls for an immediate shift in the malaria control policy in Peru from detecting and controlling clinical malaria to targeting and eliminating all malaria infections (both asymptomatic and symptomatic). The combination of molecular and serological tools have repeatedly proved their added value in accurately understanding the local malaria epidemiology, determining risk factors, describing spatial heterogeneity in transmission, detecting malaria hotpots for targeted interventions as FSAT, and identifying recent changes in malaria transmission in the study areas.

1. INTRODUCTION

1.1. Rationale

Despite several decades of intense control efforts, malaria remains an important public health problem in Peru [1]. After malaria resurgence in the late 90s, with a peak of more than 200,000 slide confirmed cases in 1998 associated with the El Niño Southern Oscillation (ENSO) [1,2], the annual incidence decreased and stabilized at around 70,000-90,000 cases between 2000 and 2005 [3]. Between 2005 and 2010, increased support from international donors, e.g. the Global Fund-PAMAFRO Project [4], allowed the scale-up of comprehensive malaria control strategies [5,6]. During this period, malaria declined drastically in Peru from 87,805 reported clinical cases in 2005 to 29,355 and 23,075 cases in 2010 and 2011, respectively. Since 2011, following the reduction of financial support allocated to the malaria control, malaria started to increase again with a doubling of clinical cases in 2013, highlighting the potential risk of rapid malaria resurgence, especially in the Amazon Region [7].

In Peru, the significant overall reduction in malaria incidence over the past decade has modified the epidemiological profile of the disease in the country, especially in their most affected regions (i.e. Pacific northern coast and the Amazon region) [1] where transmission is seasonal and currently highly concentrated in a small proportion of their total communities [7,8]. Implementing malaria control measures in low endemic areas with seasonal and increasingly focal transmission, represents a major challenge to the National Malaria Control Programmes (NMCPs) [9]. Single communities or groups of households within communities in which the malaria transmission risk is significantly higher as compared to surrounding areas (called “hotspots”) need to be identified and targeted in order to efficiently eliminate the remaining parasite reservoirs [9,10].

Moreover, targeted interventions should be evaluated to demonstrate whether they achieved expected outcomes and impact.

Both the identification of hotspots for targeting the malaria control, and the monitoring and evaluation (M&E) of targeted interventions require that malaria transmission is accurately measured [11,12]. Historically, NMCPs have used the number of reported malaria cases (microscopically confirmed infections in Peru) to stratify the malaria risk, prioritize intervention areas, and monitor the impact of control interventions [13]. However, as malaria transmission decreases, measuring the incidence of clinical malaria becomes increasingly difficult and insensitive [14], mainly due to the substantial proportion of asymptomatic infections not identified by passive case detection (PCD) [15].

Accurately measuring malaria transmission in low endemicity areas is not a straightforward undertaking, and different factors (such as costs, sensitivity-specificity, feasibility, acceptability, seasonal variations) must be taken into account before choosing adequate metrics [11,16]. Though the entomological inoculation rate (EIR) is usually considered as the gold standard for quantifying malaria transmission, this metric has proven to be inaccurate and non-cost-effective in low transmission areas [16,17]. Although determining the malaria parasite rate (i.e., the prevalence of malaria infections, both symptomatic and asymptomatic) through a large community survey seems to be a more accurate and cost-effective method, only repeated surveys using the same methods in the same population at similar time periods would provide sufficient precision for monitoring changes in malaria transmission [16,18]. Additionally, low-parasite-density infections would only be detected by highly sensitive tests such as polymerase chain reaction (PCR) [19]. Conversely, cross-sectional surveys using serological markers are increasingly proposed as useful alternatives for estimating malaria transmission intensity in low transmission settings. Age-stratified seroprevalence data have been used to model seroconversion rates (SCR) which closely correlated with EIR, parasite prevalence [20-

22] as well as related changes over time following specific interventions [22], seasonal or behavioural changes [23].

In addition to tracking the progress of control efforts with appropriate malaria transmission metrics, NMCPs should be able to anticipate and evaluate diverse threats that can appear during the scale-up of malaria control interventions (e.g., drug resistance, insecticide resistance, sub-optimal coverage, sub-optimal use of preventive measures, etc.) for an opportune programme reorientation [14]. Moreover if the programme reorientation involves the incorporation of new malaria control strategies or tools, NMCPs should be able to evaluate them in terms of geographic scope and long-term feasibility [14,24]. Since these types of evaluation cannot always be done experimentally, mathematical modelling has emerged as an important tool for understanding malaria transmission dynamics and assessing short and long term impacts of different control measures [25,26]. Modelling can build on available data, test multiple scenarios and combination of interventions, and make verifiable predictions on the expected impact and timeline of different strategies.

This thesis research aimed to characterize malaria transmission in two different ecological settings in Peru (i.e., the northern coast and the Amazon region), and to assess the elimination potential of different interventions using modelling approaches. The two main topics addressed in the thesis (i.e., malaria transmission metrics in low transmission areas, and modelling potential impact of different strategies for malaria decision-making) are in line with important needs identified by the research community and decision-makers in preliminary forums about the relevance and feasibility of a national malaria elimination program in Peru: the need to redefine surveillance and control strategies in the perspective of low transmission, and to strengthen M&E activities in order to better inform malaria control and elimination efforts [27].

1.2. Objectives

The main objective of the thesis is:

- To characterize malaria transmission in two different ecological settings in Peru and to assess the elimination potential of different interventions using modelling approaches.

The specific objectives of the thesis are:

- To measure malaria transmission intensity and identify related risk factors in the Pacific northern coast and the Amazon region of Peru using parasitological and serological metrics;
- To characterize the heterogeneity in malaria transmission in the Pacific northern coast and the Amazon region through the use of spatial analysis, and identify past changes in transmission using age-specific seroconversion rates;
- To develop a simple mathematical model able to simulate an existing baseline scenario of seasonal malaria transmission in an endemic community of the Amazon region, and to improve this model to predict the potential for elimination of focal screening and treatment (FSAT) in addition to the routine PCD.

1.3. Thesis outline

The thesis is organised in seven chapters.

Chapter 1 presents the rationale and the objectives of the thesis.

Chapter 2 describes the global malaria burden and revises briefly the past and current control and elimination efforts. Then, it provides a background on transmission metrics and on advantages and disadvantages of their use in low transmission settings. Next, it focuses on the basic malaria mathematical models and how these can be used in describing the influence of control and elimination interventions on the transmission of the disease. And finally, it presents a detailed overview of the malaria control strategies in Peru.

Chapter 3 reports on population-based estimates of *P. falciparum* and *P. vivax* prevalence and seroprevalence in an area of low endemicity in the north-west coast of Peru, providing insights into the residual transmission dynamics.

Chapter 4 characterizes the malaria transmission dynamics in three sites of the Peruvian Amazon region through the use of a single cross-sectional survey that combined parasitological and serological metrics. Current malaria transmission patterns, hotspots of high malaria risk and changes in species-specific malaria transmission are identified in the study area.

Chapter 5 describes a mathematical model developed to simulate an existing baseline scenario of seasonal malaria transmission in an endemic community of the Amazon Region, and how it was improved to predict the potential effect of adding FSAT interventions to ongoing PCD.

Chapter 6 includes an ongoing work that complements the characterization of malaria transmission in the northern coast (Chapter 3) by identifying spatial clusters of malaria

risk using survey data, and evaluates whether these spatial clusters are able to detect future malaria hotspots of clinical malaria incidence.

Chapter 7 discusses the main findings of the thesis research, the implications of them for the malaria control in Peru, and provides perspectives for future research.

Chapter 8 lists the bibliographic references, and **chapter 9** includes the chapter annexes.

2. BACKGROUND

2.1. Malaria, the global burden

The remarkable increase of financing and coverage of NMCPs overall has resulted in a wide-scale reduction in the reported malaria incidence and mortality rates since 2000 (Figure 2.1). Based on an assessment of trends of reported malaria cases, 64 of a total of the 106 countries with malaria transmission in 2000 were meeting the Millennium Development Goal (MDG) target of reversing the incidence of malaria in 2013. Of these 64 countries, 55 were on track to meet the World Health Assembly (WHA) and Roll Back Malaria (RBM) targets of reducing malaria case incidence rates by 75% in 2015. However, most of those 55 countries had low numbers of cases in 2000, accounting for only 6% of the total estimated cases in that year [28].

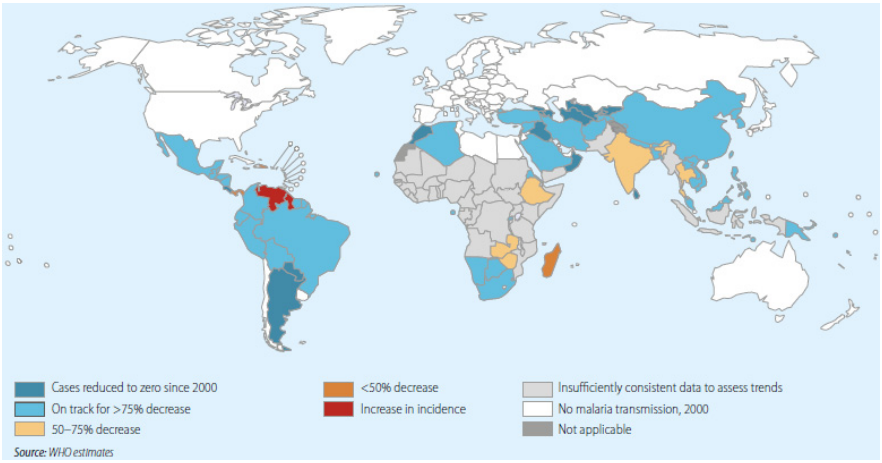


Figure 2.1. Projected changes in malaria incidence rates, by country, 2000-2015.
Source: World Malaria Report 2014 [28].

Considering the WHO classification of countries by stage of malaria elimination (Table 2.1), among the 106 countries with malaria transmission in 2000, ten countries are currently in the pre-elimination phase (Capo Verde, Belize, El Salvador, Costa Rica, Mexico, Ecuador, Paraguay, Bhutan, Democratic People's Republic of Korea, Malaysia),

nine countries are in the elimination stage (Algeria, Argentina, Iran, Saudi Arabia, Azerbaijan, Tajikistan, Turkey, Sri Lanka, Republic of Korea), seven countries in the prevention of malaria reintroduction phase (Egypt, Oman, Iraq, Syrian Arab Republic, Georgia, Kyrgyzstan, Uzbekistan), and four countries have recently been certified as malaria free countries by WHO (Morocco in 2010, United Arab Emirates in 2007, Turkmenistan in 2010, and Armenia in 2011).

Box 2.1. Definitions for stages towards malaria elimination [29,30]

Malaria control programmes are implemented in countries for reducing the malaria disease burden (incidence, prevalence, morbidity, and mortality rates) to a level at which it is no longer a public health problem. Continued intervention measures are required to maintain the reduction.

Pre-elimination programmes are implemented in countries to reorient their NMCP between the sustained control and elimination stages. During this period, high coverage with good-quality laboratory and clinical services should be reached, reporting and surveillance should be reinforced, followed by other programme adjustments to halt transmission nationwide.

Prevention of reintroduction programmes are implemented in countries that have either recently achieved zero cases and aim to maintain the situation, or in countries that are generally considered non-endemic, having been malaria-free for well over a decade, that have experienced recent outbreaks of locally acquired malaria subsequent to importation of parasites. The main activity is vigilance (surveillance and response) by the general health services, which can be combined with vector control and other interventions to reduce receptivity in vulnerable areas.

Malaria elimination is the complete interruption of the local mosquito-borne malaria transmission, i.e., reduction to zero of the incidence of infection caused by human malaria parasites in a defined geographical area as a result of deliberate efforts. Continued measures are required to prevent reestablishment of transmission.

Certification of malaria elimination is the official recognition of malaria-free status granted by WHO after it has been proven beyond reasonable doubt that the chain of local human malaria transmission by Anopheles mosquitoes has been fully interrupted in an entire country for at least 3 consecutive years.

Re-introduction of transmission is the renewed presence of a constant measurable incidence of cases and mosquito-borne transmission in an area over a succession of years. An indication of the possible re-establishment of transmission would be the occurrence of three or more introduced and/or indigenous malaria infections in the same geographical focus, for 2 consecutive years for *P. falciparum* and for 3 consecutive years for *P. vivax.*

Malaria eradication is the permanent reduction to zero of the worldwide incidence of infection caused by a particular malaria parasite species as result of deliberate efforts. Intervention measures are no longer needed once eradication has been achieved.

Despite the good progress over the past decade, malaria remains the most important human arthropod-borne disease worldwide. Over three billion people live at risk of being infected with malaria and developing disease. According to the last estimates, 198 million

cases and about 584,000 deaths occurred globally in 2013 representing a decrease in malaria case incidence and mortality rates of 30% and 47% since 2000, respectively [28].

However, the distribution is not equal: the African continent is a highly-endemic region accounting for most of the worldwide overall malaria cases (82%) and deaths (about 90%) compared to regions like the American continent which contributed with only a minor percentage of the global malaria burden with 427,000 confirmed cases (about 700,000 estimated cases) and 82 reported deaths (about 800 estimated deaths) in 2013 [28].

Approximately 120 million people in 21 American countries live at risk of malaria infection within the context of hypo-endemic and unstable transmission [28,31,32]. The Amazon basin shows the greatest transmission involving five countries (Brazil, Venezuela, Colombia, Guyana, and Peru) accounting for about 90% of the malaria burden in the continent (Figure 2.2). *P. vivax* accounts for 74% of the cases, *P. falciparum* for about 26%, and a limited number of cases (0.1%) are caused by *P. malariae* [28].

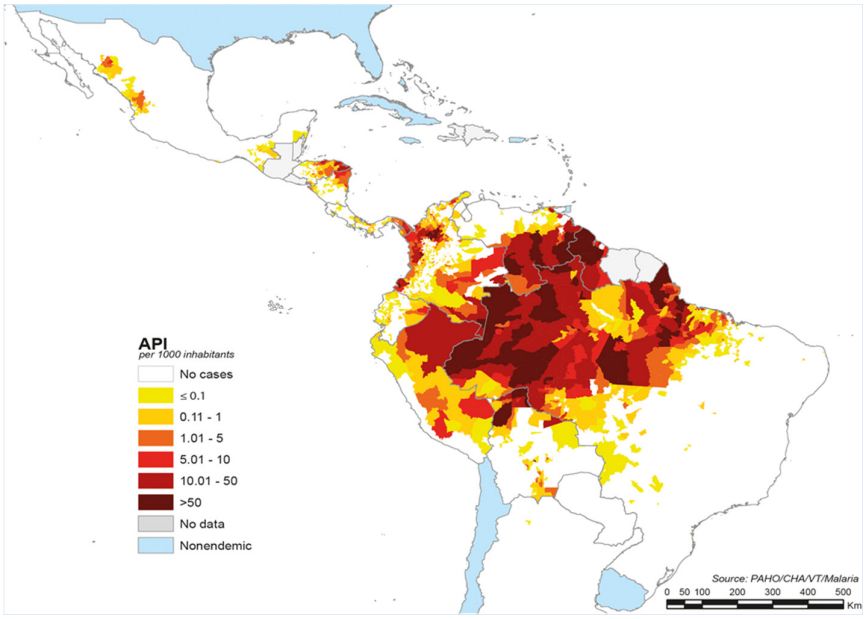


Figure 2.2. Distribution of malaria in the Americas according to the Annual Parasite Index in 2012. Source: Pan American Health Organization (PAHO)

2.2. Global malaria control and elimination efforts

A mathematical model demonstrating the effective interruption of parasite transmission through adult mosquito control was the scientific basis for the past malaria eradication efforts [33,34]. Encouraged by the early success of using dichloro-diphenyl-trichloroethane (DDT) against malaria vectors, WHO embarked on the Global Malaria Eradication Program (GMEP) in 1955. Eradication was defined as "the ending of the transmission of malaria and the elimination of the reservoir of infective cases in a campaign limited in time and carried out to such a degree of perfection that when it comes to an end, there is no resumption of transmission" [35]. Taking into account the particular outcomes of regional malaria control campaigns evidencing almost complete interruption of malaria transmission in selected areas (Greece, Italy, Cyprus, Venezuela, Argentina and the United States) [36], WHO designed a four-phased Eradication plan for NMCPs which was limited in duration (5-10 years) and in geographic scope. First as preparatory phase, a National Malaria Eradication Service (NMES) was established in each participating country to coordinate and execute all phases of the programme. Second, an attack phase was implemented in endemic regions involving deployment of antimalarial drugs (primarily chloroquine (CQ)) to reduce infection prevalence in human populations, and extensive spraying of DDT to shorten mosquito vector viability. Third, once the surveillance data confirmed the interruption of transmission, a consolidation phase based on intense active case detection (ACD) allowed the identification and elimination of the remaining malaria infections. If no autochthonous cases appeared for three years, malaria was recognized as being eradicated from that region. At that point, the region would enter the maintenance phase, during which malaria surveillance responsibilities were transferred to the national health services.

GMEP campaigns succeeded in altering the global distribution of malaria, reducing radically the morbidity and mortality in many endemic areas of Europe, North America,

the Caribbean, Asia and South-Central America [37] and eliminating the disease in over 30 countries (mostly developed countries) [38]. The remarkable success in these areas was aligned with the improvement of the local public health infrastructure, the commitment of financial and human resources that were not realized until after the Second World War, and the sustained political commitment. However their limited effectiveness in high transmission areas such as the sub-Saharan Africa demonstrated that the total eradication of malaria through single strategies was not feasible [39]. In addition, the development of insecticide resistance, the operational and financial limitations to sustain the intensive eradication efforts in some regions, and the reallocation of funds to more pressing public health problems in other regions where malaria had successfully been reduced but not eliminated resulted in the WHO abandoning the global eradication program 1969 [36].

From 1969 to 1991 the malaria control strategy changed to containment which was mainly focused on technical issues against a background of rising malaria cases, on the research and development of new tools (e.g. vector control and insecticide-treated nets), as well as on the better understanding of the social, economic and cultural dimensions of malaria. During this post-eradication period, newly independent states of Africa struggled to establish broad-based health systems and primary health care, and did not receive enough support for the malaria control.

By 1992, the combination of a worsening malaria situation and promising technical developments led to renewed global focus on malaria control. In 1998, WHO launched Roll Back Malaria (RBM) initiative as a mechanism for improving control interventions and attracting money for malaria control [35]. In 2000, the Abuja Declaration defined the progressive intervention coverage targets for reducing the malaria disease burden, emphasizing the fact this could only be achieved through vastly strengthened local health systems [37].

Since 2003, the Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM), the World Bank's Booster Program (WBBP), the US President's Malaria Initiative (PMI) and many other donors have been supporting the widespread implementation of malaria control measures such as long-lasting insecticide-treated bed nets (LLINs), indoor residual spraying (IRS), the use of malaria rapid diagnostic tests (RDTs) and the provision of efficacious anti-malarial treatments based on artemisinin-based combination therapy (ACT). The early evidence of decreasing malaria transmission in regions with high coverage of interventions renewed the optimism for malaria elimination programmes. Thus, following declarations at the Malaria Forum in 2007 convened by the Bill & Melinda Gates Foundation, and subsequent support voiced by the WHO, the RBM Partnership, and many other organizations, the paradigm of malaria control and elimination has been extended to encompass an ultimate goal of malaria eradication [29,40].

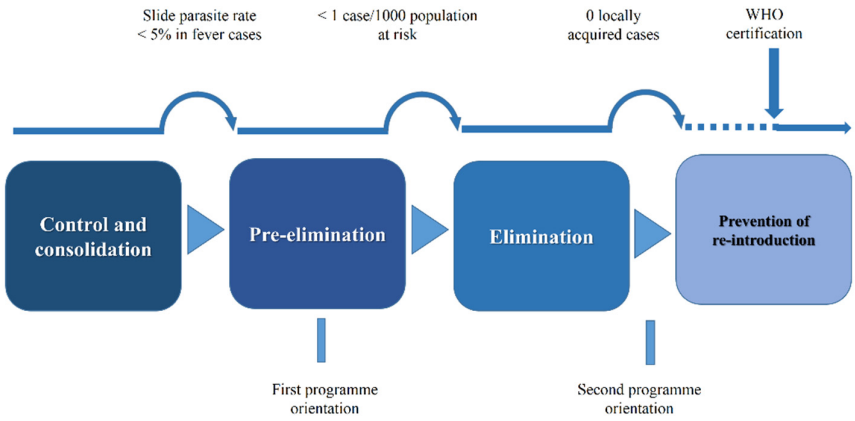


Figure 2.3. WHO malaria elimination continuum
Source: Global Malaria Action Plan. RBM, 2008.

In 2008, the RBM unveiled the Global Malaria Action Plan (GMAP) [29], which became the first single comprehensive blueprint for global malaria control and elimination [41]. The Plan characterizes different phases of malaria control as NMCPs progressively

reduce transmission, though it is understood that these phases are part of a continuum rather than abrupt shifts (Figure 2.3). At high levels of transmission, initial efforts are focused on scaling up control strategies for impact. LLINs, IRS, preventive treatment for pregnant women and access to effective treatment with ACT are considered as primary strategies. Sustained control efforts subsequently lead to further transmission reduction. As very low levels of transmission are reached, NMCPs move from control to pre-elimination and elimination, and finally the prevention of reintroduction once elimination is reached.

In 2015, WHO aims to launch a new technical strategy for 2016–2030 [42] which will set milestones and goals for burden reduction and elimination beyond 2015, the WHO vision being "A world free of malaria". This can be achieved through country-by-country (and later regional) elimination of malaria infection, followed by global malaria eradication.

2.3. Transmission metrics in low transmission areas

Many factors have been described as potential causes of the limited success of GMEP campaigns, including the collapse of funding [36,38]. Retrospective analysis of eradication efforts have also revealed that there were failures of national monitoring and evaluation (M&E) systems in providing guidelines for establishing quantitative (i.e., metrics) and operationally definitions and milestones for measuring progress towards control in a range of contexts [14].

As NMCPs reduce malaria transmission in their respective countries, they will need to use metrics that ensure an accurate description of the malaria heterogeneity and measurement of changes in the malaria transmission intensity across appropriate geographical scales. Those metrics should be chosen taking into account different factors (such as costs, sensitivity-specificity, feasibility, acceptability, seasonal variations)

[11,16]. Table 2.1 summarizes the advantages and disadvantages of four different transmission metrics in low transmission settings.

Table 2.1. Advantages and disadvantages of the use of transmission metrics in low transmission areas

Metrics	Advantages	Disadvantages
Entomological inoculation rate (EIR)	-Gold standard for measuring malaria transmission intensity. -Familiar to decision-makers	-Validity affected by the spatial, temporal and seasonal variability in vector density. -Variation in sampling methods for mosquitoes. - Data collection is resource-intensive and time-consuming.
Incidence rate of clinical malaria confirmed by microscopy	-Obtained from routine surveillance. -Able to be integrated with other programmatic data. -Familiar to decision-makers.	-Validity affected by factors that influence PCD effectiveness and case notification, e.g. high proportion of asymptomatic infections, access to health facilities, treatment-seeking behaviour, quality of microscopic diagnosis, information systems, etc.
Parasite rate (PR)	-Common used as metric of endemicity. -Metric obtained from cross-sectional surveys. -Familiar to decision-makers -Provide information about current infection.	-Validity affected by the sensitivity of the malaria diagnostic test used. -Affected by seasonal variations. -Large sample sizes are needed at low levels of infections (increase in costs and resources).
Seroconversion rate (SCR)	-Easy sample collection during cross-sectional surveys. -Cheap laboratory techniques. -Provide information about past infection. -Less affected by seasonal variations.	-Not used by decision-makers. -Require previous validation of antigens (multiple) for detection of antibodies.

The **Entomological inoculation rate (**EIR) is the number of infectious bites received per person per period of time (typically year) [43], and can be estimated using direct measurements of the human biting rate (HBR, the number of bites per person per time unit) and the sporozoite rate (SR, the proportion of mosquitoes that are infectious) supplemented by models of mosquito populations. Measuring HBR involves capturing and counting the number of mosquitoes that attempt to feed on a human, while the determination of SR requires the identification of sporozoites in salivary glands of the captured mosquitoes.

EIR is usually considered as gold standard for measuring malaria transmission and commonly used by NMCP managers. However, the precision and accuracy of EIR estimates can be affected by the spatial, temporal and seasonal variability in the vector density, as well as by the lack of standardization of available methods for measuring both HBR (i.e. human-landing catches, exit traps, CDC light traps) and SR (i.e., dissection, enzyme-linked immunosorbent assays-ELISA, Polymerase chain reaction-PCR) [17]. Although repeated sampling over time and at multiple locations can overcome the heterogeneity in the mosquito dispersal and feeding behaviour, and increase the sensitivity of EIR [44]; this sampling strategy would not be cost-effective in low transmission areas given the large number of mosquitoes to collect before finding one that is infected [17].

The **incidence rate of clinical malaria** is the rate at which new episodes of clinical malaria (fever plus confirmed parasitaemia) occur in a population. Throughout the thesis, the term "incidence" refers only the number of new cases, while the term "incidence rate" takes into account the variable time periods during which individuals are disease-free and thus "at risk" of developing malaria. In its calculation, the numerator is the number of new malaria episodes that occur in a defined time period, and the denominator is the population at risk of experiencing the event during this period [45]. Since it may not be possible to measure disease-free periods precisely, the denominator is often calculated approximately by multiplying the average size of the study population by the length of the study period. This is reasonably accurate if the size of the population is large and stable and incidence is low. NMCPs frequently use a proxy measure of the incidence rate, i.e., the **annual parasite index** (API), which is expressed as the total number of cases per 1000 person per year at risk. It results from the product of the slide positivity rate (SPR) and the annual blood examination rate (ABER) divided by 10 [11,36]. SPR is the proportion of individuals examined by light microscopy (LM) or RDTs who test

positive for parasitaemia, while ABER is the proportion of the total population examined for parasitaemia.

Since SPR and ABER can be obtained from the routine surveillance data, they allow NMCPs to rapidly detect changes in incidence rates, and to easily integrate this metric with other types of data to help evaluate and improve programmes [16]. API ascertained through passive case detection (PCD) alone only records those symptomatic individuals who are detected at health facilities, underestimating the intensity of transmission for the entire population. Other factors related to PCD that can affect the accuracy of API include the access to the health facilities with LM or RDTs [46], the quality of microscopy-based diagnosis [47], and social and cultural factors that influence treatment seeking behaviors at community level [48]. Adding ACD to detect symptomatic individuals who do not seek for attention as well as asymptomatic infections may generate more accurate API, but is costly and logistically more challenging since it involves repeated scheduled visits of targeted populations [49].

Parasite rate (PR), the proportion of individuals with parasitaemia at given point in the time, is one of the most widely used metrics of endemicity [50]. PR is commonly measured in cross-sectional surveys by examining blood samples using LM or RDTs of a representative sample of the population. While the operational utility of these diagnosis tests cannot be discounted, there is enough accumulated data that suggest that LM and RDT diagnoses underestimate the PR [51]. Epidemiological studies using molecular methods such as PCR have detected on average two fold more infections than detected by LM, uncovering an important proportion of very low-parasite-density infections particularly in low transmission areas [52-54].

Besides the method used for parasite detection, the accuracy of PR can also be affected by the distribution of both parasite infections and parasite densities in the population at the time of the blood sample collection. Since that distribution can vary according to the malaria transmission season [16,18], the level of immunity at the population [55], and the

recent history of anti-malarial drug use, it is unlikely that a single cross-sectional could accurately capture PR [56]. Repeated surveys using the same methodology in the same population at similar time periods can give enough precision for monitoring changes in malaria transmission, but not in all epidemiological contexts. The detection of the impact of malaria control efforts in low transmission areas become very difficult when PR estimates are below 1-5% [11]. In addition with low levels of positive tests, surveys will require large sample sizes to achieve an appropriate degree of statistical power; thereby increasing the resources and costs required for the measurement.

Seroconversion rate (SCR) is defined by the percentage of population that develop antibodies against malaria over a fixed time period. It is calculated by fitting a reverse catalytic model to age-specific prevalence of antibody responses to a single or defined set of recombinant malaria proteins expressed by various stages of the parasite lifecycle [20]. More details about the reverse catalytic model are presented in Box 2.2. Blood samples for generating seroprevalence data can be collected in cross-sectional surveys [57], school surveys or in health facilities taking into account the advantages and disadvantages of each survey type. Experimentally, the quantification of antibodies on blood samples collected on tubes or filter paper is relatively easy to perform using simple and cheap laboratory techniques such as ELISA [58].

Since seroprevalence represents cumulative exposure to infection [59], SCR is able to reconstruct the history of exposure in the study population even without knowing previous baseline data [21,22]. Its accuracy increases when serological surveys include enough individuals of different ages, and the detection of antibodies is performed using multiple antigens given the possibility of heterogeneous immunity in the population [16,60]. SCR as metric for malaria transmission intensity may be particularly suited to low endemic areas as antibodies remain in the blood longer than malaria parasites and are thus easier to detect and less subject to seasonal variations than other measures [57].

Box 2.2. Catalytic models

Catalytic models were first used to model age-specific changes in the force of infection, the rate at which individuals who are susceptible (S) become infected (I). For common viral diseases such as measles the presence of antibodies indicates that the person has been infected and become immune to subsequent infection, then the seroconversion rate (SCR, λ) or force of infection can be estimated directly from the age-seroprevalence data using **a simple catalytic model** and maximum likelihood methods.

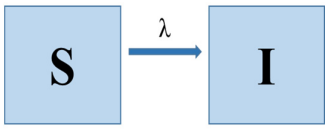
Reversible catalytic models describe the infectious process where a susceptible (S) host becomes infected (I), and then reverts to the state of susceptibility after the infection is self-limited or cured by medical interventions.

The model generates a seroconversion rate (SCR, λ), which represents the rate at which people become seropositive, and a rho (ρ) which represents the rate at which people revert back to seronegativity (seroreversion rate). Despite its simplicity, this model have been frequently used for estimate the force of infection of malaria in well-defined areas.

The main assumptions of the model are: population is constant, migration is negligible, there are no individual differences in SCR between subjects, and the disease transmission in the population is at an endemic steady-state; i.e., SCR is assumed constant over time.

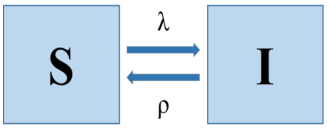
Formulas and schematic representations of catalytic models

Simple catalytic model



$$P_t = 1 - \exp(-(\lambda)t)$$

Reversible catalytic model



$$P_t = \frac{\lambda}{\lambda + \rho}(1 - \exp(-(\lambda + \rho)t)$$

P_t is the proportion of individuals aged t that are seropositive

2.4. Modelling for malaria control and elimination

A considerable number of modelling studies have addressed the transmission of infections throughout populations [61]. The traditional epidemiological approach consists of compartmentalizing the total host (human) population into susceptible (S), exposed (E), infected (I) and recovered (R) sub-populations, and/or the total vector population into susceptible (U), exposed (L), and infected (V) sub-populations [62]. Many variations of this paradigm have been proposed over the past century.

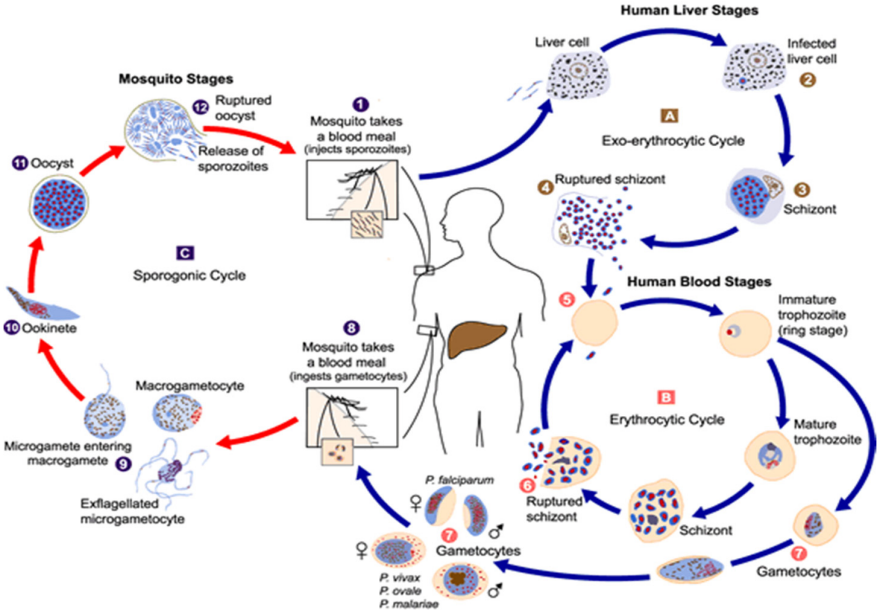


Figure 2.4. **Malaria life cycle**. During a blood meal, a malaria-infected female *Anopheles* inoculates sporozoites into the human host (1). Sporozoites infect liver cells (2) and mature into schizonts (3), which rupture and release merozoites (4). In *Plasmodium vivax* a dormant stage "hypnozoites" can persist in the liver and cause relapses weeks, or even years later. After this initial replication in the liver (exo-erythrocytic schizogony [A]), parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony [B]). Merozoites infect red blood cells (5). The ring stage trophozoites mature into schizonts, which rupture releasing merozoites (6). Some parasites differentiate into sexual erythrocytic stages (gametocytes) (7). Blood stage parasites are responsible for the clinical manifestations. The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal (8). The parasites' multiplication in the mosquito is known as the sporogonic cycle [C]. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes (9). The zygotes in turn become motile and elongated (ookinetes) (10) which invade the midgut wall of the mosquito where they develop into oocysts (11). The oocysts grow, rupture, and release sporozoites (12), which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle (1). Source: *Parasite Image Library*.

Modelling malaria transmission require a good knowledge and understanding of the malaria life cycle (Figure 2.4) and the first malaria transmission models originated with Sir Ronald Ross. While working at the Indian Medical Service in 1890's, Ross demonstrated the life-cycle of the malaria parasite in mosquito, and was one among the first to publish a series of papers using mathematical functions to study malaria transmission in the early 1900s [63-65]. In 1915, Ross introduced the first deterministic differential equation model intended to explain the relationship between the incidence of malaria in the human and mosquito population [66].

After about forty years, George Macdonald [34] in 1950s expanded the Ross model by introducing information about the time Plasmodium spends growing and multiplying inside the female mosquito before it harbours infectious sporozoites in its salivary glands ("sporogonic cycle"= about 10 days). Since the mosquito needs to survive beyond this latent period to be able to transmit the disease, mosquito survival was recognized as the most critical parameter to maintain malaria transmission. This approach provided the rationale for GMEP campaigns focused on the use of DDT to kill mosquitoes [33,36]. Then in 1991, Anderson and May added to Macdonald's model the time (about 21 days) lag a person exposed to *P. falciparum* needs to become infectious with gametocytes in the blood ("latency") [67].

Summarizing, the three basic models use the simplest scenario for predicting the progression of malaria infections in the host and mosquito population through the compartmentalization of populations and the incorporation or not of the latency periods of *Plasmodium* in the mosquito and the human, assuming that total populations remain constant over time (Box 2.3). As shown in Figure 2.5, the inclusion of latency periods in mosquitoes and humans not only reduces the long-term prevalence of infected mosquitoes (V) and humans (I), but it also reduces the rates of progression to these infections. See Annex (Chapter 9, text S2.1) for R codes of basic malaria models.

Box 2.3. Basic malaria models

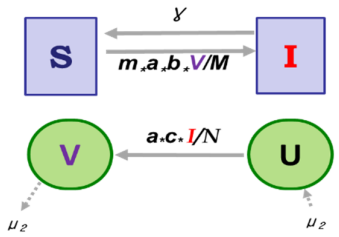
Ross model divides the human population (N) in susceptible (S) and infected (I) sub-populations, and the mosquito population (M) in susceptible (U) and infected mosquitoes (V). Individuals in S become I at a rate that is determined by the mosquito density (*m*), the human feeding rate per mosquito (*a*), the infectiousness of the mosquito population (V/M), and the susceptibility of an individual after being bitten by an infectious mosquito (*b*). In absence of treatment, infected individuals (I) recover completely without conferring long-lasting immunity at a rate (γ) that is determined by the reciprocal of the average time for the spontaneous clearance of parasites from blood. Regarding the mosquito population (M), S become V at a rate determined by: the human feeding rate per mosquito (*a*), the proportion of infectious individuals in the human population (I/N), and the mosquito susceptibility to infection after biting an infectious individual (*c*). Mosquitoes do not recover from infection due to their short life span. Both infected humans and mosquitoes are infectious since the time they acquired the infection.

Unlike Ross model, **Macdonald model** divides the mosquito population (M) into three compartments (U, L, V). Mosquitoes are not infectious immediately after infection; they remain in a latent state (L) for a specific time period before developing sporozoites and becoming infectious (V). The estimation of surviving mosquitoes by the end of the latent period requires an adjustment by the factor $e^{-\mu 2*n}$ which incorporates a constant mosquito mortality rate (μ_2) and the average mosquito latent period (*n*).

Anderson and May model added the latent period in humans. Then, the model allows the human population (N) to move between three different states (S, E, I). After being bitten by an infectious mosquito, individuals progress to the latent state (E), from which they develop gametocytes in the blood and become infectious after a latent period (*l*). The estimation of individuals who move from E to I at the end of latent period requires an adjustment by the factor $e^{-(\mu 1+\gamma)*l}$ which incorporates a constant human mortality rate (μ_1) and the average human latent period (*l*).

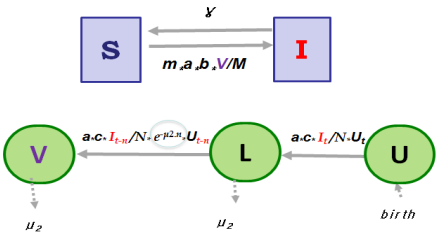
Schematic representation of models and formulas for basic reproductive ratio (R_0) are presented in the right side of the box.

Ross model



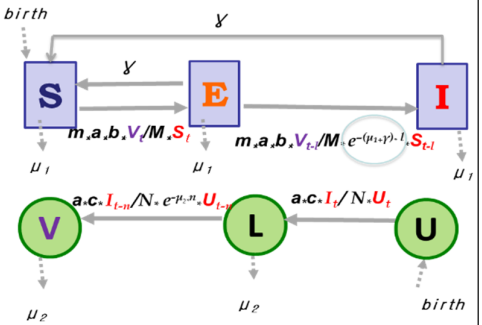
$$R_o = \frac{m * a^2 * b * c}{\gamma * \mu_2}$$

MacDonald model



$$R_o = \frac{m * a^2 * b * c * e^{-\mu 2*n}}{\gamma * \mu_2}$$

Anderson and May model



$$R_o = \frac{m * a^2 * b * c * e^{-(\mu_1+\gamma)*l} * e^{-\mu_2 * n}}{\gamma * \mu_2}$$

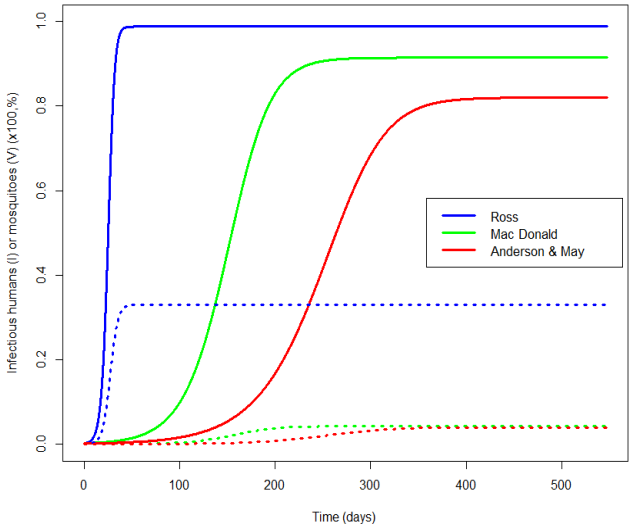


Figure 2.5. Effect of the incorporation of latency periods in the prevalence of infection in humans and mosquitoes. Graph generated using R considering the following parameters*: N=500, M=15000, I=1, m=30, a=0.2 day^{-1}, b=0.2, c=0.5, γ=1/210 day^{-1}, μ_2=0.2 day^{-1}, n=10 days* (used in MacDonald and Anderson &May model), *μ_1=0.00005 day^{-1}, l=21 days* (used only in Anderson &May model*)*. Continuous lines represent prevalence of infected individuals (*I*), while dotted lines prevalence of infected mosquitoes (*V*).

The basic reproductive number (R_0) can also be used to describe and compare these basic models. Ro is the number of infections that one infection generates on average over the course of its infectious period in a population with no previous exposure to malaria and no malaria control [67,68]. Regarding compartmental models, R_0 is defined as the number of secondary infections arising from a single infection in an otherwise uninfected population [34] and can be used to determine whether or not an infectious disease can spread through a population: malaria spreads in a population only if $R_0>1$ (epidemic), remains in an endemic stable state if $R_0=1$, or disappears if $R_0<1$. The incorporation of latency periods of mosquitoes and human involves the adjustment of the Ross R_0 formula by negative exponential functions, resulting therefore in further reductions of R_0 values (see Box 2.3).

Despite their relative simplicity, basic models can be used in describing how the control and elimination interventions can influence the transmission of the disease [25]. Parameters that regulate the fraction of human population that will enter into the exposed (E) and infectious compartments (I) (m, a, b, μ_2) may be modified by specific interventions resulted in changes in the intensity of the disease transmission, which can be measured though the Ro. Similarly, changes in the duration of the infection (γ) will also affect Ro estimates.

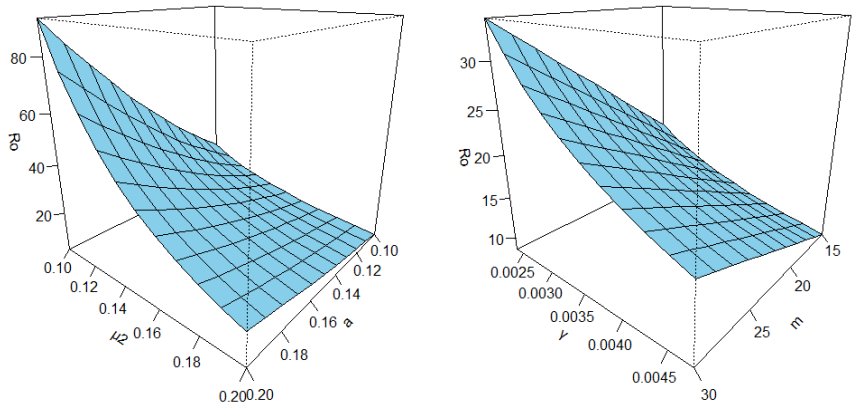


Figure 2.6. Effect of modifying the parameters values used in figure 2.5 for Macdonald model on the Ro predictions. Baseline values: N=500, M=15000, I=1, m=30, a=0.2 day^{-1}, b=0.2, c=0.5, γ=1/210 (0.0047) day-1, μ2=0.2 day^{-1}, n=10 days (used in MacDonald and Anderson &May model), μ1=0.00005 day^{-1}, l=21 days (used only in Anderson &May model). Continuous lines represent prevalence of infected individuals, while dotted lines prevalence of infected mosquitoes.

Figure 2.6 shows the effect of modifying the parameters values used in Figure 2.5 for the Macdonald model on the R_0 predictions. It is not surprising that the square dependence of the human feeding rate (a) implies that halving the biting rate is more effective than halving the mosquito density (m). However according to the model, interventions that reduce the survival of mosquitoes (i.e., increasing the mortality, μ_2) would be the most effective in reducing malaria transmission. As mentioned before, this approach provided

the rationale for GMEP campaigns focused on the use of DDT. This strategy not only increased the mosquito mortality rate (μ_2), but also decreased the mosquito density (m) and the human feeding rate (a).

Several models have been developed that expand the basic models by considering factors, such as age-related differential susceptibility to malaria in human populations, acquired immunity [69], and spatial and genetic heterogeneity of the host and parasite [70,71]. Basic and complex models have been widely used to evaluate effects of different strategies such as vector control [72], the use of future vaccines [73], the use of artemisinin-based combination therapies, as well as different levels of anti-malarial resistance [74].

Modelling to support malaria control and elimination

Despite providing the rationale for the eradication, the overall role of mathematical modelling in the GMEP was limited. Modelling informed the design of the attack phase of malaria eradication [34,35], but not the design or implementation of other phases, and there were no provisions made to evaluate or update the design of the GMEP despite its obvious limitations. Modelling as part of M&E could have helped to anticipate, analyse, and adjust for some problems that developed during the eradication efforts [14], such as the reduced impact of GMEP campaigns due to the spread of mosquito resistance to DDT.

Since the GMEP, substantial advances have been made in the theory of simulation modelling of malaria transmission, but NMCPs are still not able to integrate these models with M&E activities and with the revision of national plans. As countries scale up malaria control efforts and reach high intervention coverage targets, they are faced with the question of what to do next. The strategy for maintaining and enhancing the achieved reductions in transmission is not obvious. It is often not clear whether current coverage

levels of interventions would continue to reduce transmission, or whether new interventions are necessary to keep the reduction trend and even to accelerate progress toward the elimination of the disease. Mathematical modelling can build on available data, test multiple scenarios and combinations of intervention strategies, and make verifiable predictions on what can be expected from these strategies.

Modelling can help inform decision-making around malaria control because of the disease's complex biological systems, the considerable infrastructural and cost requirements of prevention and elimination strategies, and the rapid pace of change in global planning and national programming to halt malaria. Findings from modelling can provide valuable data to help inform a range of decision-making on issues such as identifying potential effective combinations of interventions, setting feasible coverage targets, devising target product profiles for new interventions, anticipating the implications of introducing new interventions, understanding the potential for malaria resurgence; and establishing how malaria surveillance and M&E systems can best enhance the country progress [75].

Like with all mathematical models of biology, limitations of malaria models are associated with the trade-off between parsimony and the fitting of details to what is known about the dynamics being modeled. Although a model is only useful if it represents a simplification, indicating which elements of the processes being analyzed are important, the better the fit to reality, the more likely are the predictions to be accurate. Model predictions should be interpreted taking into account their underlying assumptions, some of which are intended to simplify the complex malaria transmission dynamics, while others attempted to fill the knowledge gap in the transmission of the disease.

Mathematical modelling should involve partnership and good communication between technical experts in mathematical modelling, experts in malaria field and laboratory science, and health policy decision-makers. Models produce the most useful information when they are formulated with important biological, economic, and practical realities in mind and when their results are interpreted with care considering the model's limitations and underlying assumptions.

2.5. Peru – country profile

Peru, located along the South America's central Pacific coast, is home to about 30 million inhabitants living in 1,838 municipalities in 25 regional governments (24 departments and the constitutional province of Callao) [76]. With a total surface area of 1,285,220 km^2, Peru has the third greatest biodiversity in the world, hosting 84 of the 117 recognized ecosystems [77]. Three very different natural regions are identified with contrasted climates: the semi-desert Coast, the rugged Andean Mountains, and the tropical Amazon Basin.

Despite the recent world financial crisis, Peru has logged fifteen consecutive years of positive economic growth. Under successive three presidential transitions (2001, 2006 and 2011), the Peruvian economy has proven resilient, driven by free markets and grounded in clear macroeconomic policies. Peru's gross national income (GNI) per capita was 3,990 US dollars (USD) in 2008 and its economy has been classified as upper-middle-income country since 2009 [78]. However, while poverty significantly declined nationwide from 54% in 2001 to 25.8% in 2012, a large part of the population is still unable to benefit from such economic growth, with poverty rates exceeding 50% in rural areas, mainly in the Andean Mountains and the Amazon Region [79].

Income levels in the country remain highly unevenly distributed with an estimated Gini coefficient of about 0.5 [80]. Furthermore, socio-economic inequalities between groups have increased over the recent years, thereby reinforcing traditional ethnic and geographical divisions [81]. The differences are most pronounced between the coast, which covers only 16% of the territory but holds 60% of the population and 70% of the wealth, and the Amazon Basin, which covers 50% of the territory, yet contains only 12% of the population and 7% of the wealth. Such disparities have resulted in substantial variations in life expectancy. While a newborn in one of the poorest districts in the Amazon Basin, can expect to live to around 60 years, in the more affluent areas of the Coast, such as Lima, newborns are anticipated to live almost 15-20 years longer [76].

The good economic performance achieved in recent years, together with targeted social spending and social programs has made significant progress possible in the fulfilment of some MDGs, such as: reduction in the extreme poverty from 23% to 7.6% (1991-2010); reduction of infant mortality from 53 per 100 live births to 17 per 100 live births (1993-2012); and reduction in chronic child malnutrition from 26.5% to 15.2% NCHS (1999 – 2011) [82]. Despite these achievements, the health sector still faces important big challenges: a) the total health expenditure continues to be among the lowest in Latin America (4.8% of National Gross Domestic Product-GDP) [80]; b) the health and health care inequity remains mainly in preventive services [83]; c) the decentralization process and sub-national capacity building for management of social production of healthcare are still incomplete [84]; d) the increased risk of non-communicable chronic diseases and external causes (e.g., road crashes); and e) the persistence of communicable diseases, such as: tuberculosis, dengue and malaria.

2.6. Malaria control in Peru

Malaria remains one of the most important public health problems in Peru. As shown in Figure 2.7, the incidence of cases has fluctuated dramatically over the last 75 years, however the history of malaria control suggested on two different occasions that malaria elimination in the country could be feasible. Indeed, this was observed during the dramatic drop of malaria burden during the eradication efforts in the 1960s, and more recently, the early success in decreasing the malaria incidence following the implementation of comprehensive control strategies during the PAMAFRO project in the 2000s.

After peaking with over 95,000 cases in 1944 [1], malaria dropped drastically in the following years, and between 1960 and 1970 annual incidence rates remained below 1 case per 1,000 inhabitants with the minimum levels registered in 1965, when only about 1,500 cases were reported [85]. This achievement can be attributed to the use of DDT for vector control campaigns since 1946, and to the shift of malaria control to eradication since 1957 [1]. Malaria eradication efforts were implemented by the Peruvian NMES and supported by the National Health Fund and Social Welfare, the Interamerican Public Health Service (IPHS) and UNICEF. It was initially planned that five years of aggressive antimalarial and insecticide application would eradicate malaria [86]. The major vector in the Amazon (i.e., *An. darlingi*) was eliminated, and almost the entire coast, the inter-Andean valleys, and the Southern Amazon were malaria free by 1970 [86,87]. However, the development of DDT resistance, the decrease of funds to maintain the interventions, and the transfer of malaria control responsibilities to the health services did not allow for keeping the malaria control progress [86].

During the 1980s, the Peruvian malaria control program was unstructured [86]. DDT use was first halted in the Amazon in 1988, and then stopped across the country. The reintroduction and later spread of *An. darlingi* in the Amazon [85], as well as the

occurrence of drug-resistant *Plasmodium falciparum* strains to CQ in the northern coast [88] and to CQ and sulphadoxine-pyrimethamine (SP) in the Amazon [89,90], likely enhanced the huge malaria epidemic associated with El Niño Southern Oscillation (ENSO) climatologic phenomenon in the late 1990s [2]. After heavy rains and flooding, malaria increased dramatically reaching a peak of more than 200,000 cases in 1998, of which almost half were due to *P. falciparum*. The Amazon and the northern coast were the most affected regions, accounting respectively for approximately 45% and 40% of the total malaria cases, and for 45% and 50% of the total *P. falciparum* cases in the country during the 1997-1998 epidemic period [91].

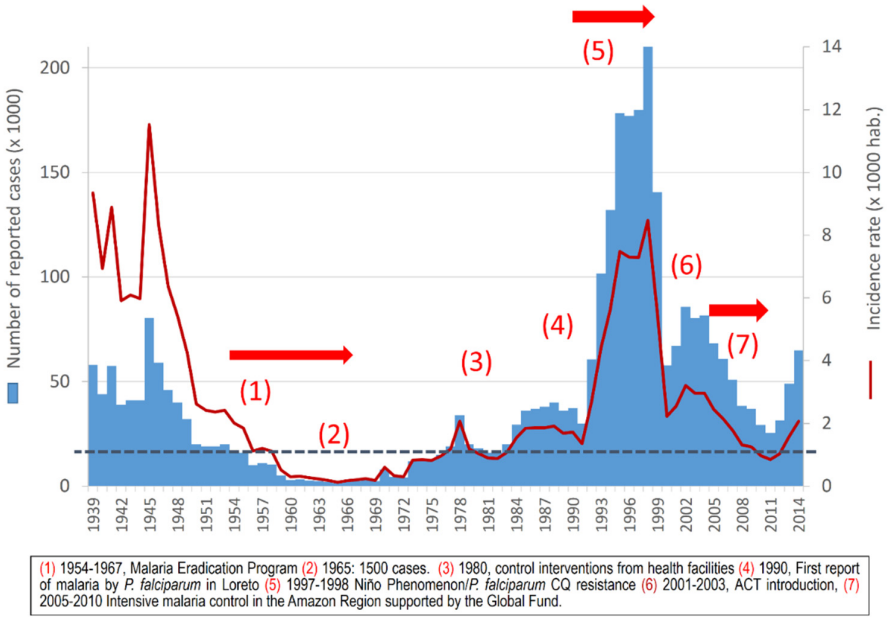


Figure 2.7. Historical trends of reported malaria incidence in Peru: 1939-2014.
Source: Peruvian Ministry of Health.

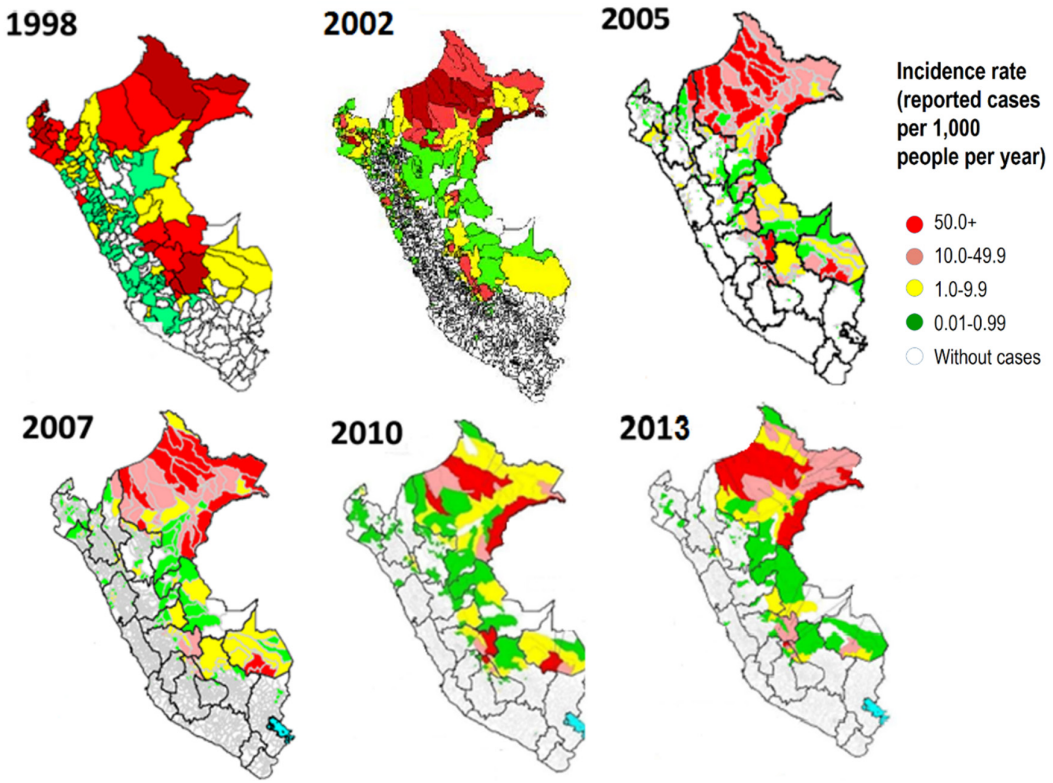


Figure 2.8. Malaria risk map of Peru based on annual incidence rates (annual parasitological index, API).
Source: Peruvian Ministry of Health

After the epidemic, annual incidence dropped markedly, and between 2000 and 2005 it remained between 70,000 and 80,000 cases (20%-25% due to *P. falciparum*), followed again by a steady decrease until 2010 and 2011, when only 29,355 and 23,075 cases were reported, respectively. The significant reduction in malaria incidence (around 90%) from 1998 to 2011 changed completely the malaria risk map in the country, with each year a larger predominance of low transmission areas over high and moderate transmission areas (Figure 2.8). After almost 40 years and for the second time in the history of malaria in Peru, the malaria incidence rate was below 1 case per 1,000 inhabitants in those two consecutive years (2010 and 2011) (Figure 2.7). This achievement can be attributed to the strengthening of the NMCP, the changes in the malaria treatment policy, and the implementation of comprehensive malaria control interventions in the most endemic areas with strong support of international donors (e.g., US Agency for International Development (USAID), and the GFATM.

Indeed, following drug efficacy studies in the late 1990s and early 2000s and showing increasing level of *P. falciparum* resistance to CQ or SP in the Amazon, and the northern coast), the NMCP decided to introduce two different ACTs as the first line treatments for *P. falciparum, i.e.* mefloquine (MQ)-artesunate (AS) in the Amazon Region, and SP-AS in the Pacific northern coast [89]. With these changes, Peru became the first country in the Americas to use ACTs for *P. falciparum* malaria and the first country in the world to use two different ACTs in different regions [91]. Regarding *P. vivax* malaria, treatment guidelines were also adjusted following adherence problems to the radical regimen based on 14 days Primaquine (PQ) together with CQ (3 days). Thus the PQ regimen was shortened to 7 days at increased daily dose (0.5- instead of 0.25mg/kg/day). In addition, guidelines encouraged health facilities to offer directly observed therapy (DOT) for malaria, and started a 28-day follow up for all patients diagnosed with malaria. Despite their inclusion in the previous treatment guidelines of 1994 [89], both DOT and the 28-day follow up of confirmed malaria cases had been only partially accomplished.

The new malaria treatment policy was implemented faster in the northern coast than in the Amazon, mainly due to the better accessibility to the malaria endemic areas (i.e., peri-urban communities) in the northern coast that facilitated the training and supervision of the local health facilities and staff. PCD and treatment of confirmed malaria infections with ACT, together with the implementation of community-based environmental management strategies to control the predominant vector *An. albimanus* (such as the shift of rice cultivation patterns), have probably contributed to the steady decline in malaria incidence observed in the northern coast since the early 2000s.

In the Amazon Region, the new treatment policy took more time to be efficiently implemented. Since 2005, the implementation process was powered by the Global Fund's Malaria Project "PAMAFRO" [4] by increasing the accessibility and quality of microscopy-based diagnosis, ensuring the availability of the antimalarial drugs, and the periodical training and supervision of local health workers, even in the most remote endemic areas. PAMAFRO was simultaneously applied at the border areas of Venezuela, Colombia, Ecuador, and Peru between October 2005 and September 2010, with common control strategies, including early diagnosis and treatment, ACD interventions in communities of high malaria risk, mass distribution of LLINs, community participation, and intensified health education and promotion campaigns. During this period, similarly to the majority of intervention areas of the project, malaria declined drastically in Loreto, from 54,291 reported cases in 2005 to 11,446 in 2010 [3].

Alarmingly since 2010, the number of malaria cases is rising again in Loreto, with the total number of reported *P. falciparum* and *P. vivax* malaria cases increasing from 2,238 to 10,243 and from 9,208 to 52,323 cases, respectively, from 2010 to 2014. Currently, Loreto reports the vast majority of malaria cases (94%) in the country. The resurgence of malaria in the Peruvian Amazon after 2010 is thought to be associated with the limited capacity of NMCP to keep up with the intense control interventions implemented during the PAMAFRO project ended. The reallocation of funds to more pressing public health

issues in the region (particularly dengue) after assuming that malaria was "under control", and the unusual heavy rains since 2011, causing damage and flooding to many rural communities located along the Amazon river and all its tributaries, further worsened the situation. Currently, five strategies are prioritized for malaria control in the country: a) surveillance, case identification and effective, free, and timely treatment of confirmed infections; b) distribution of LLINs when available; c) behaviour change communication (BCC) activities; d) community participation in environmental management; and e) entomological surveillance and integrated and selective vector control.

Malaria surveillance relies mainly on PCD with microscopy. Patients presenting with fever or any other symptoms compatible with malaria are systematically tested by microscopy at health facilities and treated with CQ for 3 days (10mg/g on days 1 and 2, and 5mg/kg on day 3), plus PQ for 7 days (0.5 mg/kg/day) if *P. vivax* malaria is confirmed, or with MQ (12.5 mg/kg/day for 2 days) plus AS (4 mg/kg/day for 3 days) if *P. falciparum* malaria is confirmed. According to treatment guidelines all health facilities should perform DOT with these treatment combinations [92].

According to the current guidelines [92], any unusual recent increase of malaria cases detected by PCD at local level (local outbreak) should trigger reactive case detection (RACD) which consists of systematic screening for malaria by microscopy of all individuals living in close proximity to passively detected cases, and the treatment of confirmed infections irrespectively of symptoms. However, due to the lack of evidence-based guidelines, the criteria and procedures to implement RACD interventions have often been arbitrarily decided and are subject to annual variations in logistical and financial resources. In addition, the NMCP has recently introduced focal screening and treatment (FSAT) to further decrease the malaria burden in the Amazon Region. FSAT interventions are implemented based on prior information about monthly malaria incidence and specific high-risk groups in geographical areas where transmission is known to be highest (i.e., hotspots).

2.7. Peruvian malaria endemic regions

About half the total population is exposed to malaria or live in areas with ecological factors favourable to transmission which are represented by the Amazon region and the Pacific northern coast (Table 2.2)

Table 2.2. Characteristics of malaria transmission in the Amazon region and northern coast

Characteristics	Amazon region	Northern coast
Period of transmission	Perennial transmission with marked seasonality.	Seasonal transmission
Environmental conditions	Tropical rainforest, heavy rains (November-April)	Dry desert area, rainfall (December-March) influenced by ENSO.
Other ecological conditions	Deforestation	Rice farming
Vector	*An. darlingi*, *An. benarrochi.*	*An. albimanus*
Parasite	*P. vivax* (80%), *P. falciparum* (20%)	*P. vivax* (98%), *P. falciparum* (2%)
Risk areas	Rural, peri-urban	Urban, peri-urban.
House characteristics	Open houses without or rudimentary walls	Closed houses with completed walls
Economical activities	Subsistence-scale agriculture, logging, fishing, hunting.	Commerce, fishing, agriculture.
Accessibility to health services	Limited	Accessible

The Loreto department, the largest department of the country (28.7% of the Peruvian territory), covers most of the **Amazon Region** in the northeast part of Peru, and borders with Brazil, Colombia and Ecuador. According to national statistics, the Loreto population totalled about one million inhabitants (including more than one third (38.6%) below the age of 15 years), and about one third (34.6%) living in rural areas [76]. The local economy varies according to the different areas with indigenous and rural villages mainly depending on natural resources exploitation such as agriculture, wood logging and fishing [93], and bigger localities focusing on commercial activities [94]. The region is situated between 61 to 220 metres above sea level, and is covered by tropical rainforest and an extensive fluvial network connecting to the Amazon River.

The climate in Loreto is warm and humid with a heavy rainy season from November to May and a mild rainy season from June to October [85]. The annual average temperature is around 27°C, the relative humidity above 80%, and an average annual rainfall of 4 meters [95]. Malaria transmission in the region is perennial, with a peak between February and July [96]. *P. vivax* and *P. falciparum* infections occur at a ratio 5:1, and mostly affect rural and remote villages along the main Amazon River and its tributaries. Most people in these villages live in open or semi-closed wood houses. All age groups are at risk of infection, though adults more than children. *An. darlingi* is the main malaria vector and is highly anthropophilic [97].

Despite the fact that Loreto is considered as low endemic area [94], routine surveillance data and epidemiological studies suggest that malaria transmission in the region is highly heterogeneous. More than 80% of the total malaria clinical cases detected by PCD in Loreto are concentrated in only 10-20% of the total communities of the department [7]. Moreover, cross-sectional studies using LM for malaria diagnosis have also showed that malaria parasite prevalence varied considerably across communities around Iquitos (capital of Loreto) [94,96], and those differences remained or even increased when using more sensitive diagnostic tests [94,98]. Indeed, the recent use of PCR in epidemiological studies have shown that an important proportion of the total infections are asymptomatic and with low and sub-microscopic parasite densities [94,96,98]. Similarly, entomological studies have also evidenced heterogeneity in the transmission by finding a wide variation of EIR estimations at micro-geographical level in riverine campsites about 50 km of Iquitos and along the unique highway of Loreto (i.e., Iquitos-Nauta road) [97,99,100]. Ecological factors that provided optimal conditions for mosquito breeding and resting sites associated with deforestation, natural and man-made water bodies (e.g. fish ponds), and surrounding secondary vegetation are among the potential explanations for the spatial malaria heterogeneity. Besides ecological factors, human socio-economic differences such as poorer housing conditions (open or semi-closed houses) and outdoor economic

activities (such as farming, logging or fishing) may also play an important role in increasing exposure to mosquito bites [93,101].

The northern coast, which includes Tumbes, Piura, Lambayeque and La Libertad departments, has historically been the second most important region for malaria transmission after the Amazon rainforest [1]. It is characterized by the presence of coastal valleys receptive to the vector and by human migration for labour-intense agricultural activities [102]. The region has winter seasons from April to November with cool and dry days, and hot and humid summers with moderate rains from December to March. However, this seasonal climate pattern has been altered in the past by the ENSO phenomenon [2] with torrential rains and strong winds causing flooding and landslides which have been associated with malaria outbreaks (e.g. malaria epidemic in 1997-1998).

The significant decline in malaria incidence over the past decade has modified the epidemiological profile of the disease in the region [7]. Currently, reported malaria cases are only due to *P. vivax* and concentrated in a few localities of Piura and Tumbes. Transmission occurs in epidemic and sporadic patterns between February and June, the months of highest density of the main vector, *An. Albimanus* [103]. The most affected people live in peri-urban localities in close proximity to irrigated fields and irrigation canals which provide the favourable environment for the development of anopheline mosquitoes [103,104]. Though houses in those localities have complete walls (closed houses), they are mainly made of precarious materials leaving many small openings for mosquitoes to enter.

3. ASSESSING MALARIA TRANSMISSION IN A LOW ENDEMICITY AREA OF NORTH-WESTERN PERU

Authors: Angel Rosas-Aguirre, Alejandro Llanos-Cuentas, Niko Speybroeck, Jackie Cook, Juan Contreras-Mancilla, Veronica Soto, Dionicia Gamboa, Edwar Pozo, Oscar J Ponce, Mayne O Pereira, Irene S Soares, Michael Theisen, Umberto D'Alessandro, Annette Erhart.

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3.1. Abstract

Background: Where malaria endemicity is low, control programmes need increasingly sensitive tools for monitoring malaria transmission intensity (MTI) and to better define health priorities. A cross-sectional survey was conducted in a low endemicity area of the Peruvian north-western coast to assess the MTI using both molecular and serological tools.

Methods: Epidemiological, parasitological and serological data were collected from 2,667 individuals in three settlements of Bellavista district, in May 2010. Parasite infection was detected using microscopy and polymerase chain reaction (PCR). Antibodies to *Plasmodium vivax* merozoite surface protein-1_{19} ($PvMSP1_{19}$) and to *Plasmodium falciparum* glutamate-rich protein (PfGLURP) were detected by ELISA. Risk factors for exposure to malaria (seropositivity) were assessed by multivariate survey logistic regression models. Age-specific antibody prevalence of both *P. falciparum* and *P. vivax* were analysed using a previously published catalytic conversion model based on maximum likelihood for generating seroconversion rates (SCR).

Results: The overall parasite prevalence by microscopy and PCR were extremely low: 0.3 and 0.9%, respectively for *P. vivax,* and 0 and 0.04%, respectively for *P. falciparum,* while seroprevalence was much higher, 13.6% for *P. vivax* and 9.8% for *P. falciparum*. Settlement, age and occupation as moto-taxi driver during previous year were significantly associated with *P. falciparum* exposure, while age and distance to the water drain were associated with *P. vivax* exposure. Likelihood ratio tests supported age seroprevalence curves with two SCR rather for both *P. vivax* and *P. falciparum* indicating significant changes in the MTI over time. The SCR for PfGLURP was 19-fold lower after 2002 as compared to before ($\lambda1 = 0.022$ *versus* $\lambda2 = 0.431$), and the SCR for $PvMSP1_{19}$ was four-fold higher after 2006 as compared to before ($\lambda1 = 0.024$ *versus* $\lambda2 = 0.006$).

Conclusion: Combining molecular and serological tools considerably enhanced the capacity of detecting current and past exposure to malaria infections and related risks factors in this very low endemicity area. This allowed for an improved characterization of the current human reservoir of infections, largely hidden and heterogeneous, as well as providing insights into recent changes in species specific MTIs. This approach will be of key importance for evaluating and monitoring future malaria elimination strategies.

Keywords: Malaria transmission intensity, low endemicity, elimination, polymerase chain reaction, serology, Peru.

3.2. Background

Despite recent reduction in its estimated incidence, malaria remains the most important human arthropod-borne disease, worldwide and in the Americas [105]. In Peru, malaria incidence has fluctuated dramatically over the last 50 years. Between 1954 and 1967, malaria was well under control by coordinated eradication efforts (resulting in only 1,500 cases in 1965) [106], but returned to high levels when eradication campaigns ceased. Following the occurrence of drug-resistant *Plasmodium falciparum* strains to chloroquine (CQ) and sulphadoxine-pyrimethamine (SP) [88,107] and the spread of *Anopheles darlingi* in the Amazon Region [85], malaria re-emerged in the 1990s, reaching a peak of more than 200,000 cases in 1998 after the El Niño Southern Oscillation (ENSO) climatologic phenomenon [2]. Between 2000 and 2005, the annual incidence fluctuated between 70,000 and 80,000 cases followed by a steady decrease until 2011, when 22,877 cases were reported [7]. This achievement can be attributed to the strengthening of the National Malaria Control Programme (NMCP) and to the implementation of comprehensive interventions such as the use of artemisinin-based combination therapy (ACT), the distribution of long-lasting insecticidal mosquito nets (LLINs), and health education campaigns with the strong support of international donors, e g, US Agency for International Development (USAID), and the Global Fund for Aids, Tuberculosis and Malaria (GFATM) [6,89,108].

In Peru, the significant decline in malaria incidence over the past decade has modified its epidemiological profile and consequently calls for adapted control strategies. Currently, there is the need for targeting interventions and surveillance to foci of residual transmission in the north-west coast as well as in the Peruvian Amazon region. A key element for this re-orientation will be the availability of accurate measurement of malaria transmission intensity (MTI) and its evolution in space and time [11,12]. However, it is not clear how best to monitor changes in transmission and disease burden in low endemicity areas [109].

Traditional methods to measure MTI include the collection of entomological and parasitological parameters. However, in areas of low endemicity, these measures are often subject to fluctuations for reasons other than true changes in transmission. For example, the improvement of case detection methods usually leads to an artificial increase in malaria incidence, which makes difficult the comparison between pre- and post-intervention data. Furthermore, entomological (entomological inoculation rate (EIR)) and parasitological measures (infections detected by microscopy) estimated through community surveys require very large sample sizes and are therefore extremely time and money consuming, while not able to catch seasonal variations [18,44]. Moreover, parasitological surveys using microscopy cannot detect subpatent infections which are commonly reported in areas of low transmission [110,111]. Molecular tests such as polymerase chain reaction (PCR) are much more sensitive for the detection of low parasite-density infections, and will become increasingly important for malaria elimination programmes [112,113].

Serological markers have recently been used in several endemic areas across the world to estimate MTI [20,21,59,114,115] and monitor its changes over time following interventions [22]. Serological techniques are particularly suited to low endemic areas as antibodies remain in the blood longer than malaria parasites and are thus easier to detect and less subject to seasonal variations. This paper reports on population-based estimates of *P. falciparum* and *Plasmodium vivax* prevalence and seroprevalence in an area of low endemicity in the north-west coast of Peru. The combination of molecular and serological tools is aimed at improving the detection of current malaria infections as well as recent exposure, in order to get insights into the residual transmission dynamics.

3.3. Methods

Study area

The study was conducted in Bellavista, a small district of Sullana province located in Piura department, north-western coast of Peru (Figure 3.1). Piura is historically considered an important focus of malaria transmission in Peru [3]. Bellavista district is a peri-urban settlement (37,000 inhabitants in 2010) directly connected to Sullana city, at 30 km from Piura, the fifth largest city in Peru. Three contiguous squatter settlements were selected for the study: Pavletich (PAV, nine blocks of houses), Jose Carlos Mariátegui (JCM, 12 blocks) and Nuevo Porvenir (NP, 13 blocks) based on their malaria endemicity (between 2008 and 2010 most of the district malaria cases occurred in these settlements) and their proximity to the largest water drain called *Boqueron* (=big hole). Situated at the eastern edge of the settlements, this artificially created drain flows south to north for about 2 km (maximum width 100 m, depth 30 m) to end into the Chira River.

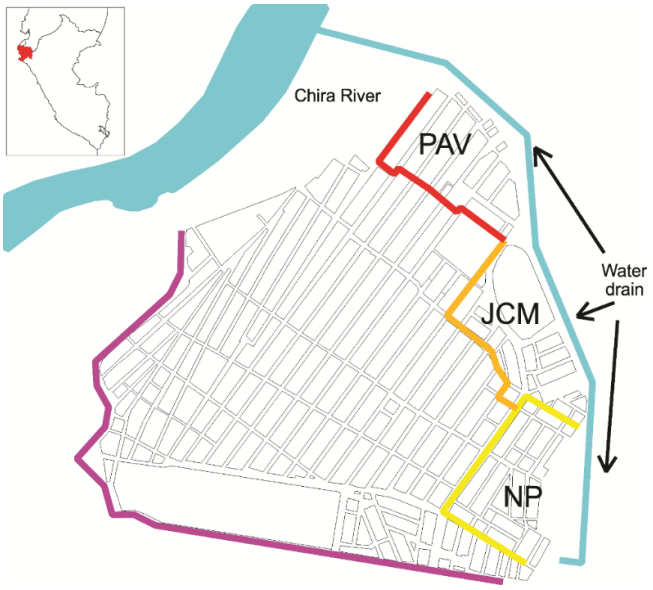


Figure 3.1. Study area in Bellavista district (Piura department, northwestern coast of Peru) including three settlements. Pavletich (PAV), Jose Carlos Mariátegui (JCM) and Nuevo Porvenir (NP)

The winter season is from May to November and the hot and rainy summer from December to April. This seasonal pattern is periodically altered by the ENSO phenomenon [2] with torrential rain and strong winds that can cause flooding and landslides. In 2010, annual rainfall, average relative humidity and average temperature were 103 mm, 76% and 24.2°C, respectively [95]. The main vector is *Anopheles albimanus* and the vast majority of malaria cases are due to *P. vivax* [116]. The main occupations in the area are informal trade, agriculture and small animal farming.

Data collection

A full census of the study population was carried out in April 2010, collecting individual data on sociodemographic (age, gender, education, economic activities, income) as well as on malaria prevention characteristics. Each house was identified with a unique code number and georeferenced using a handheld global positioning system (GPS) device (Garmin's GPSMAP 60CSx) that also calculated the closest distance to the water drain. Each individual was given a seven-digit unique code number combining the village, household and individual code.

The cross-sectional survey was carried out in May 2010 and included all residents in the study area. Each household was visited, and a written informed consent was sought from each participant. If part of, or the whole household, was absent at the time of survey, the study team would return within the next two days to maximize subject participation. Each participant was examined for fever and malaria symptoms, and a finger-prick blood sample was taken for immediate microscopy (thick and thin blood smears) and later serological and molecular tests at Institute of Tropical Medicine Alexander von Humboltd, Lima (ITM-AvH). Filter paper (Whatman grade 3, Whatman, Springfield Mill, USA) dried blood samples were individually stored at 4°C with desiccant until processed at the ITM-AvH. Infected individuals (presence of *Plasmodium* trophozoites) were treated according to the national guidelines.

Additionally, retrospective data on annual malaria incidence by species from 1990 to 2010, registered by the Office of Epidemiology at Regional Health Direction of Piura, were obtained from the National Institute of Statistics and Informatics [7]. In Peru, weekly notification of confirmed malaria cases (by standard microscopy) at health facilities and data aggregation at district and at departmental level are mandatory. Patients presenting with symptoms compatible with malaria at health facilities are systematically tested by microscopy and treated following national guidelines.

Laboratory procedures

Microscopy

Thick and thin smears were stained for 10 min with a 10% Giemsa solution, and parasite density was expressed as the number of parasites/µl, after counting a total of 200 white blood cells (WBC) (or 500 WBCs if less than ten parasites/field), and assuming an average of 6,000 WBCs/µl according to the national guidelines [117]. Microscopy examination was performed immediately after sample collection at the Reference Laboratory in Sullana, and later quality control was done blindly on all positive slides and 10% of randomly chosen negative slides by a senior technician at ITM-AvH. Any discordant results were reread by a second senior technician until agreement.

Species-specific polymerase chain reaction (ss-PCR)

Parasite DNA was extracted using the saponin Chelex 100 method [118]. Briefly, filter-paper blood spots containing approximately 20 µl of blood were cut into pieces of approximately 5 sq mm, and incubated with 20 µl of 0.05% saponin at room temperature for four hours. Then, 10 µl of 20% Chelex 100 solution was added, and the sample was incubated for 10 min at 95°C, followed by a centrifugation at 11,000 g. The supernatant (DNA) was transferred into a new tube and stored at −20°C until PCR was performed. The DNA was amplified by a semi-nested multiplex PCR method targeting the 18S rDNA region, as described by Rubio *et al* [119]. PCR products were analysed in a 2% agarose

gel with a standard 100 bp DNA lader, using ethidium bromide staining (0.5 µg/ml) and a data image Analyzer with UV trans-illuminator.

Serology

Plasmodium falciparum glutamate-rich protein (PfGLURP) and *P. vivax* merozoite surface protein1$_{19}$ (PvMSP1$_{19}$) antibodies were detected using an ELISA protocol published elsewhere [120]. Briefly, dried blood filter-paper samples (5 mm diameter disc/sample) were eluted overnight at 4°C in 2 ml of PBS-Blotto-Tween. Two hundred µl of the eluate were added in duplicate to blocked ELISA plates (Plate Chamaleon, Hydex) coated separately with *P. falciparum* GLURP R2 [121] and *P. vivax* MSP1-$_{19}$ [122]. Pooled sera from five *P. falciparum*- or *P. vivax*-infected patients, and from five non-infected control group were diluted at 1:400 in PBS-Blotto, as positive and negative control, respectively. Goat anti-human IgG (H + L) peroxidase (Sigma, affinity purified) diluted to 1: 20,000 in PBS-Tween was used as conjugate and incubated for one hour before development of the ELISA using 200 µl ABTS substrate-chromogen solution. Optical densities (ODs) were read at 405 nm, and corrected OD values were computed by subtracting the mean OD of the antigen negative control wells from the mean OD of the corresponding antigen containing wells. To ensure a standardization of the sample results across ELISA plates, the percent positivity (PP) of each specimen was calculated using the OD of the positive control serum as 100%. Quality control was done blindly at ITM-AvH on 5% of randomly chosen samples. The cut-off for positivity was generated using a mixture model as previously described by Corran *et al* [58].

Data management and statistical analysis

Data were double entered, validated and cleaned in Excel (Microsoft Corp, USA), and data analysis was performed with Stata v.11 (Stata Corp, College Station, USA) and R v.2.15 software (R Development Core Team, R Foundation for Statistical Computing, Austria).

Malaria infection by PCR was defined as an individual with a positive PCR result, regardless of symptoms. Clinical malaria was defined as a patient with fever (body temperature >37.5°C), and/or history of fever in the previous two days, and positive for malaria by microscopy and/or PCR. As maternally derived antibodies can be found in young infants [123], malaria exposure was defined as an individual older than six months with a positive ELISA test for either or both *P. falciparum* and *P. vivax*. Descriptive statistics were used to calculate malariometric indices (parasite and antibody prevalence). Uni- and multivariate adjusted analyses were performed using survey logistic regression to determine risk factors for malaria infection (PCR positive) or malaria exposure (serology positive) adjusting for all potential confounders such as: settlement, gender, age, income, education, occupation during previous year, bed net use, distance to the water drain, and movements outside the settlement during previous year. A p-value <0.05 was considered significant for risk factors to be included in the multivariate adjusted model. Interactions were systematically checked for up to order two. All analyses took into account the survey design characteristics, using settlement as strata and household as primary sampling unit.

A simple reversible catalytic conversion model was used to fit the dichotomized serological results, using maximum likelihood methods as published by Drakeley *et al* [20]. Briefly, this model generates age-seroprevalence curves, allowing for the estimation of the force of infection, or seroconversion rate (SCR, λ), and a seroreversion rate (ρ). Upon visual examination, if age-seroprevalence data suggest an obvious step at a certain age (time point), a model allowing for two forces of infection (λ_1 and λ_2) and related profile likelihood plots are run to determine the most likely time point for change in SCR, as previously described by Stewart *et al* [21]. The identified time point is subsequently incorporated into the catalytic model to fit a seroprevalence curve with two forces of infection (before and after time cut-off)[57], which is then compared -using the likelihood ratio (LR) test ($p < 0.05$)- with a sero-prevalence curve with only one force of infection.

Ethical issues

Permission was received from health and local authorities after explaining the purpose and procedures of the survey. Signed, informed consent was obtained prior to participation by all adults and the parents of all participating children <18 years. In addition to their parent's consent, children ≥ seven years old provided signed informed assent prior to participating. Ethical clearance was obtained from the Ethics Review Board of the Universidad Peruana Cayetano Heredia, Lima, Peru (SIDISI code: 056736).

3.4. Results

A total of 996 households and 4,650 individuals were identified during the census, and were distributed over the three study settlements as follows: PAV (433 houses; 2,163 individuals), JCM (312; 1,358) and NP (251; 1,129). The overall sex ratio was 0.94, and the age distribution showed that half of the population was <20 years old. A total 2,733 individuals (58.8%) living in 871 households (87.4%) were available at the time of the survey and 97.6% (2,667/2,733) of them accepted to participate. Females (61%) slightly outnumbered males, but the age distribution was very similar to the one in the total population. Among adults aged ≥18 years old, 60% had completed secondary education level. Only about 20% of the respondents had a regular income, almost all below the minimum national vital wage. The most commonly reported occupations were housewife (27.1%), labourer (9.0%) and trader (6.4%), while preprofessionals (children, students) and retired represented half of the participants. Very few respondents reported having been working as farmer (2.4%) or moto-taxi driver (2.6%) in the past year. More than half (52.6%) of the participants stated they were regularly sleeping under an untreated net, and 11.5% had experienced at least one confirmed *P. vivax* episode during the previous year (Table 3.1).

Table 3.1. Baseline characteristics of the study population (N = 2,667)

	n	%
Settlement		
Pavletich (PV)	1,128	42.3
José Carlos Mariategui (JCM)	797	29.9
Nuevo Porvenir (NP)	742	27.8
House distance to water drain (m)		
<100	183	6.9
100-250	1401	52.5
>250	1,083	40.6
Gender		
Female	1,628	61.0
Male	1,039	39.0
Age groups (years)		
0-5	447	16.8
6-10	404	15.1
11-19	491	18.4
20-39	889	33.3
> = 40	436	16.4
Education (> = 18 years, n = 1,421)		
None	44	3.1
Primary	517	36.4
Secondary	689	48.5
Superior	171	12.0
Income amount*		
None	2,081	78.0
< 650 PEN	458	17.2
> = 650 PEN	83	3.1
Missing	45	1.7
Current economic activity		
None (children, students, retired)	1,319	49.5
Farmer	41	1.5
Moto-taxi driver	74	2.8
Housewife	723	27.1
Trader	172	6.4
Laborer (indoor, manual worker)	240	9.0
Others	98	3.7
Occupation as farmer (previous 12 months)		
No	2,604	97.6
Yes	63	2.4
Occupation as moto-taxi driver (previous 12 months)		
No	2,598	97.4
Yes	69	2.6
Bed net use		
Never/Sometimes	1,250	46.9
Always	1,402	52.6
Missing	15	0.5
Confirmed *P. vivax* malaria (previous 12 months)		
No	2,360	88.5
Yes (1 episode)	273	10.2
Yes (> = 2 episodes)	34	1.3

*Income expressed in Peruvian nuevos soles (PEN). The official vital minimum wage in Peru is 650 PEN (approx 230 USD).

Parasite prevalence determined by microscopy was 0.3% (total eight *P. vivax* infections) while by PCR it was estimated at 1% including one *P. falciparum* and 25 *P. vivax* infections (Table 3.2). Malaria seroprevalence for *P. vivax* and *P. falciparum* was estimated at 13.6 and 9.8%, respectively, with 9.0% of the total seropositive individuals having antibodies against both antigens. A very weak correlation between antibody responses (percent positivity = PP) to the two species were found in individuals older than ten years ($r = 0.1$, $p < 0.001$), but not in younger children ($p > 0.1$). Most of the *P. vivax* PCR-positive cases were submicroscopic (17/25 = 68.0%) and asymptomatic (22/25 = 88.0%) at the time of the survey (Table 3.3). PCR positivity to *P. vivax* was significantly associated with the presence of *P. vivax* parasites (by microscopy) ($p < 0.001$) or antibodies ($p < 0.001$), as well as with symptoms ($p < 0.001$). Association between PCR and sero-positivity to *P. falciparum* could not be explored since only one *P. falciparum* infection was detected by PCR. This infection was asymptomatic and submicroscopic, and was found in a 16-year-old girl who also had antibodies against *P. falciparum*.

Table 3.2. Malaria prevalence (by microscopy and PCR) and seroprevalence

		n	N	%	95% CI
Prevalence by microscopy	*P. vivax*	8	2,667	0.3	[0.2; 0.6]
Parasite density/µl, median [range]	432 [112; 1,529]				
Prevalence by PCR	*P. vivax*	25	2,667	0.9	[0.7; 1.4]
	P. falciparum	1	2,667	0.04	[0.01; 0.3]
	Any species	26	2,667	1	[0.6; 1.4]
Seroprevalence	$PvMSP1_{19}$	360	2,653	13.6	[12.2; 15.1]
	PfGLURP	259	2,653	9.8	[8.6; 11.1]
	Total	567	2,653	21.4	[19.7; 23.1]

Table 3.3. Association between *Plasmodium vivax* PCR results and microscopy, serology and symptoms

	***P. vivax* PCR Negative (N = 2642)**		***P.vivax* PCR Positive (N = 25)**		**p-value**
	n	**%**	**n**	**%**	
P. vivax microscopy					
Negative	2,642	100	17	68.0	<0.001
Positive	0	0	8	32.0	
***P. vivax* MSP_{1-19} serology**					
Negative	2,282	86.8	11	44.0	<0.001
Positive	346	13.2	14	56.0	
***P. falciparum* GLURP serology**					
Negative	2,369	90.2	22	88.0	0.71
Positive	256	9.8	3	12.0	
Fever and/or history of fever (previous 2 days)					
No	2,592	98.1	22	88.0	<0.001
Yes	50	1.9	3	12.0	

Given the small number of PCR-positive individuals, a multivariate adjusted risk factor analysis was not carried out, and the univariate analysis showed that distance to water drain was significantly associated with the risk of *P. vivax* infection (no association could be found with age, gender, income, education, bed net, or previous travelling outside the community). Individuals living at <100 m from the water drain) had increased odds of *P. vivax* infection than those living farther (≥100 m) (OR=3.4, 95% CI [1.3–9.0]).

Distance to water drain, age, education, income, occupation as farmer in the previous year, and history of *P. vivax* malaria in the previous year were significantly associated to *P. vivax* exposure in the univariate analysis (Table 3.4). The multivariate analysis (excluding history of malaria in the previous year) showed that only age and distance to water drain remained independently associated with *P. vivax* seropositivity. Individuals older than ten years had increasingly higher odds of *P. vivax* seropositivity compared to younger children (test for trend p<0.001), while those living at closer distance to the water drain had increasingly higher odds of exposure to *P. vivax* compared to those living

farther than 250 m. A significant interaction (OR=5.7; p=0.014) between age groups > ten years and distance <100 m was found, showing that the effect of age (>ten *versus* ≤ ten years) was significantly higher in those living closer (<100 m) to the drain (OR=11.7; 95 CI [3.0; 45.1]; p<0.001) compared to those living farther (OR=2.0; 95 CI [1.5; 2.7], p<0.001). Conversely, the effect of distance to water (<100 m *versus* ≥100 m) was significant only in the older age group (OR=2.4, 95 CI [1.6; 3.7], p<0.001).

Significant risk factors for *P. falciparum* exposure identified in the univariate analysis were the following: settlement, age, education, prior work as farmer or moto-taxi driver, and bed net use (Table 3.5). The multivariate-adjusted analysis showed that only settlement, age and previous work as moto-taxi driver remained independently associated with exposure to *P. falciparum* (no interaction was found). Individuals living in JCM (AOR: 1.5, 95% CI [1.02–2.1]) and in NP (AOR: 2.2, 95% CI [1.6–3.2]) were more likely to be exposed to *P. falciparum* compared to those living in PAV. In addition, individuals older than five years had increasingly higher odds (test for trend p < 0.001) of *P. falciparum* seropositivity compared to younger children, and those having worked as moto-taxi driver in the previous year were two times more exposed to *P. falciparum* (AOR: 2.1, 95% CI [1.1–3.8]) compared to those who did not.

Note: Two tables (S3.1 and S3.2) were added as supplementary files in the Annex (Chapter 9). Table S3.1 show the distribution of *P. vivax* infections across age groups (p=0.87) and between adult sub-groups with and without outdoor occupations (i.e., farmers, moto-taxi drivers) (p=0.47, p=0.53). Table S3.2 shows the prevalence of *P. falciparum* exposure between adult sub-populations with and without occupation as moto-taxi drivers (p=0.008).

Table 3.4. Multivariate analysis for the risk of exposure to *P. vivax* using survey logistic regression

Risk factors	Positive PvMSP1-19				Unadjusted		Adjusted ≈	
	%	95% CI	n	N	OR	95% CI	OR	95% CI
Settlement								
PAV	12.7	[10.8; 14.9]	143	1,123	1.0		-	
JCM	15.6	[13.1; 18.5]	123	789	1.3	[0.95; 1.7]	-	
NP	12.7	[10.0; 15.9]	94	741	1.0	[0.7; 1.4]	-	
House distance to water drain (m)								
>250	9.5	[7.8; 11.6]	103	1,080	1.0		1.0	
100-250	15.6	[13.6; 17.9]	217	1,391	1.8*	[1.3; 2.3]	1.8*	[1.4; 2.4]
<100	22.0	[16.2; 29.2]	40	182	2.7*	[1.7; 4.1]	2.8*	[1.8; 4.4]
Age groups (years)								
0.5-5	6.7	[4.7; 9.4]	29	433	1.0		1.0°	
6-10	8.9	[6.5; 12.1]	36	404	1.4	[0.8; 2.2]		
11-19	13.9	[10.7; 16.7]	68	491	2.2*	[1.4; 3.5]	2.1*[∞]	[1.6; 2.8]
20-39	15.8	[13.4; 18.4]	140	889	2.6*	[1.7; 3.9]		
> = 40	20.0	[16.5; 24.0]	87	436	3.5*	[2.2; 5.4]	3.1*	[2.2; 4.4]
Education								
None	8.1	[6; 10.8]	39	483	1.0		-	
Primary	13.3	[11.3; 15.7]	140	1,199	1.8*	[1.2; 2.5]	-	
Secondary	16.3	[14; 19]	153	829	2.2*	[1.5; 3.2]	-	
Superior	15.1	[10.7; 20.9]	27	141	2.0*	[1.2; 3.4]	-	
Income amount								
None	12.6	[11.1; 14.3]	261	2,067	1.0		-	
< 650 PEN	17.9	[14.7; 21.7]	82	458	1.5*	[1.2; 2]	-	
> = 650 PEN	14.5	[8.4; 23.8]	12	83	1.3	[0.6; 2.2]	-	
Occupation as farmer (previous 12 months)								
No	13.3	[11.9; 14.8]	344	2,590	1.0		-	
Yes	25.4	[16.3; 37.3]	16	63	2.2*	[1.3; 3.9]	-	
Occupation as moto-taxi driver (previous 12 months)								
Never/Sometimes	13.4	[12.1; 14.9]	347	2,584	1.0		-	
Always	18.8	[11.2; 30.0]	13	69	1.5	[0.8; 2.8]	-	
Bed net use								
No	13.0	[11.1; 15.2]	162	1,244	1.0		-	
Yes	14.1	[12.2; 16.2]	196	1,395	1.1	[0.9; 1.4]	-	
Confirmed *P. vivax* malaria (previous 12 months)+								
No	10.5	[9.3; 12]	247	2,346	1.0		-	
Yes (1 episode)	33.3	[27.9; 39.3]	91	273	4.2*	[3.2; 5.7]	-	
Yes (> = 2episodes)	64.7	[46.7; 79.3]	22	34	15.6*	[7.3; 33]	-	

* $p < 0.05$. + Not considered in the multivariate analysis.
° Baseline category = 0.5-10 years. [∞] Include ages between 11 and 39 years.
≈ A significant interaction (OR=5.7; 95CI [1.4; 22.7], p=0.014) was found between age>10 y and distance <100 m.

Table 3.5. Multivariate analysis for the risk of exposure to *P. falciparum* using survey logistic regression

Risk factors	Positive Pf GLURP-19				Unadjusted		Adjusted	
	%	95% CI	n	N	OR	95% CI	OR	95% CI
Settlement								
PAV	7.4	[5.9; 9.3]	83	1,123	1.0		1.0	
JCM	9.6	[7.6; 12.2]	76	789	1.3	[0.9; 1.9]	1.5*	[1.02; 2.1]
NP	13.6	[11; 16.5]	100	738	2.0*	[1.4; 2.8]	2.2*	[1.6; 3.2]
House distance to water drain (m)								
>250	10.6	[8.7; 12.8]	114	1,080	1.0		-	
100-250	8.7	[7.3; 10.4]	121	1,388	0.8	[0.6; 1.1]	-	
<100	13.2	[8.7; 19.5]	24	182	1.3	[0.8; 2.2]	-	
Age groups (years)								
0.5-5	3.7	[2.9; 7.4]	16	432	1.0		1.0	
6-10	7.4	[3.4; 8.0]	30	404	2.1*	[1.1; 3.9]	2.1*	[1.1; 3.8]
11-19	12.5	[9.7; 15.9]	61	490	3.7*	[2.1; 6.5]	3.2*[∞]	[1.9; 5.3]
20-39	10.3	[8.4; 12.5]	91	888	3.0*	[1.8; 5.0]		
> = 40	14.0	[11.0; 17.6]	61	436	4.2*	[2.4; 7.4]	4.7*	[2.6; 8.3]
Education								
None	6.0	[4.2; 8.5]	29	483	1.0		-	
Primary	10.0	[8.3; 12]	105	1,051	1.7*	[1.1; 2.7]	-	
Secondary	11.1	[9.2; 13.4]	104	937	2.0*	[1.3; 3]	-	
Superior	11.8	[7.8; 17.6]	21	178	2.1*	[1.2; 3.8]	-	
Income amount								
None	9.2	[8; 10.6]	190	2,064	1.0		-	
< 650 PEN	11.8	[9.1; 15.1]	54	458	1.3	[0.96; 1.8]	-	
> = 650 PEN	14.5	[8.4; 23.8]	12	83	1.7	[0.9; 3.1]	-	
Occupation as farmer (previous 12 months)								
No	9.6	[8.4; 10.9]	248	2,587	1.0		-	
Yes	17.5	[9.9; 29]	11	63	2.0*	[1.03; 3.9]	-	
Occupation as moto-taxi driver (previous 12 months)								
No	9.5	[8.3; 10.8]	245	2,581	1.0		1.0	
Yes	20.3	[12.4; 31.4]	14	69	2.4*	[1.3; 4.4]	2.1*	[1.1; 3.8]
Bed net use								
Never/Sometimes	11.3	[9.5; 13.4]	141	1,244	1.0		-	
Always	8.5	[7.1; 10.2]	118	1,392	0.7*	[0.6; 0.96]	-	

* $p < 0.05$.
[∞] Included all ages between 11 and 39 years.

Age seroprevalence plots for PvMSP1$_{19}$ and PfGLURP are shown in Figure 3.2 (A and B), and confirm previous results: increasing *P. vivax* and *P. falciparum* seroprevalence with age. Likelihood ratio (LR) tests supported models with two SCR (forces of infection) rather than one SCR for both *P. vivax* (p = 0.028) and *P. falciparum* (p = 0.046), indicating significant changes over time in the MTI for both species. For PvMSP1$_{19}$, best estimates indicated a significant four-fold increase in the SCR approximately four years prior to the survey (year 2006): post-2006 $\lambda_1 = 0.024$ (95% CI [0.018–0.032]) and pre-2006 $\lambda_2 = 0.006$ (95% CI [0.002–0.017]). For PfGLURP, best estimates indicated a 19–fold drop in the SCR approximately eight years prior to the survey (year 2002): post-2002 $\lambda_1 = 0.022$ (95% CI [0.014–0.033]), and pre-2002 $\lambda_2 = 0.431$ (95% CI [0.041–4.54]).

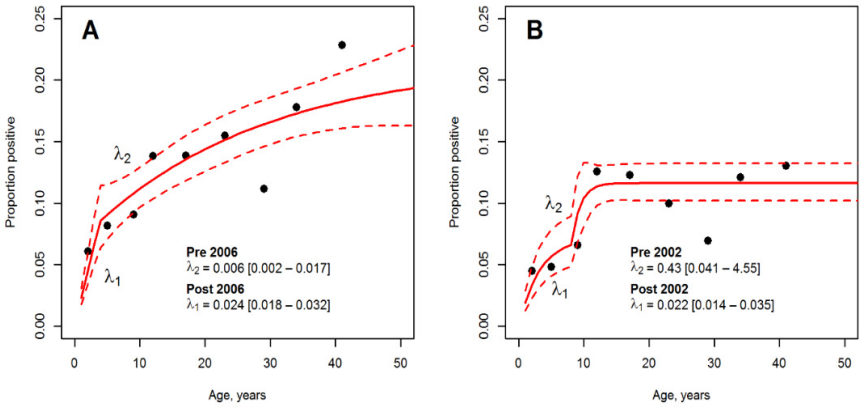


Figure 3.2. Malaria seroprevalence curves. A: Seroprevalence curve for *Plasmodium vivax*. B: Seroprevalence curve for *Plasmodium falciparum*. Points represent observed data points, whilst the blue lines represent the maximum likelihood model and 95% confidence intervals. Estimated force of infection (λ) is plotted on the graphs. Two forces infection are plotted by likelihood ratio tests indicating change at a certain point in calendar time.

The retrospective analysis of 20-year annual malaria incidence and rainfall from 1990 to 2010 in Piura department showed that after the El Niño-Southern Oscillation (ENSO) phenomenon in 1997–1998 (Figure 5.3), when nearly 50,000 *P. falciparum* cases were recorded (1998), *P. falciparum* incidence dropped and stabilized around 4,000-5,000

cases annually in 2000–2001. This was followed by a steady decrease, and since 2006, autochthonous *P. falciparum* reported cases have become scarce (six and three reported cases in 2006 and 2008, respectively; and no cases in 2007, 2009 and 2010). Similarly to *P. falciparum*, *P. vivax* incidence decreased gradually after the ENSO (around 23,000 cases in 1998) reaching very low levels during the four-year period of intense droughts, with only 700, 315, 168, and 606 reported cases, respectively from 2004 to 2007. However, since then, *P. vivax* cases have increased with 4,185, 2,735 and 2,153 cases in 2008, 2009 and 2010, respectively. During the same period, annual rainfall in Piura dramatically dropped from 1998 (1,687 mm) to minimum values (between 14.3 and 59.4 mm) during the drought period, but subsequently increased since 2008 (193.5 mm in 2008).

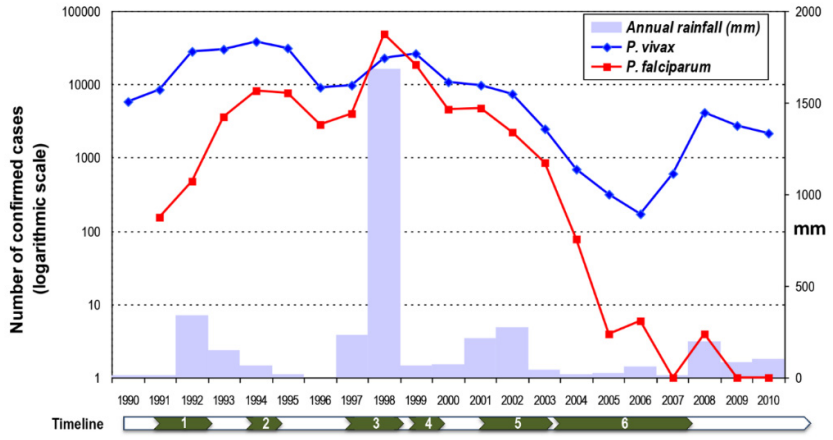


Figure 3.3. Evolution of the annual malaria incidence, and rainfall, in Piura department: 1990–2010. Information on annual malaria cases, registered by Regional Health Direction of Piura, was obtained from the National Institute of Statistics and Informatics [27]. Numbers in the timeline point out important events that influenced malaria incidence: 1) moderate ENSO phenomenon (1991–1992), 2) first reports of chloroquine (CQ) failure for *Plasmodium falciparum* (since 1994), 3) very strong ENSO phenomenon (1997–1998), 4) confirmed CQ resistance for *Plasmodium falciparum* (1999), 5) ACT implementation (2001–2003), 6) intense droughts (2004–2007).

3.5. Discussion

The combination of molecular and serological tools, in addition to standard microscopy, allowed for an in-depth characterization of the current malaria transmission pattern as well as recent changes in species-specific MTI in this peri-urban area of the Peruvian northern coast. Most of the malaria infections were submicroscopic (70%), as well as asymptomatic (89%) despite the low level of transmission. Though *P. vivax* was predominant, species-specific seroprevalence showed that exposure to *P. falciparum* still occurs in the study area. Interestingly, over the past ten years, both species experienced significant but opposite changes in MTI: while *P. falciparum* MTI dropped after 2002, the one of *P. vivax* significantly increased after 2006.

Despite having been conducted at the end of rainy season, when the health information system usually records the highest malaria incidence [7], few malaria-infected individuals were identified and most of them had not had malaria-related symptoms within the two days prior to the survey. These results are in line with findings from other areas in the northern coast [103] and the Peruvian Amazon region [96,124] and support the hypothesis that this asymptomatic and submicroscopic reservoir of infections contributes to malaria transmission [125].

In areas of low malaria transmission with seasonal patterns affected by climatic conditions, such as the Peruvian northern coast region, parasitological indicators determined by microscopy or PCR can be insensitive. In these areas, serological measures can be used as a proxy for malaria transmission intensity [22,58,59,115]. Indeed, seroprevalence reflects the cumulative exposure to malaria and, since antibodies persist longer in the blood than parasites, it is more sensitive and less affected by seasonality or unstable transmission [10,20,115]. Though it may be limited in detecting discrete seasonal variations in transmission, it is a good indicator of long-term transmission potential [20,21].

Even though *P. falciparum* reported cases in Piura department have been extremely rare in the past five years, *P. vivax* and *P. falciparum* seroprevalence indicated ongoing exposure to both species. The low PfGLURP seropositivity in young children and the only one *P. falciparum* case detected by PCR suggest that a residual *P. falciparum* transmission is still present in the study area. This residual transmission may be due to asymptomatic *P. falciparum* infections that remain undetected, unless a population-wide survey (such as the present study) is carried out. However, the implementation of mass screening as a public health strategy in low transmission settings remains a matter of debate [96,112,124], as the cost-effectiveness of such an intervention remains difficult to establish. The role of asymptomatic infections in maintaining malaria transmission as well as the impact of mass screening and treatment should be further evaluated through modelling techniques [126].

One might argue that an alternative explanation for the observed *P. falciparum* seropositivity could be the presence of antibodies cross-reacting with *P. vivax* or other infectious agents' antigens. Limited information is available about the cross-reactivity between *P. falciparum* and *P. vivax* antigens [122,127], and this has not been reported for PfGLURP. In addition, the lack of association between confirmed *P. vivax* infections by PCR and PfGLURP seropositivity, and the absence of correlation between antibody responses to the two species suggest that cross-reactivity is probably minimal. A few studies have suggested the cross-reactive potential of PfGLURP with *Schistosoma haematobium* [128], which is unlikely inthe study area since the latter has not been reported in Piura.

Serologic markers can also be used to determine risk factors for malaria. This is particularly important in low endemicity areas as the one reported here, where the low malaria prevalence jeopardizes the risk factor analysis. In the study area, age was the only independent risk factor that was associated to both species. The age-dependent PvMSP119 and PfGLURP seropositivity indicates cumulative *P. vivax* and *P. falciparum*

exposure with increasing age. As antibody responses will persist for prolonged periods, the rate of acquisition in young age groups can be used to determine current malaria transmission intensity [20-22,115] as well as to identify recent changes in MTI.

The higher seroprevalence in individuals with several *P. vivax* malaria episodes also confirmed that PvMSP119 antibody detection measures cumulative exposure. However, the history of *P. vivax* malaria in the past year was not included in the multivariate risk factor analysis for *P. vivax* exposure, since it is not a risk factor for *P. vivax* infections *per se* but rather a consequence of previous exposure to a given risk factor. *Plasmodium vivax* infections are on the pathway between risk factors and the presence of *P. vivax* antibodies.

Differences in the risk factors for *P. vivax* and *P. falciparum* seropositivity reflect the heterogeneity of malaria transmission intensity between species. Age and distance to the water drain were the only two independent risk factors significantly associated with *P. vivax* exposure. As mentioned above, the effect of age on seroprevalence usually reflects the cumulative exposure to malaria infections occurring with age rather than a higher risk of exposure in adults as compared to children. However, the significant interaction found between age and house distance to the drain, beside the cumulative effect of age on seroprevalence, indicates a significant lower exposure in children aged < ten years. Indeed, the effect of distance (<100 m *versus* ≥100 m) on *P. vivax* seropositivity was only significant in adults, indicating that children living close to the drain were not exposed any more to infectious bites compared to children living farther. Water accumulation in the drain provides breeding habitats for *Anopheles* malaria vectors after rains [116,103], resulting in a higher risk of exposure to infectious vectors for people living close to the drain. This is also supported by the results of univariate analysis for the risk of *P. vivax* infection. However, since adults are more likely to have outdoor evening activities, this could explain why children below ten, were no more at risk of malaria while living close to the drain.

Besides age, settlement and prior work as moto-taxi driver in the previous year, there were two additional independent risk factors for *P. falciparum* seropositivity. Historically, the three study settlements were important foci of *P. falciparum* transmission during the epidemic of 1998 and the early 2000s [129]. Therefore, higher seroprevalence in JCM and NP, compared to PAV again suggest differential reduction rates in MTI between villages (heterogeneity). Occupational activities outside settlements (as moto-taxi driver) that were associated with increased *P. falciparum* exposure support the hypothesis that transmission occurs not only in the immediate house environment as previously described by Guthmann *et al* [116], but outside the community as well. Moto-taxi drivers in the study area usually work in evening times, when exposure to *An. albimanus* bites increases. Occupational risk for malaria has also been found in the Peruvian Amazon Region, where malaria morbidity has been clearly associated with forest-related activities such as farming, land clearing and wood extraction [93,100].

The catalytic model used to generate PfGLURP seroprevalence curves suggested that a significant decline in *P. falciparum* transmission occurred in the study area approximately eight years before the current study (around 2002). This supports the idea that the wide scale implementation of ACT since 2001 has significantly contributed to the reduction of *P. falciparum* MTI. Indeed, following the 1998 epidemic and the subsequent intensified control interventions implemented [108], *P. falciparum* incidence returned to the pre-epidemic levels (1997) by 2000–2001 (around 5,000 cases), and since 2002 it steadily decreased to reach almost undetected levels from 2007 onwards (Figure 3). In addition, the absence of *P. falciparum* surge after the drought period in 2007, as observed with *P. vivax,* advocates for the additional significant effect of the ACT deployment on *P falciparum* MTI in the northern coast region [89].

Since the end of the drought period and the resuming of the rains, *P. vivax* incidence substantially increased [7] (Figure 3) which is concordant with the fitted results from the model, i.e., significant increase in *P. vivax* MTI after 2006. *Plasmodium vivax*

transmission in this area seems to be more sensitive than *P. falciparum* to changes in climatological and environmental conditions, especially since the implementation of ACT. The recent changes in species specific MTIs are also in line with the results of the multivariate risk factor analysis. While children under-five were significantly at lower risk of *P.falciparum* exposure compared to older children age 6–10, this was not the case for the *P. vivax* exposure where the under five were at similar risk of exposure compared to the 6–10 years old.

The number of antigens used in the two serological tests could be a limitation of the study. Due to the degree of sequence variability, it is recommended to use multiple antigens for each species in order to optimize the sensitivity of the antibody detection and to improve the identification of changes in malaria transmission over time in low transmission settings [57,59]. However, the observation that the antigens used in the study are relatively conserved [130] combined with results from recent studies performed in Cambodia, Vietnam and Peru suggests that PfGLURP and $PvMSP1_{19}$ are highly suitable for the detection of antibodies against *P. falciparum* and *P. vivax*, respectively [120,131]. Multiplexing the serological tests as well as standardizing the antigens used is of crucial importance for monitoring elimination efforts and comparing trends across countries and regions.

3.6. Conclusions

Monitoring MTI in areas of low endemicity and seasonal patterns, such as the north-western coast of Peru, poses a major challenge to national malaria control programmes. In this setting, molecular tools confirmed the current malaria transmission pattern characterized by low parasite rates, mainly due to *P. vivax* with a majority of asymptomatic and sub-microscopic infections. Additionally, serological markers reflected exposure to malaria and allowed for species-specific risk factor analysis, as well

as showing recent changes in respective MTIs. In conclusion, the combination of both serological and molecular tools improved detection sensitivity, and provided new insights into recent changes in transmission intensity for both species. This invaluable information can help decision-makers to adjust malaria control strategies in order to maintain current achievements, avoid outbreaks, and possibly move towards the elimination of malaria.

Authors' contributions

AR contributed to the study design, analysed the data and wrote the paper; ALL and VS contributed to the study design and paper review; NS and JC contributed to the data analysis and paper review; JCM contributed to the fieldwork supervision and processed the blood samples with ELISA and PCR; EP and OJP carried out and coordinated the fieldwork; DG contributed to the supervision of the sample processing and paper review; MOP, ISS and MT contributed antigen to the project and reviewed the article; UDA contributed to the study design and reviewed the manuscript; AE contributed to the study design, the data analysis and reviewed the paper. All authors read and approved the final manuscript.

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4. HOTSPOTS OF MALARIA TRANSMISSION IN THE PERUVIAN AMAZON: RAPID ASSESSMENT THROUGH A PARASITOLOGICAL AND SEROLOGICAL SURVEY.

Authors: Angel Rosas-Aguirre, Niko Speybroeck, Alejandro Llanos-Cuentas, Anna Rosanas-Urgell, Gabriel Carrasco-Escobar, Hugo Rodriguez, Dionicia Gamboa, Juan Contreras-Mancilla, Freddy Alava, Irene Soares, Edmond Remarque, Umberto D´Alessandro, Annette Erhart.

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4.1. Abstract

Background: With low and markedly seasonal malaria transmission, increasingly sensitive tools for better stratifying the risk of infection and targeting control interventions are needed. A cross-sectional survey to characterize the current malaria transmission patterns, identify hotspots, and detect recent changes using parasitological and serological measures was conducted in three sites of the Peruvian Amazon.

Material and Methods: After full census of the study population, 651 participants were interviewed, clinically examined and had a blood sample taken for the detection of malaria parasites (microscopy and PCR) and antibodies against *P. vivax* (PvMSP1$_{19}$, PvAMA1) and *P. falciparum* (PfGLURP, PfAMA1) antigens by ELISA. Risk factors for malaria infection (positive PCR) and malaria exposure (seropositivity) were assessed by multivariate survey logistic regression models. Age-specific seroprevalence was analyzed using a reversible catalytic conversion model based on maximum likelihood for generating seroconversion rates (SCR, λ). SaTScan was used to detect spatial clusters of serology-positive individuals within each site.

Results: The overall parasite prevalence by PCR was low, i.e. 3.9% for *P. vivax* and 6.7% for *P. falciparum,* while the seroprevalence was substantially higher, 33.6% for *P. vivax* and 22.0% for *P. falciparum*, with major differences between study sites. Age and location (site) were significantly associated with *P. vivax* exposure; while location, age and outdoor occupation were associated with *P. falciparum* exposure. *P. falciparum* seroprevalence curves showed a stable transmission throughout time, while for *P. vivax* transmission was better described by a model with two SCRs. The spatial analysis identified well-defined clusters of *P. falciparum* seropositive individuals in two sites, while it detected only a very small cluster of *P. vivax* exposure.

Conclusion: The use of a single parasitological and serological malaria survey has proven to be an efficient and accurate method to characterize the species specific heterogeneity in malaria transmission at micro-geographical level as well as to identify recent changes in transmission.

Keywords: Malaria; transmission; PCR; Serology; hot-spot; heterogeneity; Peruvian Amazon.

4.2. Background

Despite several decades of intense control efforts, malaria remains an important public health problem in Peru [1], mainly in the department of Loreto in the Amazon region, which has historically accounted for most of the malaria burden within the country [7]. After malaria resurgence in the late 90s, with a peak of more than 120,000 slide confirmed cases in 1997 [1,2], the annual incidence in Loreto following intensified control activities decreased and stabilized at around 45,000-55,000 cases between 2002 and 2005 [7]. Between October 2005 and September 2010, increased support from international donors, e.g. the Global Fund-PAMAFRO Project [4], allowed the scale-up of comprehensive malaria control strategies in the Peruvian Amazon [5,6]. During this period, malaria declined drastically in Loreto from 54,291 reported clinical cases (25% due to *P. falciparum*) in 2005 to 11,604 cases (20% due to *P. falciparum*) in 2010, [7]. Since October 2010, malaria control activities are mainly supported by the Ministry of Health (MoH) budget, prioritizing passive and reactive case detection using standard light microscopy (LM) and treatment of confirmed infections. Unfortunately since 2012, malaria incidence in Loreto is rising again, with more than 25,000 cases reported [7].

Although Loreto is considered as a low endemic area [94], previous studies around Iquitos have reported high heterogeneity in malaria transmission at micro-geographical scale [96,132]. Even though this heterogeneity creates opportunities for targeted control interventions [9], the identification of hotspots of high malaria transmission intensity (MTI) is not straightforward. Choosing an appropriate method to measure MTI is key in assessing the risk of infection. Though the entomological inoculation rate (EIR) is usually considered as the gold standard for measuring MTI, in low transmission area this measure has proven to be insensitive and non cost-effective [16,17]. Incidence of confirmed clinical malaria has frequently been used for malaria risk stratification; however, its validity is affected by the differential acquisition of immunity inside and outside hotspots as well as contextual factors such as treatment seeking behaviors [9]. Although

determining malaria parasite carriage (both symptomatic and asymptomatic infections) through large community surveys seems to be a more accurate method, this is still unable to capture seasonal variations [16,18]. Additionally, low-parasite-density infections would only be detected by highly sensitive diagnosis tests such as polymerase chain reaction (PCR) [19].

Cross-sectional surveys using serological markers are increasingly proposed as useful alternatives for estimating MTI in low transmission settings, especially for the identification of malaria hotspots and related risk factors [10,59,115,120,133]. Age-stratified seroprevalence data have been used to model seroconversion rates (SCR) which closely correlated with EIR, parasite prevalence rates [20,21] as well as related changes over time following specific interventions [22], seasonal or behavioral changes [23].

Following the recent changes in malaria control activities in the Peruvian Amazon region, we conducted a cross-sectional survey combining molecular and serological markers with the aim of characterizing the current malaria transmission patterns, detecting hotspots, and identifying recent changes in species-specific MTI in three sites around Iquitos city.

4.3. Methods

Study area

The study was conducted in three different sites in San Juan (south of Iquitos city) (Figure 4.1), one of the most malaria endemic district in Loreto over the past five years. Study sites included communities which were established after the 1980s following the intensification of the deforestation and the extension of the road connecting Iquitos to Nauta district [134]. Site A included three contiguous peri-urban communities, i.e. Aguaje (AG), Agua Blanca (AB) and Pampachica (PA), located about 500 meters from the Nanay river, just on the administrative border between San Juan and Iquitos districts. The communities have well-constructed wooden houses (with 24 hour electric service)

built on stilts as the river floods the area during the rainy season. Site B included three rural communities, i.e. El Milagro (EM), Villa Buen Pastor (BP) and San Carlos (SC), situated at 21 kilometers (km) from Iquitos on the Iquitos-Nauta district road, and then at 2, 4 and 9 km, respectively via a rural trail. The surrounding area is mainly composed by dense secondary growth vegetation with natural water bodies (swamps, ground pools, etc.) that enlarge during the rainy season. Among the three communities, only SC has direct access to the Itaya River. Site C was the farthest, consisting of two rural communities, i.e. San Lucas (SL) and El Triunfo (ET), with houses located on both sides of the Iquitos-Nauta road between the 42nd and 48th km. Several fish ponds are found along this section of the road. Unlike site A, communities in sites B and C have an important proportion of semi-closed wooden houses and no electricity. The study population was predominantly mestizos - referring to all Peruvians that cannot be clearly identified as belonging to any ethnic minority population- living mainly from small-scale agriculture, fishing, and small commercial activities.

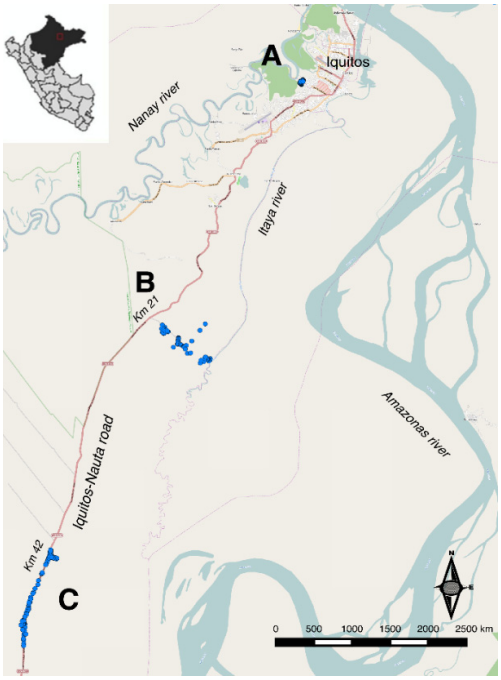


Figure 4.1. **Study area in San Juan district, Loreto department, Northern Peruvian Amazon.**

Eight communities in three sites were included: Site A: Aguaje (AG), Agua Blanca (AB) and Pampachica (PA); Site B: El Milagro (EM), Villa Buen Pastor (BP) and San Carlos (SC); and Site C: San Lucas (SL), El Triunfo (ET).

The climate is tropical, warm and humid with a rainy season from November to May and a dry season from June to October. The annual average temperature is around 27°C, the relative humidity above 80%, and an average annual rainfall of 4 meters [85]. Malaria transmission in the area is perennial, with a peak between February and July [96]. *Anopheles darlingi* is the main malaria vector and is highly anthropophilic. A recent entomological study carried out in some communities near the study area [135] showed that *An. darlingi* was now mainly biting outdoors while it was previously known to bite equally in- and outdoors. *P. vivax* and *P. falciparum* infections at district level occur at a ratio 5:1 and all age groups are at risk of infection, though adults more than children. Malaria surveillance relies on passive case detection (PCD) with microscopy. Patients presenting with fever or any other symptoms compatible with malaria are systematically tested by microscopy at health facilities and treated with chloroquine (CQ) for 3 days (10mg/g on days 1 and 2, and 5mg/kg on day 3), plus primaquine (PQ) for 7 days (0.5 mg/kg/day) if *P. vivax* malaria is confirmed, or with mefloquine (MQ) (12.5 mg/kg/day for 2 days) plus artesunate (AS) (4 mg/kg/day for 3 days) if P. falciparum malaria is confirmed. These treatment guidelines are in place since 2001, and all health facilities should perform directly observed therapy (DOT) [92].

Data collection

A complete census of the study population with collection of information on socio-demographic variables (age, gender, education, occupation, socio-economic status) and malaria preventive measures (bed net use), was conducted two weeks prior to the survey. Each house was identified with a unique number and geo-referenced using a handheld Global Positioning System (GPS) device (Garmin's GPSMAP 60CSx, Garmin International Inc., USA).

The survey was done in November 2012. Each household was visited and all available children between six months and seven years old, plus one randomly selected individual above 7, were enrolled after providing written informed consent/assent. Whenever

selected individuals were absent, the household was revisited within the next two days to maximize subject participation. Each participant had the axillary temperature taken, malaria symptoms were recorded, and a finger-prick blood sample collected for immediate microscopy (thick and thin blood smears) and on filter paper (Whatman grade 3, Whatman, Springfield Mill, USA) for later serological and molecular tests. Filter paper dried blood samples were individually stored at 4°C with desiccant until processed at Institute of Tropical Medicine Alexander von Humboldt, Lima (ITM-AvH). All individuals with a malaria infection detected by LM were treated according to the national guidelines.

Laboratory procedures

Microscopy

Thick and thin smears were stained for 10 minutes with a 10% Giemsa solution, and parasite density was computed after counting the number of asexual parasites for 200 white blood cells (WBC) in the thick smear and assuming a concentration of 8,000 WBCs/µl. Slides were read during the survey and then a day later by an expert at microscopy at the reference laboratory in Iquitos. A slide was declared negative if no malaria parasite was found after examining 100 fields. Quality control was done blindly on all positive slides and 10% of randomly chosen negative slides by a senior technician at ITM-AvH. Any discordant results were reread by a second senior technician until agreement.

Species-specific Polymerase Chain Reaction (ss-PCR)

Parasite DNA was extracted using the Saponin lysis Chelex 100 method and stored at 4°C for immediate use in PCR reactions or at -20°C for later use. All samples were first analyzed by ssPCR following a protocol published elsewhere [119]. Briefly, this ssPCR consisted of a primary PCR with primers directed to specific Plasmodium sequences, and

obtained amplicons were used for a second PCR using *P. falciparum* and *P. vivax* specific primers.

Serology

Antibodies for specific IgG responses to *P. vivax* merozoite surface protein-1(19-kDa C-terminal region, PvMSP-1_{19}) [122] and apical membrane antigen-1 (PvAMA-1) [136], and to *P. falciparum* glutamate-rich protein-fragment R2 (PfGLURP-R2) [121] and apical membrane antigen-1 (PfAMA-1) [137] were detected using ELISA. Although data on the kinetics of antibody responses are limited, some studies using similar antigens (such as AMA-1 and MSP1-19) have reported that these antibodies are not short-lived (lasting from months to several years) and that the half-live of those responses can be influenced by differences in the background immunity of populations and in the level of malaria transmission [138]. According to the ELISA protocol [120], dried blood filter paper samples (5mm diameter disc/sample) were eluted overnight at 4°C in 2 ml of PBS-Blotto-Tween. Two hundred microliters of the eluate were added in duplicate to blocked-ELISA plates coated separately with each antigen. Pooled sera from five *P. vivax* and five *P. falciparum* infected patients, and from five control individuals without recent (negative PCR) and past history of malaria infection were diluted at 1:400 in PBS-Blotto, as positive and negative control respectively. Goat anti-human IgG (H+L) specific peroxidase (Merck Millipore, USA) diluted to 1:20,000 in PBS-Tween was used as conjugate and incubated for 1 h before development of the ELISA using 200µl ABTS substrate-chromogen solution. Optical Density (OD) values at 405 nm (Lector Multi-Modal Synergy H1, BioTek, USA), were corrected by subtracting the mean OD of the antigen negative control wells from the mean OD of the corresponding antigen containing wells. Analyses with duplicate OD values that differed by more than 1.5-fold were rejected and rerun. To ensure a standardization of the sample results across ELISA plates, the percent positivity (PP) of each specimen was calculated using the OD of the positive control serum as 100%. Quality control was done blindly at ITM-AvH on 5% of randomly chosen

samples. The criterion for positivity was determined for each antigen of each species applying a mixture model to the PP data which assumed two inherent Gaussian distributions: a narrow distribution or seronegatives, and a broader distribution of seropositives. Cut-off value was calculated as the mean plus 3 standard deviations of the narrow distribution [21].

Data analysis

Survey data were double entered and cross-checked in Excel (Microsoft Corp, USA), and data analysis was performed with R v.2.15 software (R Development Core Team, R Foundation for Statistical Computing, Austria). Baseline characteristics between sites were compared using the Chi-squared test. Species-specific parasite prevalence (by LM and by PCR) as well as seroprevalence were estimated overall and by site.

Uni- and multivariate adjusted analyses were performed using survey logistic regression to assess risk factors (i.e., site, age, gender, out-door occupation, predominant material in walls and roof, availability of electricity, and past history of malaria) for species-specific malaria outcomes (i.e. malaria infection, and malaria exposure), adjusting for potential confounders. Malaria infection was defined as an individual with a positive PCR result, regardless of symptoms; while malaria exposure to any of the two species was defined as an individual with a positive serology to any of the two antigens for each species. Factors with p-values <0.1 for the likelihood ratio test in the univariate analysis (intended to assess enough potential risk factors) were considered for inclusion in the multivariate adjusted model. Using the manual backward methods, final models retained all factors that were significantly associated with the malaria outcome ($p<0.05$). Interactions were systematically checked for up to order two. All analyses took into account the following survey design characteristics: community as strata, household as primary sampling unit, and corresponding sampling weights (w) for children <7 years (w=1) and for individuals ≥7 years (w=1/n, where n=number of individuals ≥7 years at household).

The QGIS™ software QGIS v.2.6 (QGIS developer team, Open Source Geospatial Foundation) was used to map all surveyed individuals [139] and the SaTScan™ software v.9.3 (M Kulldorff and Information Management Services Inc, USA) [140] to detect spatial clusters of individuals with malaria infection as well as with malaria exposure in each site, using the following characteristics: purely spatial analysis, Bernoulli probability model [141], latitude/longitude coordinates, report of most likely clusters with no geographical overlap of secondary clusters, Gini optimizer cluster collection, and maximum spatial cluster size equal to 50% of total population. The main analysis was done without adjustment for covariates, and a secondary analysis included age as covariate. SaTScan applies multiple circular windows with varying size (from zero to a maximum radius of 50% of total population) across the study area, each circle representing a possible cluster. Clusters were assessed based on 999 Monte Carlo simulations to determine the probability of observed frequency of infected/exposed individuals being due to chance relative to expected frequency under the null hypothesis of no clustering. The null hypothesis was rejected if any resulting p-value of assessed clusters was <0.05 and the window with the maximum log likelihood ratio (LLR) was identified as the most likely cluster. The relative risk (RR) reported for each identified cluster was the estimated risk within the cluster divided by the estimated risk outside the cluster [141]. Moreover, uni- and multivariate survey logistic regression analysis were performed in sites with detected spatial clusters to determine association between the residence of individuals (inside or outside the spatial cluster) and potential risk factors.

Seroprevalence was stratified into yearly age-decile groups and then analyzed using a reverse catalytic conversion model [20] which allowed the estimation of two parameters: *a) a seroconversion rate (SCR, λ)* or force of infection (FOI) defined as the annual rate at which individuals change from seronegative to seropositive); and *b) a seroreversion rate (SRR, ρ)* defined as the annual rate at which seropositive individuals revert to a seronegative state. The main assumptions of the model are the following [142]: all

individuals spent their entire live in the community, migration is negligible, there are no individual differences in SCR between subjects of a same community, and the disease transmission in the community is at an endemic steady-state, i.e. SCR is assumed constant over time.

Since study communities were established in the early 1980s, the first analysis excluded individuals older than 30 years old (21.2% of total population) in order to comply with the first assumption mentioned above. The dataset of all sites was first analyzed using a reversible catalytic model that assumed a stable transmission throughout time estimating SCR and SRR for the study population up to age 30 years. Then, the specific data set from each site was analyzed using two reversible catalytic models, one that assumed a constant-SCR over time and another that assumed a change in SCR at a given time point. All these models included the population SRR as common parameter. Profile likelihood plots were applied to determine the most likely time point for change in the transmission prior to survey [21]. The latter was subsequently incorporated into the catalytic model to fit a seroprevalence curve with two SCRs (before and after time cut-off) [22], which was then compared with a single SCR seroprevalence curve using the likelihood ratio (LR) test ($p<0.05$). Bootstrapped 95% confidence intervals for SCR and SRR were estimated using the bias-corrected and accelerated (BCa) bootstrap resampling method [143]. Later, a sensitivity analysis was done including individuals of all ages in order to assess the influence of the individuals older than 30 years old on the age-seroprevalence curves and on the identification of most likely time points for change in the transmission according to the model.

Additionally, routine surveillance data, namely reported clinical malaria episodes through PCD provided by the Regional Direction of Health Loreto, were used to explore the association between malaria incidence trends in the department (between 1990 and 2013) [7] and the changes in malaria transmission obtained from catalytic models. Since routine malaria surveillance data since 2002 could be reliably disaggregated only to the district

level but not to the village level, an retrospective revision of all registered individuals detected by PCD between January and December 2012 was done in all health facilities near the study communities in order to estimate the annual parasite index for *P. vivax* (API*v*) and *P. falciparum* (API*f*) per site in the year of the survey.

Ethical issues

Ethical clearance was obtained from the Ethics Review Board of the Universidad Peruana Cayetano Heredia, Lima, Peru (SIDISI code: 059745). Permissions were received from health and local authorities after explaining the purpose and procedures of the study. Signed informed consent was obtained prior to participation and blood sampling by all adults and the parents of all participating children <18 years. Besides their parent's consent, children older than 7 years provided a signed informed assent.

4.4. Results

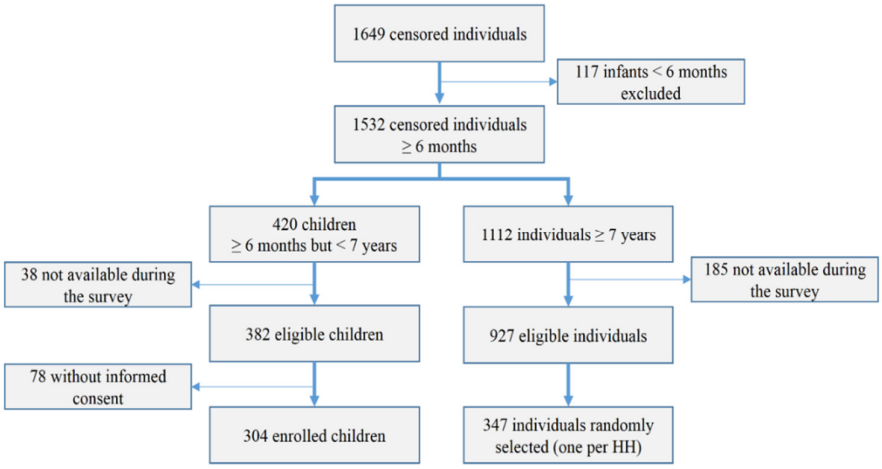


Figure 4.2. Flowchart of study participants

A total of 348 houses and 1,649 individuals were identified during the census, with the following site specific distribution: Site A=129 houses (H) and 655 individuals (Ind); Site

B=80 H and 377 Ind; Site C=139 H and 617 Ind. The overall ratio of female to male was 1.04; half of the population was less than 15 years old. The survey enrolled 651 individuals: 304 (72.4%) of the total 420 censored children between 6 months and 7 years, and 347 (31.2%) individuals of the total individuals ≥ 7 years. Figure 4.2 presents the flowchart of participants and Annex (Chapter 9)-Table S4.1 shows the survey coverage by site and age group.

Table 4.1. Baseline characteristics of the study population

	Site A (AG, AB, PC)		**Site B (SC, BP, EM)**		**Site C (ET, SL)**		**Total**	
	n	**%**	**n**	**%**	**n**	**%**	**N**	%
Total surveyed	238	36.6	153	23.5	260	39.9	651	100.0
Gender								
Female	137	57.6	77	50.3	134	51.5	348	53.5
Age groups (years)								
< 7	109	45.8	74	48.4	121	46.5	304	46.7
7-14.9	42	17.6	19	12.4	40	15.4	101	15.5
15-30	47	19.7	17	11.1	44	16.9	108	16.6
> 30	40	16.8	43	28.1	55	21.2	138	21.2
Education (>=18 years, n=218)*								
None	0	0.0	2	3.6	3	3.5	5	2.3
Primary	23	29.5	28	50.9	55	64.7	106	48.6
Incomplete secondary	29	37.2	11	20.0	18	21.2	58	26.6
Complete secondary/+	26	33.3	14	25.5	9	10.6	49	22.5
Occupation (≥15 years, n=246)*								
None (students, retired)	12	14.1	7	11.7	12	11.9	31	12.6
Housewife	46	54.1	23	38.3	41	40.6	110	44.7
Laborer/trader/mototaxi driver	22	25.8	3	5.0	2	2.0	27	11.0
Farmer/guard/logger/fisher	5	5.9	27	45.0	46	45.5	78	31.7
Predominant material in roof*								
Palm leaf, straw	56	22.8	88	56.0	197	79.9	341	51.5
Tin roof	182	77.2	65	44.0	56	20.1	303	48.5
Missing					7		7	
Electricity availability*								
Yes	225	96.0	91	65.3	113	47.6	429	65.9
Sleeping under bednet previous night								
Yes	227	96.7	149	99.7	259	100.0	635	98.8
Missing	4		3		1		8	
Self-report of confirmed malaria*								
Never	182	65.0	74	31.3	135	42.7	391	60.4
In the past 12 months	7	3.8	25	19.1	66	29.5	98	15.1
>12 months	48	31.1	54	49.6	56	27.8	158	24.4
Missing	1				3		4	

* p-value <0.05 for differences between sites

Of the total 651 individuals, 238 (36.6%) lived in Site A, 153 (23.5%) in Site B, and 260 (39.9%) in Site C (Table 4.1). Children under 7 represented between 45% and 48% of the participants across sites, and females slightly outnumbered males in site A (ratio female/male =1.15; p=0.27). The majority of adult participants had only primary school education level, except in site A where 70.5% had secondary or higher education level (p<0.001). Overall, the most commonly reported occupation among individuals aged ≥15 years was housewife, followed by farmers and guards in sites B and C, while activities were more diversified including traders and indoor laborers in Site A (p<0.001). In all sites, most of houses had wooden walls, with roofs of predominantly palm leaf and straw in Sites B and C, and tin sheets in Site A (p<0.001). Reported bed net use was very high (>95%) in all sites; nevertheless, most participants in site A stated of never have experienced malaria, while this was not the case in the other two sites (p<0.001).

By LM, the overall prevalence was 3.3% (7 *P.vivax* and 10 *P.falciparum* infections), and by PCR 10.9% (18 *P. vivax*, 34 *P.falciparum* and one mixed infections) (Table 4.2), with the highest figures in the two rural sites B and C. Species-specific parasite prevalence varied widely across the sites by both LM and PCR (p<0.001). By PCR, both species had similar prevalence in site C (8.0%) and A (3.0 *vs* 1.1%), while in site B *P.falciparum* was predominant (11.2%) over *P.vivax* (2.1%). The species specific seroprevalence showed similar heterogeneity between sites. *P.vivax* seroprevalence was equally high in sites B (42.3%) and C (46.7%), and relatively low in site A (16.4%) (p<0.001). *P.falciparum* seroprevalence was 36.7% in site B, 16.7% in site A and 18.7% in site C (p<0.001). Interestingly for both species, sites with the highest seroprevalence were respectively the ones with the highest prevalence by PCR, and also the ones with the highest API estimated from PCD records in 2012. Gametocytes were found in 4 (44.4%) of the total 9 microscopically confirmed *P.falciparum* infections in site B, as well as in 4 (57.1%) of the total 7 microscopically confirmed *P. vivax* infections in site C. The unique microscopically confirmed *P.falciparum* infection in site C had also gametocytes.

Table 4.2. Malaria prevalence (by microscopy and PCR) and seroprevalence by study site

	Site A			Site B			Site C			Total		
	n	%	**95% CI**	**n**	%	**95% CI**	**n**	%	**95% CI**	**n**	%	**95% CI**
Microscopy												
*P.vivax**	0	0.0		0	0.0		7	4.9	[2.1,10.8]	7	1.8	[0.8,4.1]
*P.falciparum**	0	0.0		9	6.4	[3.0,13.1]	1	0.2	[0.0,1.2]	10	1.5	[0.7,3.1]
Total*	0	0.0		9	6.4	[3.0,13.1]	8	5.0	[2.2,10.9]	17	3.3	[1.9,5.8]
PCR												
*P.vivax**	1	1.1	[0.1,7.3]	2	2.1	[0.5,8.1]	15	8.0	[4.4,14.0]	18	3.9	[2.3,6.5]
*P.falciparum**	6	3.0	[1.2,7.8]	14	11.2	[6.2,19.2]	14	8.0	[4.2,14.6]	34	6.7	[4.5,9.9]
Mixed	0	0.0		1	1.1	[0.1,7.2]	0	0.0		1	0.2	[0.0,1.7]
Total	7	4.1	[1.7,9.6]	17	14.4	[8.6,23.0]	29	15.9	[10.5,23.5]	53	10.9	[8.0,14.6]
Seropositivity *P.vivax*												
MSP1-19*	13	8.1	[4.2,14.8]	26	24.2	[16.5,34.0]	88	38.5	[30.6,47.1]	127	23.1	[19.2,27.6]
AMA-1*	18	11.9	[7.0,19.5]	35	33.5	[24.5,43.9]	67	34.5	[27.1,42.6]	120	25.2	[21.1,29.9]
Total*	27	16.4	[10.7,24.4]	45	42.3	[32.6,52.6]	102	46.7	[38.5,55.1]	174	33.6	[29.0,38.5]
Seropositivity *P. falciparum*												
GLURP*	26	13.7	[8.8,20.8]	34	27.9	[19.9,37.7]	39	15.8	[10.8,22.5]	99	17.7	[14.2,21.8]
AMA-1*	9	4.3	[1.9,9.2]	22	20.7	[14.0,29.7]	18	8.5	[4.9,14.6]	49	9.6	[7.1,12.8]
Total*	33	16.7	[11.5,23.8]	46	36.7	[27.9,46.5]	47	18.7	[13.2,25.8]	126	22.0	[18.3,26.3]
Incidence rate (2012)												
API*v* (x1000)$^{+}$		7.6			66.3			205.8				
API*f* (x1000)$^{+}$		4.6			148.5			14.6				
API *total* (x1000)$^{+}$		12.2			214.8			220.4				

*p-value<0.05 for significant differences between sites**.**

+ APIv= Annual parasite index for *P. vivax*, APIf= Annual parasite index for *P. falciparum*, APItotal= Annual parasite index

Most of PCR-positive infections were sub-microscopic (>70%) and asymptomatic (>75%) at the time of the survey, regardless of the *Plasmodium* species (Table 4.3). Species-specific PCR positivity was significantly associated with species-specific seropositivity both overall and for each specific antigen ($p < 0.05$).

Table 4.3. Association between PCR results and microscopy, serology and symptoms

	PCR results*						
	Negative		***P. vivax***		***P. falciparum***		**p-value**
	n	%	n	%	n	%	
Total analyzed	596		18		34		
Microscopy							
Negative	594	99.7	13	72.2	24	70.6	<0.001
P. vivax	2	0.3	5	27.8	0	0	
P. falciparum	0	0	0	0	10	29.4	
***P. vivax* MSP-119 serology**							
Negative	485	81.4	10	55.6	26	76.5	0.043
Positive	111	18.6	8	44.4	8	23.5	
***P. vivax* AMA-1 serology**							
Negative	493	82.7	9	50	26	76.5	0.004
Positive	103	17.3	9	50	8	23.5	
Combined *P. vivax* serology							
Negative	445	74.7	8	44.4	21	61.8	0.011
Positive	151	25.3	10	55.6	13	38.2	
***P. falciparum* GLURP serology**							
Negative	513	86.1	15	83.3	21	61.8	0.003
Positive	83	13.9	3	16.7	13	38.2	
***P. falciparum* AMA-1 serology**							
Negative	554	93	18	100	27	79.4	<0.001
Positive	42	7	0	0	7	20.6	
Combined *P. falciparum* serology							
Negative	489	82	15	83.3	18	52.9	<0.001
Positive	107	18	3	16.7	16	47.1	
Fever/history of fever (previous 2 days)	33	5.6	3	16.7	8	23.5	<0.001

* One mixed infection not shown (asymptomatic individual seronegative both for*P. falciparum* and *P. vivax*

Given the small number of PCR-positive infections by species, multivariate adjusted risk factor analysis was carried out only for any parasite infection. Only site and age remained independently associated with malaria infection: while individuals living in Site B (AOR: 3.9, 95% CI [1.4–11.5]) and in Site C (AOR: 4.5, 95% CI [1.6–12.7]) were more likely to be infected than those living in Site A, individuals >30 years had higher odds of malaria infection than children < 7 years (AOR: 2.4, 95% CI [1.0–5.7]). Considering *P. vivax* exposure, the multivariate adjusted risk factor analysis showed that site and age remained independently associated with the *P.vivax* seropositivity: individuals living in Site B (AOR: 3.9, 95% CI [2.0–7.7]) and in Site C (AOR: 5.1, 95% CI [2.8–9.3]) had higher odds of *P. vivax* seropositivity than those in Site A, and those older than seven years had increasingly higher odds (test for trend $p < 0.001$) of *P. vivax* seropositivity compared to younger children (Table 4.4).

For *P. falciparum* exposure, site, age and outdoor occupation remained independent risk factors in the final model: individuals living in Site B (AOR: 2.3, 95% CI [1.2–4.4]) were more likely to be exposed to *P. falciparum* than those in Site A, adults >30 years (AOR: 2.1, 95% CI [1.1–4.0]) were more exposed than children<7 years, and people working outdoor were three times more exposed to *P. falciparum* (AOR: 3.1, 95% CI [1.5–6.6]). Despite being significantly associated with both *P. vivax* (OR: 6.8, 95% CI [3.7–12.5]) and *P. falciparum* exposure (OR: 2.7, 95% CI [1.5–4.8]) in the univariate analysis, the history of malaria in the past year was not included in the multivariate analysis, since it is not a risk factor for malaria infections per se but rather a consequence of previous exposure to given risk factors.

Table 4.4. Univariate and multivariate risk factor analysis for species-specific malaria exposure

	Univariate								Multivariate					
	P. vivax					*P. falciparum*					*P. vivax*			*P. falciparum*		
	%	n/N	OR		[95% CI]	%	n/N	OR		[95% CI]	AOR		[95% CI]	AOR		[95% CI]
Site																
A	16.4	27/238	1			16.7	33/238	1			1			1		
B	42.3	45/152	3.7	*	[2;7.1]	36.7	46/152	2.9	*	[1.6;5.2]	3.9	*	[2;7.7]	2.3	*	[1.2;4.4]
C	45.0	102/259	4.4	*	[2.4;8.1]	18.7	47/259	1.1		[0.6;2.1]	5.1	*	[2.8;9.3]	0.8		[0.4;1.6]
Age groups (years)																
< 7	13.1	40/303	1			13.7	39/303	1			1			1		
7-14.9	27.0	27/101	2.5	*	[1.5;4.2]	18.0	16/101	1.4		[0.7;2.6]	2.9	*	[1.7;5]	1.5		[0.8;2.8]
15-30	34.8	35/107	3.6	*	[2.1;6.1]	18.3	20/107	1.4		[0.7;2.7]	4.3	*	[2.4;7.7]	1.3		[0.7;2.6]
> 30	52.6	72/138	7.4	*	[4.5;12]	34.7	51/138	3.3	*	[1.9;5.7]	8.8	*	[5.2;14.8]	2.1	*	[1.1;4]
Gender																
Female	29.6	80/346	1			16.9	54/346	1								
Male	38.7	94/303	1.5	º	[1;2.3]	28.6	72/303	2	*	[1.2;3.2]						
Outdoor occupation (farmer, guard, logger, fisher)																
No	29.0	127/571	1			18.1	91/571	1						1		
Yes	63.3	47/78	4.2	*	[2.4;7.6]	47.5	35/78	4.1	*	[2.3;7.5]				3.1	*	[1.5;6.6]
Predominant material in roof																
Palm leaf, straw	25.1	52/302	1			19.0	43/302	1								
Tin roof	41.5	120/340	2.1	*	[1.3;3.4]	24.6	82/340	1.4		[0.8;2.3]						
Electricity availability																
Yes	29.3	95/428	1			20.9	76/428	1								
No	44.1	79/221	1.9	*	[1.2;3.0]	24.7	50/221	1.2		[0.7;2.1]						
Malaria episode in the past 12 months																
No	26.3	108/547	1			18.9	89/547	1								
Yes	70.8	66/98	6.8	*	[3.7;12.5]	38.3	37/98	2.7	*	[1.5;4.8]						

*p<0.05, º p<0.1

Figures 4.3 and 4.4 show the spatial distribution of infected (positive PCR) and exposed (positive serology) individuals by species and for each site. Despite some aggregation of cases within adjacent houses in sites B and C, no significant spatial clusters of infections were identified by SaTScan, mainly because of the low number of infections. However, the purely spatial analysis without adjustment for covariates confirmed that malaria exposure mainly to *P. falciparum* was not randomly distributed. In site A, the most likely spatial cluster of *P. falciparum* seropositive individuals (RR=6.3, p<0.001), located on the western part of the village close to Nanay river (Figure 4.3A), had a 120m radius area which included 114 surveyed individuals (47.9% of total) in 59 households (45.7% of total). This cluster represented 85.3% (29/34) of all *P. falciparum* seropositive individuals, but only two of the six PCR-confirmed infections by species. In site B, the most likely *P. falciparum*-exposure cluster (RR=2.9, p=0.001) had a radius of 1.3 km including 50 individuals (32.7% of the total) in 30 households (37.5% of the total) (Figure 4.3B). This cluster represented 58.7% (27/46) of all 46 *P. falciparum* seropositive individuals in the site, and 9 (64.2%) of the 14 *P. falciparum* mono-infections detected by PCR. Site C had no significant spatial cluster of *P. falciparum* exposure but was the only site where a most likely cluster of *P. vivax*-exposure (RR=2.9, p=0.001) could be identified (Figure 4.4C). This was a small cluster of 68m radius that included 10 households, all surveyed individuals were *P. vivax* seropositive but none of them was infected (PCR negative). Adding age group as covariate in SaTScan analysis to locate species-specific exposure clusters had no effect on the results.

The multivariate survey logistic regression analysis for risk factors independently associated with the most likely *P. falciparum*-exposure cluster in site B identified outdoor occupations and electricity availability. Indeed people living inside the most likely cluster were more likely to have outdoor activities (AOR: 4.4, 95% CI [1.6–11.8]), and a house with electricity (AOR: 4.5, 95% CI [1.6–12.9]) compared to people not living in that cluster. No significant risk factors were found with the residence inside the detected clusters in sites A and C (see Annex, Chapter 9, Table S5.2).

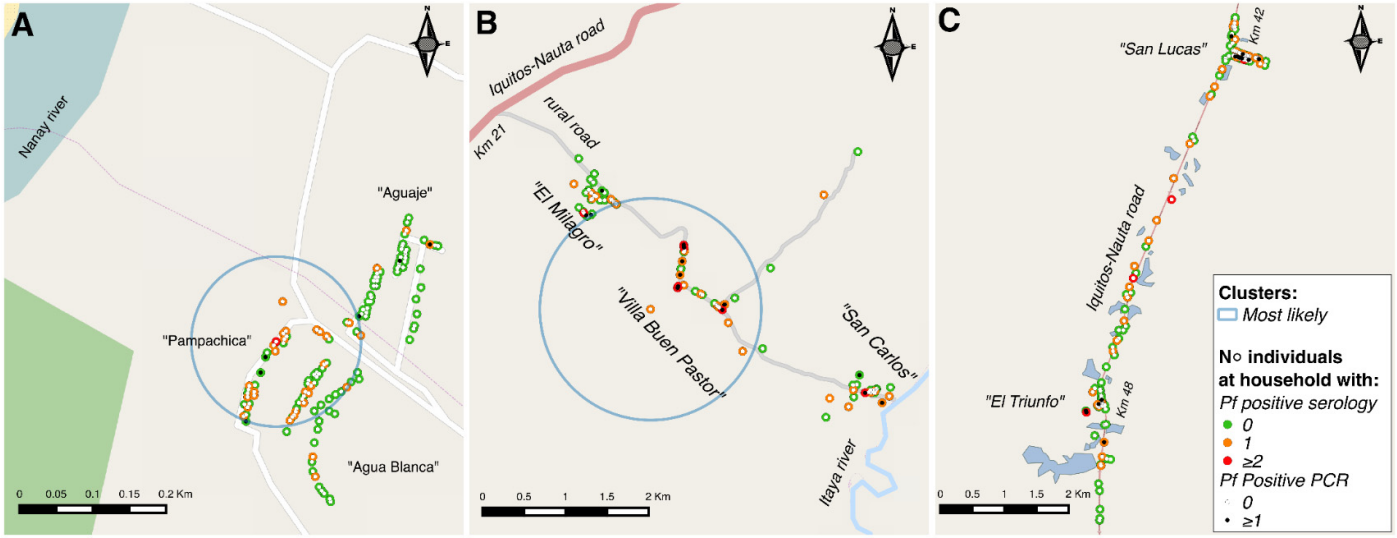


Figure 4.3. Distribution of *P. falciparum* infected and exposed individuals by study site (A, B, C) with respective significant spatial clusters for *P. falciparum* seropositivity.



Figure 4.4. Distribution of *P. vivax* infected and exposed individuals by study site (A, B, C) with respective significant spatial clusters for *P. vivax* seropositivity

Age seroprevalence curves of individuals aged ≤ 30 years confirmed a significant increase of malaria seroprevalence with age, but with different species specific patterns (Figure 4.5). The overall (all sites) *P. falciparum* age seroprevalence was better described by a model with only one SCR (λ=0.08) and one SRR (ρ=0.384), while the overall *P. vivax* age seroprevalence was best modeled with two SCR (pre-2010 $\lambda 1$=0.046, post-2010 $\lambda 2$=0.122) and one SRR (ρ=0.096) indicating an increase in MTI in 2010 for this species (p=0.01). The reversible catalytic models for *P. falciparum* confirmed a stable transmission, with higher transmission intensity in Site B (λ=0.129) than in sites A (λ=0.061) and C (λ=0.075). Conversely, best models for *P. vivax* differed by site. While site C had a change in *P. vivax* MTI in 2010 with a significant threefold increase (pre-2010 $\lambda 1$=0.09, post-2010 $\lambda 2$=0.241, p=0.02), sites A and B experienced significant drops in SCR but at different times: 13-14 years before the survey in site A (pre-1998-1999 $\lambda 1$=0.023, post-1998-1999 $\lambda 2$=0.007, p=0.04), and 7-8 years prior survey in site B (pre-2005 $\lambda 1$=0.028, post-2005 $\lambda 2$=0.008, p=0.03). Moreover for both species, sites with the highest SCRs (SCR after the time cut-off in models with two SCRs) were respectively the ones with the highest API.

Although the inclusion of seroprevalence data of individuals >30 years did not change the assumption of a stable transmission over time for *P. falciparum* per site (site A, λ=0.024; site B, λ=0.063; site C, λ=0.033), it allowed identifying a decrease in SCR for *P. falciparum* 32 years before the survey when the analysis included all the sites (pre-1980 $\lambda 1$=0.05, post-1980 $\lambda 2$=0.031, p=0.03) (Figure 4.6). For *P. vivax*, the incorporation of antibody responses of individuals >30 years had different effects. While in all sites and site C it was identified an increase in SCR for *P. vivax* two years before the survey (all sites: pre-2010 $\lambda 1$=0.035, post-2010 $\lambda 2$=0.117, p=0.002; site C: pre-2010 $\lambda 1$=0.061, post-2010 $\lambda 2$=0.231, p=0.003), *P. vivax* seroprevalence curve in site A was best described by a model assuming a decrease in malaria transmission 37 years prior survey (pre-1975 $\lambda 1$=0.031, post-1980 $\lambda 2$=0.009, p=0.008). Conversely, model results for site B indicated a constant *P. vivax* SCR over time (λ=0.04).

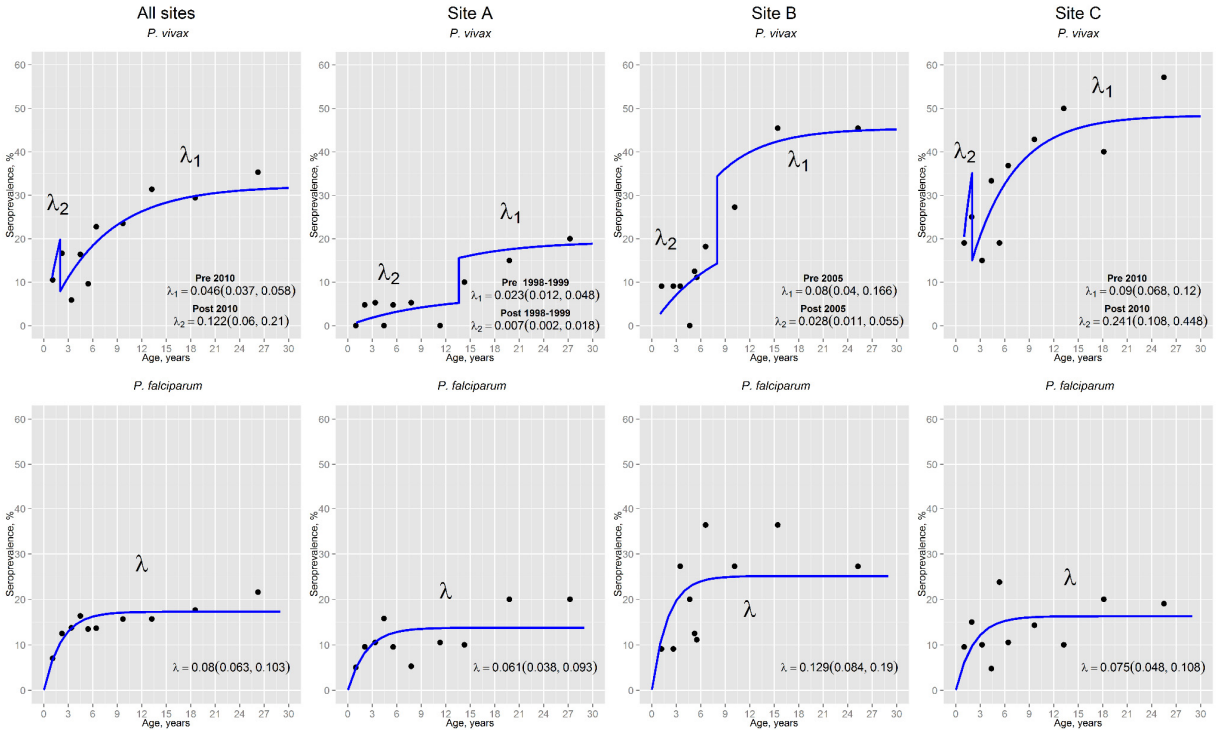


Figure 4.5. *P. vivax* and *P. falciparum* age-seroprevalence curves for individuals under 30 years old in each site. Black dots represent observed prevalence, whilst the blue lines represent the maximum likelihood model. Seroconversion rates (λ, (95%CI)) are plotted on the graphs. Two seroconversion rates (λ_1 and λ_2) are plotted in graphs if likelihood ratio tests indicate change at a certain point in calendar time.

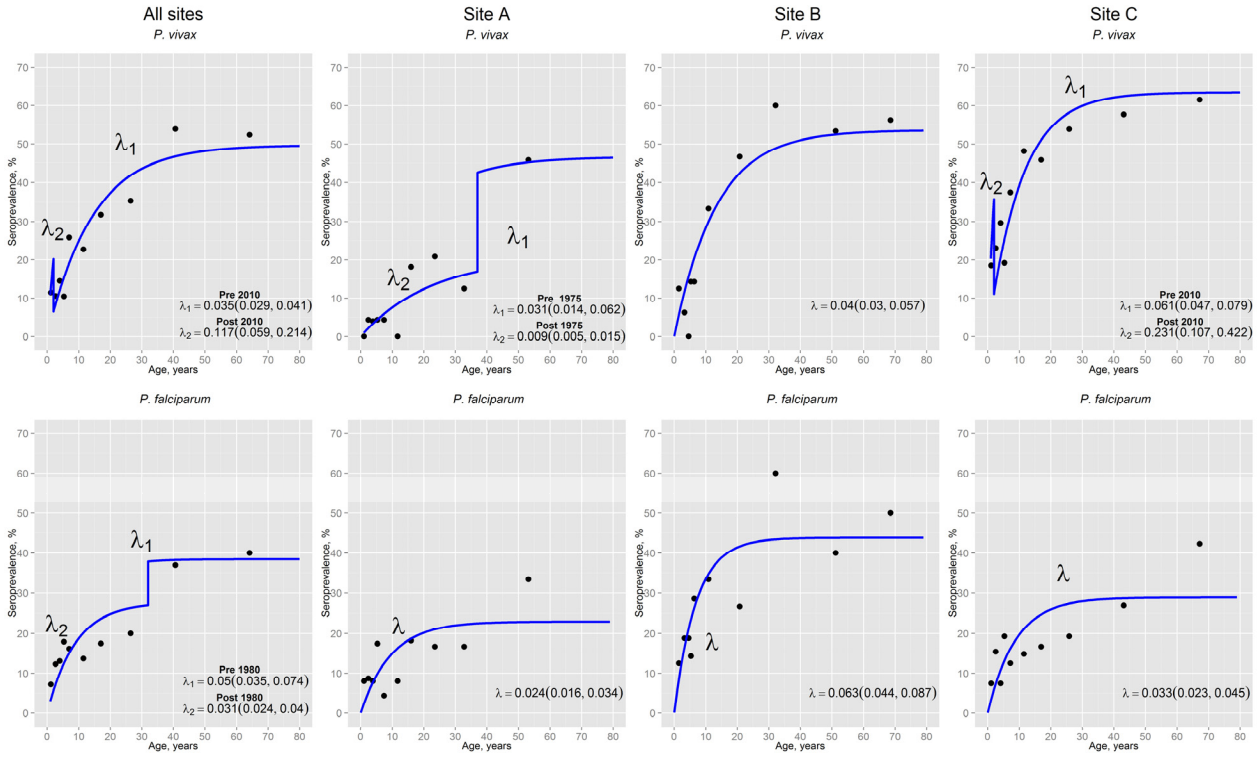


Figure 4.6. *P. vivax* and *P. falciparum* age-seroprevalence curves for individuals of all ages in each site. Black dots represent observed prevalence, whilst the blue lines represent the maximum likelihood model. Seroconversion rates (λ, (95%CI)) are plotted on the graphs. Two seroconversion rates (λ1 and λ2) are plotted in graphs if likelihood ratio tests indicate change at a certain point in calendar time.

The retrospective analysis (1990-2013) of annual malaria incidence in Loreto department (Figure 4.7) showed a peak during the malaria epidemic (1996-1998) associated with the ENSO phenomenon when more than 65,000 *P. vivax* and 50,000 *P. falciparum* cases were recorded. After the epidemic, incidence dropped drastically and stabilized between 2000 and 2001 at around 30,000 total annual cases, and at 45,000 and 55,000 cases between 2002 and 2005. During this post-epidemic period, a new antimalarial treatment policy (2001-2004) was progressively implemented as previously described. During the Global Fund-PAMAFRO Project (2005-2010), intense control efforts resulted in a marked decline of malaria morbidity from 54,291 reported clinical cases in 2005 to 11,604 cases in 2010. However, since 2011 (11,703 cases), malaria incidence is doubling each year. Noteworthy, *P. falciparum* infections usually followed similar trends than *P. vivax*, accounting for 20-25% of the total malaria cases, with exception of 1996-1998 when they represented almost 45% of the cases in Loreto. Between 2002 and 2013, though San Juan and Iquitos districts followed similar trends of malaria incidence than Loreto department (See Annex, Chapter 9, Figure S4.1), *P. falciparum* cases started to increase two years earlier (2009) in these two districts.

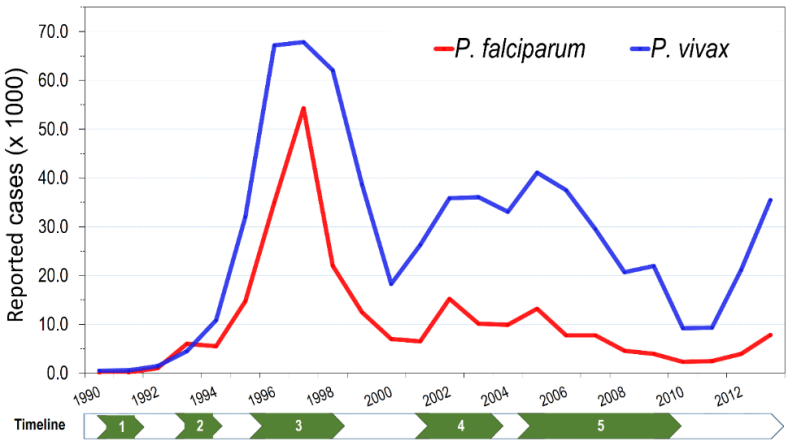


Figure 4.7. Evolution of the annual malaria incidence in Loreto department: 1990–2013. Information on annual malaria cases was obtained from the Regional Health Direction of Loreto. Numbers in the timeline point out important events that influenced malaria incidence: 1) First report *P. falciparum* in Loreto (1990), 2) first reports of chloroquine resistance (CQR) in *P. falciparum* (since 1994), 3) very strong ENSO phenomenon (1996–1998), 4) Implementation of new antimalarial treatment policy for *P. falciparum* and *P. vivax* (2001–2004), 5) Global fund-PAMAFRO project (2005–2010).

4.5. Discussion

The combined analysis of parasitological and serological measures collected during a single cross-sectional survey allowed for an in-depth characterization of the malaria transmission patterns in three different sites in an endemic district of the Peruvian Amazon. Most malaria infections detected by PCR were sub-microscopic and asymptomatic. The site specific analysis of both prevalence and seroprevalence data showed a high degree of heterogeneity at micro-geographic level and in time for both *P. falciparum* and *P. vivax*. However, species-specific parasitological and serological metrics (i.e., seroprevalence, SCRs) were in good agreement with IPA estimates by species.

Although the survey was conducted in November, before the usual peak of clinical malaria cases, an important number of malaria-infected individuals were identified, mainly by *P. falciparum*, and most of them were undetectable by microscopy and free of malaria-related symptoms. As reported by other countries in the region, asymptomatic malaria infections (including submicroscopic infections) are common in low endemic settings, representing a "hidden" human reservoir likely to contribute to maintain transmission [54,111,124,125]. Previous cross-sectional studies using PCR in rural peri-Iquitos communities have also reported high prevalence of asymptomatic parasite carriers (5%-14%) [94,96,98]; however, the surveys were conducted at the time of high seasonal transmission and *P. vivax* was always the predominant species.

Although routine surveillance data at district and department level showed a predominance of *P. vivax* over *P. falciparum* reported cases, parasitological and serological measures indicated that species-specific malaria transmission may vary widely across sites. Indeed, site C had similar prevalence of both species by PCR, but the higher seroprevalence and SCR estimates for *P. vivax* compared to those of *P. falciparum* suggested a very recent increase in *P. falciparum* infections. Moreover, in site

B the higher *P. falciparum* seroprevalence and SCR compared to those of *P. vivax* suggested that the predominance of *P. falciparum* infections detected by PCR was not new. Additionally, the identification of *P. falciparum* gametocyte carriers by microscopy suggested ongoing transmission of this species in the site. These hypothesis were further supported by the newly generated site incidence data on *P. falciparum* (IPA*f*) and *P. vivax* (IPA*v*) in 2012. These results highlight the added value of combining parasitological and serological metrics in accurately understanding the local epidemiology since data from the health information system are often missing or not very reliable.

The differences in species-specific seroprevalence across sites were in line with findings from previous entomological studies, all reporting a high heterogeneity in malaria transmission in the peri-Iquitos region [99,100]. During the late 90s malaria epidemic, EIRs for *P. falciparum* were significantly higher in rural communities along the Iquitos-Nauta road than in periurban areas, with large monthly variations ranging from 0 to 12 infective bites per person per month [99]. More recent studies have shown a positive association between EIR and level of deforestation [45] as well as significant heterogeneity between collection sites and over time, reaching sometimes comparable EIR estimates to holoendemic African areas. Deforestation in areas of site B and C following road construction and subsequent population settlements may have resulted in favorable ecological conditions for mosquito breeding and resting sites near households. Though entomological evaluations (mosquito larval habitat mapping, mosquito density and biting patterns) were not included in the study, previous reports showed that natural water bodies and fish ponds (such those located in sites B and C) constituted ideal breeding sites for *An. darlingi*, and were significantly associated with malaria infection [100,144]; while surrounding secondary vegetation in previously deforested zones would provide resting place for adult mosquito populations [100]. Besides ecological factors, human socio-economic differences such as poorer housing conditions (mostly semi-closed houses) and higher proportion of individuals with outdoor economic activities in

rural sites B and C compared to site A, may play an important role in increasing exposure to mosquito bites [93,101].

Genetic factors may play a role in human susceptibility to malaria infection and human infectiousness to mosquitoes [145,146]. However, there is insufficient data to establish their role in determining heterogeneity of malaria transmission in the Peruvian Amazon. The later could be partly due to the biological differences between *P. falciparum* and *P. vivax* [147,148]. Even though in theory *P. vivax* relapse patterns may influence serological profiles, the quantification of their effect on the production of malaria antibodies remains unclear due to the difficulty to classify a recurrent parasitaemia as relapse, new infection or recrudescence [147,148].

Species specific heterogeneity was not only observed at small-scale geographical level but also over time as illustrated by the respective SCR estimates calculated by models including and excluding individuals older than 30 years old. A strong advantage of SCRs is the ability to reconstruct the history of malaria exposure in a population [16], but one important assumption of the model is that all individuals in a specific community have experienced the same disease transmission intensity over time. Thus, reductions in malaria transmission identified more than 30 years ago, as identified by catalytic models using seroprevalence data of all ages, may be explained by higher exposure to malaria of this age group prior to the settlement in 1980s of the study communities.

On the other hand, SCR estimates generated using seroprevalence data of individuals aged ≤ 30 years would be more accurate to identify more recent changes in malaria transmission. Indeed, reversible catalytic models used to generate *P. vivax* seroprevalence curves showed that the significant decline in malaria transmission in sites A and B occurred at different points in time. While in site A, this time point coincided with the drastic reduction in *P. vivax* incidence after the late 90s epidemic; in site B, the change was concordant with the start in 2005 of the Global Fund-PAMAFRO project which prioritized comprehensive malaria control interventions in rural communities

[5,6,149]. Since the end of this project in 2011, malaria incidence has steadily increased and already reached pre-PAMAFRO levels in 2013 and 2014, highlighting the potential risk of rapid malaria resurgence in the Amazon Region [7]. This was captured by the age-seroprevalence model for *P. vivax* in site C, i.e. increase in MTI two years before the survey. The fact that the catalytic model did not detect similar changes in SCRs for *P. falciparum* transmission as for *P. vivax*, could be due to the lack of power due to the lower levels seropositivity for *P. falciparum* and therefore insufficient sample size. Moreover, the independent association of outdoor economic activities with *P. falciparum* exposure would influence the model fit to age-seroprevalence data, since the assumption of the catalytic model that *P. falciparum* transmission is homogeneous in the community would not be fulfilled [142].

Effective methods for quantifying micro-geographical variations in malaria exposure should be able to identify stable hotspots of transmission over time [9]. The spatial analysis of clinical malaria incidence over several seasons should be a good method for detecting hotspots; provided most malaria infections are symptomatic, detected by health facilities, and accurately traced for place of infection and/or residence. However, this is rarely the case in most endemic settings, especially in low endemic area such as the one in our study, where most infections remain asymptomatic. Therefore the spatial identification of hotspots using serological measures may produce more robust results [10,150]. Serological markers can also be used to identify high risk sub-groups (or "hot-pops")[151] within communities that could be targeted by additional interventions such as focal screening and treatment (FSAT) or mass drug administration (MDA)[152], which are financially and logistically unattractive at when implemented at large scale.

Our spatial analysis using serological data allowed the identification of clusters with the highest *P. falciparum* exposure: while in site A, the cluster covered the area closest to the river which floods every year during the rainy season; in site B, the cluster location was associated with outdoor occupations (i.e. farmer, guards), as well as with electricity

availability which has been reported to influence evening outdoor activities (e.g., watching television, chating, resting, etc.) of the household members [153]. These findings correlate well with the recent entomological findings from communities near the study area that showed that *An. darlingi* mosquitoes bite mainly outdoors [135]. On the other hand, the quasi absence of clustering in site C may be explained by the local mosquito and human mobility patterns. Indeed the presence of several permanent breeding sites (i.e. fish ponds) along the Iquitos-Nauta road could result into a wider dispersal of *An. darlingi* and numerous movements of infected individuals through the unique and highly accessible road could further increase the dispersal of parasites. Although a similar explanation could be advanced for the absence of *P. vivax* clusters in site A and B, it is difficult to know how the relapse patterns can affect the spatial analysis using serological data.

Our study has some limitations. First, despite the use of two antigens of proven sensitivity for each *Plasmodium species* [57,133], regional variability in immunogenicity cannot be excluded and could be overcome by combining more antigens [22,59]. Though further research is still needed to identify the best antigens for measuring MTI [138] in Peruvian malaria endemic areas, we believe that sets used in our study were adequate and complementary since the antibody response produced was a good proxy for malaria exposure in other south-American endemic areas [133,154,155]. Ongoing cohort studies in the Peruvian Amazon will contribute to provide insights into the kinetics of antibody responses in the region. Second, the assumption by the mixture model of a bi-modal distribution of seropositives and seronegatives for the determination of the cut-off for seropositivity may not be true. However, in the absence of a good "gold standard", this approach is widely accepted for determining an event status such as "malaria exposure" [156]. Third, due to the lack of information about SRR in the Amazon region, we included a common species-specific SRR (the study population SRR) into each corresponding site model as previously reported in other serological studies [154]. Although this approach

allows comparing SCR estimates across sites, it is important to note that marked differences in SRR across sites could influence significantly the SCR estimates. Finally, despite the characteristics of the survey sampling, an enough proportion of individuals of each age group were included in each study site reducing the potential for selection bias. However, detecting the time point for change in malaria transmission as well as estimating corresponding SCRs may be slightly less accurate before than after 7 years prior the survey.

4.6. Conclusions

The use of a single parasitological and serological malaria survey has proven to be an efficient and accurate method to characterize the species specific heterogeneity in malaria transmission at micro-geographical level as well as to identify recent changes in transmission. This approach, once optimized by a validated set of best antigens by geographical area, represents a reliable and effective tool to accurately identify and characterize hotspots of transmission and consequently help decision-makers to better design and deliver targeted interventions. This is particularly important in low transmission settings moving towards malaria elimination such as the Peruvian Amazon.

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5. MODELLING THE POTENTIAL OF FOCAL SCREENING AND TREATMENT AS ELIMINATION STRATEGY FOR *PLASMODIUM FALCIPARUM* MALARIA IN THE PERUVIAN AMAZON REGION

Authors: Angel Rosas-Aguirre, Annette Erhart, Alejandro Llanos-Cuentas, Oralee Branch, Dirk Berkvens, Emmanuel Abatih, Philippe Lambert, Gianluca Frasso, Hugo Rodriguez, Dionicia Gamboa, Moisés Sihuincha, Anna Rosanas-Urgell, Umberto D'Alessandro, Niko Speybroeck.

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5.1. Abstract

Background: Focal screening and treatment (FSAT) of malaria infections has recently been introduced in Peru to overcome the inherent limitations of passive case detection (PCD) and further decrease the malaria burden. Here, we used a relatively straightforward mathematical model to assess the potential of FSAT as elimination strategy for *Plasmodium falciparum* malaria in the Peruvian Amazon Region.

Methods: A baseline model was developed to simulate a scenario with seasonal malaria transmission and the effect of PCD and treatment of symptomatic infections on the *P. falciparum* malaria transmission in a low endemic area of the Peruvian Amazon. The model was then adjusted to simulate intervention scenarios for predicting the long term additional impact of FSAT on *P. falciparum* malaria prevalence and incidence. Model parameterization was done using data from a cohort study in a rural Amazonian community as well as published transmission parameters from previous studies in similar areas. The effect of FSAT timing and frequency, using either microscopy or a supposed field polymerase chain reaction (PCR) test, was assessed on both predicted incidence and prevalence rates.

Results: The intervention model indicated that the addition of FSAT to PCD significantly reduced the predicted *P. falciparum* incidence and prevalence. The strongest reduction was observed when three consecutive FSAT were implemented at the beginning of the low transmission season, and if malaria diagnosis was done with PCR. Repeated interventions for consecutive years (10 years with microscopy or 5 years with PCR), would allow reaching near to zero incidence and prevalence rates.

Conclusions: The addition of FSAT interventions to PCD may enable to reach *P. falciparum* elimination levels in low endemic areas of the Amazon Region, yet the progression rates to those levels may vary substantially according to the operational criteria used for the intervention.

Keywords: Malaria, *Plasmodium falciparum*, Focal Screening and Treatment (FSAT), Modelling, Elimination, Peru.

5.2. Background

Despite several decades of intense control efforts, malaria remains an important public health problem in Peru, especially in the Amazon region where most (85%) of the malaria cases occur, mainly due to *P. vivax* [7]. Increased financial support from international donors, e.g., the Global Fund-PAMAFRO Project (2005–2010), allowed for the scaling-up of comprehensive malaria control strategies such as the use of artemisinin-based combination therapy (ACT) and the distribution of long-lasting insecticidal mosquito nets (LLINs) [6]. During this period, malaria declined drastically in Peru from 87,805 reported clinical cases in 2005 to 29,355 and 23,075 cases in 2010 and 2011, respectively. Since 2011, following the reduction of financial support allocated to malaria control activities, malaria incidence started to increase again with a doubling of clinical cases in 2013, highlighting the potential risk of rapid malaria resurgence in the Amazon Region [7].

In contrast to the classical statement that the development of acquired malaria immunity requires long-term exposure and intense transmission, recent studies in endemic countries of South America have reported a large proportion of asymptomatic malaria infections [54,94,96,98,111,157-161] suggesting that clinical immunity may develop as well in low-transmission settings of the subcontinent. The wider use of molecular diagnostics in epidemiological studies allows for a better knowledge on the extent of these asymptomatic infections, thus uncovering a "hidden" human infectious reservoir likely to contribute in maintaining transmission in endemic areas [111,124]. Indeed, cross-sectional surveys conducted in rural communities surrounding Iquitos city in Peru reported prevalence of asymptomatic parasite carriers ranging from 5 to 14%, mostly detectable only by polymerase chain reaction (PCR) [94,96,98].

National malaria surveillance in the Amazon Region relies on passive case detection (PCD) with microscopy [92]. Patients presenting with fever or any other symptoms compatible with malaria are systematically tested by microscopy at peripheral health facilities and treated following national guidelines. However, the major limitation of PCD

is that asymptomatic individuals do not seek medical care and therefore remain undetected and untreated [96,125], extending the period of parasite carriage [162]. Other described limitations of PCD include the quality of microscopy-based diagnosis [47], the access to health services with available microscopy [163], and social and cultural factors that influence treatment seeking behaviour at community level [48].

Active case detection (ACD) has recently been recommended in low transmission and malaria-elimination settings to overcome the inherent limitations of PCD and target the asymptomatic parasite reservoir [164]. According to the World Health Organization (WHO), ACD is defined as the detection of malaria infections through household visits in population groups that are considered to be at risk. In the Peruvian Amazon, focal screening and treatment (FSAT), has recently been introduced by the National Malaria Control Program (NMCP) to target malaria hotspots and further decrease the malaria burden. Malaria hotspots are well-delimited villages in rural areas or neighborhoods in periurban areas in which the cumulative malaria incidence over the past 3–5 years is the highest. These hotspots represent ~10-20% of the total malaria reporting-localities and account for more than 80% of the total cases in the Region. The intervention consists of systematic finger prick blood sampling of the whole population (100–1000 inhabitants) during the period of highest incidence (usually between February and May) and the treatment of all microscopically positive infections, irrespective of the presence of symptoms [92]. Following the national treatment guidelines, *P. falciparum* infections are treated with an ACT -mefloquine and artesunate (MQ-AS)-, and *P. vivax* infections with chloroquine (CQ) and primaquine (PQ) [92]. However, due to the lack of evidence-based guidelines [46,151], the criteria and procedures to implement FSAT interventions in the Amazon Region have often been arbitrarily decided and are subject to annual variations in logistical and financial resources.

Mathematical models of various levels of complexity have been used for understanding malaria transmission dynamics and informing decision-making in malaria control and

elimination efforts [25,165-167]. Here, we used a model to assess the long term impact of FSAT on the *P. falciparum* malaria transmission intensity (MTI) in a low endemic area of the Peruvian Amazon Region. Retrospective data from a cohort study in a rural community of the Peruvian Amazon were used to estimate infection rates and a time-variant seasonal vector parameter, and then to evaluate the model. Other malaria-transmission parameters were obtained from previously published studies in the Amazon region. Eventually, the model was used to predict the potential reduction in malaria transmission following the addition of FSAT to the ongoing PCD, allowing for a simulation of the effect size according to the diagnostic tools used, as well as the timing and frequency of FSAT interventions.

5.3. Methods

Baseline model

An extension of the Anderson-and-May's malaria transmission model [67] and the corresponding differential equations was used to simulate a scenario where seasonal malaria transmission remains at similar levels each year within a restricted human population with ongoing PCD and treatment of symptomatic *P. falciparum* infections (Figure 5.1 and Annex-Chapter 9: Figure S5.1). The deterministic model allows the human population (N) to move between four different states (compartments), and the mosquito population (M) between three states. Individuals initially start in a susceptible state (S) and become infected at a rate that is determined by the mosquito density (*m*), the human feeding rate per mosquito *(a*), the infectiousness of the mosquito population (V/M), and the susceptibility of an individual after being bitten by an infectious mosquito (*b*). Individuals progress to the latent state (E), from which they develop gametocytes in the blood and become infectious after a latent period (*l*). Two consecutive sub-states are distinguished in infected latent individuals (E_1 and E_2) thus dividing the latent period into two time intervals: between sporozoite inoculation and development of detectable asexual

stage parasites in blood (l_1), and between asexual parasite infection and development of gametocytes (l_2). Then, the number of individuals who move from E_1 to E_2 at time t is equal to the number of individuals who entered E_1 at time t-l_1 adjusted by the factor e^{-l1*h}, which incorporates the human mortality rate (h) and the average time interval for developing asexual blood parasites (l_1). On the other hand, infected individuals leave sub-state E_2 at a rate ($1/l_2$) which is the reciprocal of average duration of the gametocytogenesis process.

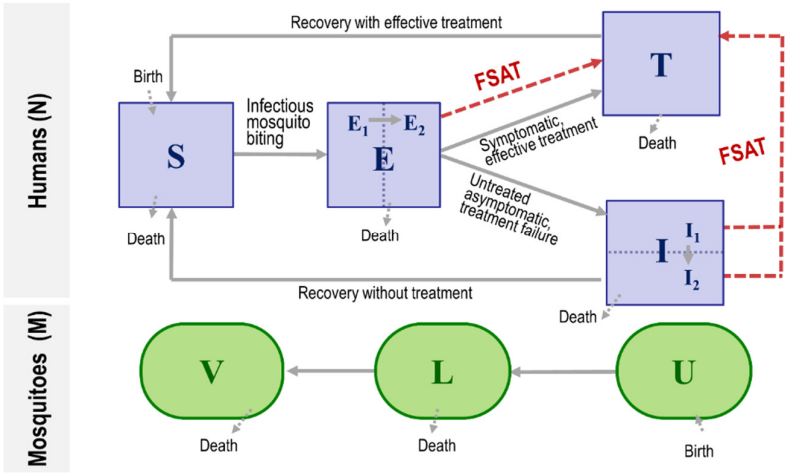


Figure 5. 1. Schematic diagram of the human and vector model states. The human population (N) is divided into 4 compartments: susceptible (S), infected latent (E), infectious treated (T) and infectious untreated (I) individuals. The latent period has 2 sub-states: latent which have not yet developed asexual blood-stage parasites (E_1) and latent with asexual blood-stage parasites (E_2). Before becoming recovered, individuals in I compartment need to pass through 2 sub-states: those who have not yet (I_1) and those who have spontaneously cleared the asexual blood stage parasites (I_2). The mosquito population (M) is divided into 3 compartments: uninfected (U), infected latent (L) and infectious (V) mosquitoes. The addition of FSAT to PCD allows for the detection and treatment of individuals in E_2, I_1 and I_2 compartments.

While a constant fraction (y) of symptomatic infections of all newly infected individuals is routinely detected by PCD and treated with an ACT of known effectiveness (ε) within the treated state (T), the remaining fraction (1-y*ε), including asymptomatic individuals and previously treated individuals with parasitological treatment failure, remains infectious in an untreated state (I) until the "spontaneous cure" of the disease (i.e. clearance of all parasite forms) unless they are actively detected and treated. Once

recovering from compartments T and I, individuals return to the susceptible state (S). While recovery rate for treated individuals ($1/r_1$) is the reciprocal of average human infectious period (i.e. length of time gametocytes remain in the bloodstream) with effective treatment, recovery for untreated individuals requires they pass through two sub-states before becoming recovered: those who have not yet (I_1) and those who have spontaneously cleared the asexual blood stage parasites (I_2). Then, the rate of movement of individuals from I_1 to I_2 ($1/r_{2a}$), and from I_2 to S ($1/r_{2b}$) are the reciprocal of the average time for the spontaneous clearance of asexual parasites from blood (r_{2a}), and the remaining average infectious period after the clearance of blood asexual parasites (r_{2b}), respectively in untreated individuals.

Regarding the mosquito population (M), the model includes an initial susceptible state (U) for all mosquitoes, becoming infected at a rate determined by the following three parameters: human feeding rate per mosquito (a), fraction of infectious individuals in the human population ((T + I)/N), and the mosquito susceptibility to infection after biting an infectious individual (c). Infected mosquitoes remain in a latent state (L) for a specific time period before developing sporozoites and becoming infectious (V). The estimation of surviving mosquitoes by the end of the latent period requires an adjustment by the factor e^{-g*n} which incorporates a constant mosquito mortality rate (g) and the average mosquito latent period (n). The model assumes that mosquitoes remain infectious until they die.

The main assumptions of this model are the following: i) human and mosquito populations remain constant (number of births and deaths are equal at any time); ii) malaria-attributable deaths are not explicitly incorporated (malaria mortality rate is similar to general mortality rate); iii) no individual differences in transmission parameters between subjects; iv) super-infections do not occur [168]; v) all infected individuals develop mature gametocytes immediately after they leave the latent sub-state E2; vi) any

gametocytemia represents potential for transmission; and vii) there is no prophylactic effect of the drugs post recovery.

Retrospective data on malaria cohort study

The model was fine-tuned by using retrospective data from a cohort study carried out between 2003 and 2008 in four rural communities of San Juan district, south of Iquitos city in the Peruvian Amazon [96,168,169]. Malaria transmission in this area typically starts in January and finishes in July with a peak of clinical malaria cases between February and March; *P. vivax* and *P. falciparum* infections occurring at a ratio 4:1. Previous reports from this area indicated that more than two thirds of *P. falciparum* infections were asymptomatic with low parasite density [94,96]. *Anopheles darlingi* is the major malaria vector with a strong anthropophilic behavior [97,170]. The initial cross-sectional survey conducted in April 2003 among cohort participants showed that most of them reported sleeping under bed nets, although none of them mentioned the use of insecticide treated nets or repellents in their homes [96]. From 2004 to 2008, biannual community screening campaigns were carried out (beginning-end of each malaria season), and in between, weekly ACD for *P. falciparum* infections was conducted, sampling each month a new group of individuals living within a 100-m radius of houses where at least one *P. falciparum* infection was detected during the previous month (index houses), either through the surveys, ACD or the routine PCD at health facilities [96]. Since high coverage during screening campaigns was achieved only in Ninarumi community, local model parameters were estimated from this community only, and taking into account malariometric indices from the year 2004 only, which was the first full year of ACD in the cohort study. The prevalence of *P. falciparum* infections by microscopy in Ninarumi was measured at 6.5% and 1.1%, respectively in January and August 2004 (Annex, Chapter 9: Table S5.1); and during that year a total of 139 new *P. falciparum* infections were detected (0.28 infections/person/year), 91 (65.4%) of them being symptomatic either at the time of sampling or during the following week. Of the

additional 48 infections which remained asymptomatic during the follow-up, 19 were confirmed only by retrospective PCR analysis on blood samples collected on filter paper from individuals during weekly ACDs. The detailed procedures for the PCR analysis are described elsewhere [169]. All individuals enrolled in the cohort study gave signed informed consent or assent. Ethical clearance was received from New York University (NYU Institutional Review Board [IRB] approval no. 08–982), the University of Alabama at Birmingham, and the Peruvian Ministry of Health and National Institutes of Health Internal Ethical Review Boards [169].

Transmission parameter values

Considering days as the time unit, the model parameterization was done in several steps, i.e. using first the available cohort data for the estimation of important local parameters, and in a second step, using published data to improve the first step estimates or to set values for the remaining transmission parameters. A systematic literature search was undertaken in different databases (i.e. PubMed, EMBASE and SciELO) prioritizing published studies on malaria in the Amazon Region in English and Spanish language, especially those from the Iquitos area when available. Additional searches were conducted in Google and through reference tracking. Third, when parameters could not be identified in previous steps, they were estimated by fitting the model to the observed clinical malaria incidence in Ninarumi. Table 5.1 summarizes key parameter values used in the baseline model (see Annex, Chapter 9: Text S5.1 for a detailed description).

FSAT model and assessment of interventions

In the scenario with FSAT interventions, the baseline model was improved by allowing movements from sub-states E_2, I_1 and I_2 to the treated state (T) (red dotted lines in Figure 5.1), at specific rates that are determined by the coverage (Δ), the reciprocal of duration of the intervention ($1/v$), the sensitivity of the diagnostic test used for malaria screening (s), and the effectiveness of treatment in confirmed infections (ε).

Table 5.1 Model parameters

Parameters	Symbol	Value	Source
Baseline model parameters			
Mosquito density (ratio female mosquitoes/humans)	m	75	Estimation from fitting cohort data; values within published range.
Human feeding rate, bites per mosquito per day	a	0.035 - 0.32 Mean: 0.088	Monthly estimations from fitting cohort data; values within published range
Susceptibility of an individual to infection after being bitten by an infectious mosquito	b	0.05	Estimation using cohort and published data
Mosquito susceptibility to infection after biting an infectious individual	c	0.41	Published data
Duration of human latent period for *Pf** in days $(l = l_1 + l_2)$	l	21	Published data
Duration from sporozoite inoculation to development of blood asexual parasites in days	l_1	11	Published data
Duration from asexual blood stage infection to development of gametocytes in days	l_2	10	Published data
Duration of mosquito latent period in days	n	11	Published data
Daily human mortality rate	h	0.00005	Published data
Daily mosquito mortality rate	g	0.16	Published data
Fraction of symptomatic infections	y	0.65	Cohort data
Treatment effectiveness	ε	0.98	Published data
Duration of infectious period in treated humans with ACT (in days)	r_1	14	Published data
Duration of infectious period in untreated humans in days $(r_2 = r_{2a} + r_{2b})$	r_2	200	Published data
Duration of presence of blood asexual parasites in untreated humans in days	r_{2a}	80	Published data
Duration of infectious period in untreated humans after clearance of blood asexual parasites in days	r_{2b}	120	Published data
FSAT parameters			
Coverage of FSAT	Δ	1	Assumption
Duration of FSAT in days	v	1	Assumption
Sensitivity of microscopy for detecting *Pf** asexual stages	s_{1a}	0.5	Published data
Sensitivity of microscopy for detecting *Pf**gametocytes	s_{1g}	0.1	Published data
Sensitivity of highly sensitive diagnostic tests for detecting asexual stages	s_{2a}	0.95	Assumption
Sensitivity of highly sensitive diagnostic tests for detecting gametocytes	s_{2g}	0.8	Assumption

**Pf = P. falciparum*

After setting initial conditions for human and mosquito compartments based on the average observed prevalence in the 2004 Ninarumi surveys and the reported *A. darlingi*

sporozoite rates in the area [97], the baseline model was run until reaching steady levels (baseline scenario), indicated by the stable cumulative number of *P. falciparum* infections recorded for 20 more simulated years. Then, the model was run for an additional year (year 0) after which interventions based on FSAT (intervention scenario) were added to the routine PCD (baseline scenario). A first intervention scenario was modeled with the following assumptions: one FSAT during the peak of the high transmission season (only in February of year 1); FSAT covers 100% of population ($\Delta = 1$) in one day ($v = 1$); microscopy used in FSAT allows for the detection of 50% of asexual blood-stage infections by *P. falciparum* (sub-states E_2 and I_1), and 10% of infections with only sexual stages (gametocytes) in blood (sub-state I_2) [52]; and all detected *P. falciparum* infections are treated with a 98% effectiveness ($\varepsilon = 0.98$).

Additional intervention scenarios were modeled to assess the impact of different operational criteria for FSAT implemented during only one year (i.e. timing, number and time intervals between consecutive interventions) on the predicted cumulative incidence and prevalence of *P. falciparum* infections. Besides microscopy, a field PCR was also considered as diagnostic test during malaria screening, assuming sensitivities of 95%, 95% and 80% for detecting infections in sub-states E_2, I_1, and I_2, respectively. The predicted cumulative incidence (i.e. cumulative number of new symptomatic and asymptomatic infections) was recorded over one-year and five-year periods from the start of the first FSAT in each intervention scenario, and was compared with the cumulative incidence found with the baseline PCD scenario. The predicted impact of each intervention was determined as the percentage difference between the cumulative number of new *P. falciparum* infections with baseline and with the respective intervention scenarios (cumulative number of infections averted). In addition, minimum and maximum predicted prevalence were determined during both the first and the fifth year after the onset of FSAT interventions. Finally, operational criteria for implementing FSAT interventions during only one year which had the strongest impact were used again

to assess the effect of the same interventions if implemented during several consecutive years.

Malaria transmission scenarios were simulated in R software version 2.15.3 (function "dede" in deSolve package) allowing to solve delay differential equations to take into account latent periods for infected individuals and mosquitoes before being infectious (see R code for baseline model in Annex, Chapter 9: Text S5.2) [171]. Baseline model performance was examined by comparing predicted and observed *P. falciparum* incidence and prevalence from the cohort study. A Wilcoxon matched-pairs signed-ranks test was used to test differences between observed and predicted monthly incidence, with $p < 0.05$ indicating statistical significance. Different initial values for the *A. darlingi* sporozoite rate (V/M) were tested and did not affect the results. Moreover, a one-at-a-time sensitivity analysis was conducted to determine the effect of the following parameters on the baseline model predictions [172]: the mosquito density (m), the human feeding rate (a), the fraction of symptomatic infections (y), and the treatment effectiveness (ε). Changes (increase and decrease) of 20% in parameter values were considered in the analysis. Finally, only human parameters (y, ε) were used later to assess their impact on the predictions of the intervention scenarios with FSAT.

5.4. Results

Baseline model predictions largely reflected *P. falciparum* incidence in Ninarumi in 2004 (Figure 5.2A), with no statistically significant difference between observed and predicted monthly incidence of symptomatic *P. falciparum* infections. While Ninarumi reported 91 symptomatic and 38 asymptomatic *P. falciparum* infections (total = 139 infections) in 2004, the baseline model predicted 99 symptomatic and 43 asymptomatic infections (total = 152 infections). Furthermore, predicted prevalence curves accurately reflected the seasonal dynamics of malaria infections (figure 5.2B), with high transmission season (HTS) between January and July, and low transmission season (LTS) between August

and December. According to the baseline model predictions, the maximum overall and microscopically determined prevalence during HTS were 12.3% and 6.2%, respectively; while the corresponding minimum values during LTS were 3.4% and 0.7%, respectively. Therefore, predicted and observed prevalence by microscopy also correlated well in Ninarumi in 2004. Furthermore, the proportion of detected infections by microscopy among the total predicted infections was significantly higher in the peak of HTS (~50%) than in LTS (~10-20%). Similarly, the proportion of asymptomatic infections among the total predicted infections varied according to the season, from a minimum of 58% during the peak of HTS to a maximum of 96% in the middle of LTS.

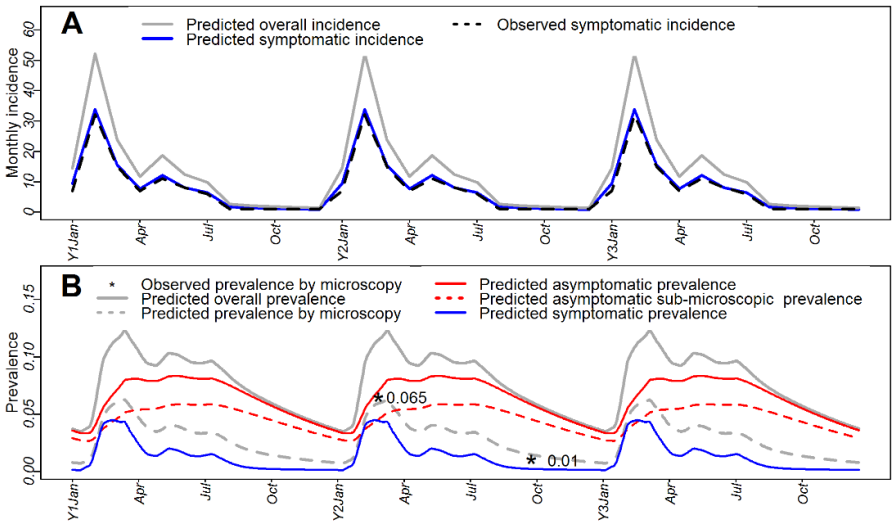


Figure 5.2. Comparison between observed and baseline model-predicted *P. falciparum* malariometric measures in Ninarumi village. Curves with data from Ninarumi in 2004 were repeated for three consecutive years: **A)** observed and predicted *P. falciparum* monthly new *P. falciparum* infections (monthly incidence); **B)** observed and predicted *P. falciparum* prevalence.

The sensitivity analysis for the baseline model (Figure 5.3) showed that with values above 0.7 for the fraction of symptomatic infections (y), malaria transmission decreased progressively until almost undetectable levels. In addition, seasonal malaria transmission persisted only when mosquito density (m) and mean expected number of bites on humans

per mosquito (*a*) are higher than 67 and 0.080, respectively. Increasing monthly values of human feeding rate (*a*) by 20% had by far the strongest effect on the annual incidence, with more than three times the baseline number of *P. falciparum* infections (~225% more infections) (Figure 5.3A). On the other hand, a decrease from 0.65 to 0.5 in the fraction of symptomatic infections (*y*) had the highest impact on the predicted incidence of asymptomatic infections (increase of baseline incidence by ~250%) (Figure 5.3B). Moreover, the same changes in both parameters (i.e. human feeding rate and fraction of symptomatic infections) had similar effects on the predicted prevalence, almost triplicating the maximum and minimum prevalence in HTS and LTS, respectively (Figures 5.3C and 5.3D). Furthermore, a reduction of the treatment effectiveness from 0.98 (baseline value) to 0.80 would increase the incidence and prevalence by more than 100% and 150%, respectively.

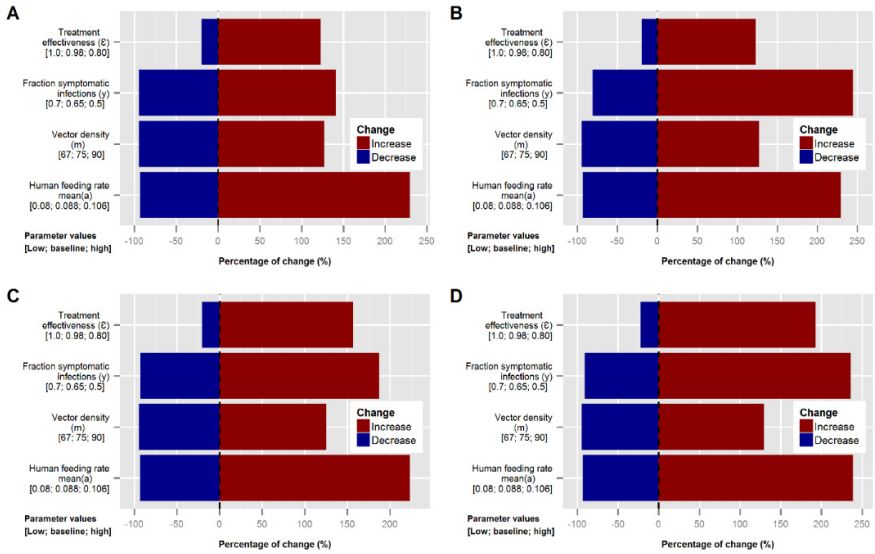


Figure 5.3. Sensitivity analysis for the baseline model. Changes in: **A)** annual overall incidence (baseline model prediction: 152 infections); **B)** annual asymptomatic incidence (baseline: 53 infections); **C)** maximum prevalence during the year (baseline: 12.3%); **D)** minimum prevalence during the year (baseline: 3.5%). Results are expressed in percentage of change (%) in comparison with baseline model predictions.

Table 5.2. One and five-year predicted cumulative number of *P. falciparum* infections averted after the addition of FSAT interventions to PCD at different timing, using either microscopy or assumed PCR

		Microscopy				PCR			
		One year		5 years		One year		5 years	
		n	%	n	%	n	%	n	%
One FSAT									
Month of implementation	January	11	6.9	41	5.4	36	23.8	145	19.0
	February	6	3.8	34	4.5	15	10.0	84	11.0
	March	23	15.1	98	12.8	45	29.7	200	26.3
	April	26	16.9	104	13.6	53	34.7	224	29.4
	May	23	15.3	95	12.4	50	32.6	213	28.0
	June	26	16.9	101	13.2	56	36.5	232	30.5
	July	29	18.7	108	14.2	64	41.8	260	34.1
	August	31	20.4	113	14.9	74	48.3	292	38.4
	September	28	18.7	102	13.5	73	48.2	290	38.2
	October	26	17.0	93	12.2	73	47.9	287	37.7
	November	23	15.4	84	11.0	72	47.1	281	37.0
	December	20	13.2	72	9.5	66	43.4	257	33.8
Two consecutive FSAT									
Time interval between FSAT	7 days	53	34.8	202	26.5	110	72.3	487	63.9
	15 days	53	34.5	201	26.3	110	72.6	489	64.3
	30 days	52	34.0	198	26.0	111	73.0	494	64.9
	60 days	50	33.0	192	25.2	111	73.0	497	65.3
	90 days	48	31.8	186	24.4	110	72.4	493	64.8
	120 days	46	30.2	176	23.1	107	70.3	477	62.6
	150 days	39	25.3	148	19.5	91	60.0	396	52.0
	180 days	35	22.7	145	19.1	80	52.4	356	46.7
Three consecutive FSAT									
Time interval between FSAT	7 days	68	45.0	271	35.7	127	83.7	598	78.5
	15 days	68	44.5	269	35.3	129	84.5	608	79.8
	30 days	66	43.5	263	34.6	130	85.1	617	81.1
	60 days	62	40.6	246	32.3	128	83.9	609	80.0
	90 days	51	33.8	217	28.5	113	74.5	535	70.3

Table 5.2 shows the predicted impact on the *P. falciparum* incidence after the addition of different FSAT interventions according to timing and number of interventions. Conducting one FSAT intervention with microscopy alone between July and September during only one year would have a larger impact on the *P. falciparum* incidence than conducting it during any other month, with a reduction of about 20% and 15%, respectively at 1- and 5 years post intervention. However, the magnitude of this effect

would be twice as high if PCR is used instead of microscopy, reaching reductions of more than 40% and 30% respectively at 1- and 5 years after intervention. Similarly, the impact on *P. falciparum* prevalence was the highest when FSAT was added in LTS (August), instead of in HTS (February) during only one year (Figure 5.4A), with a significant reduction in the maximum prevalence reached during the next HTS ($p<0.05$), i.e. from 12.3% (baseline prevalence) to 9.6% (using microscopy) or to 6.0% (if PCR is used).

The strongest decrease in *P. falciparum* malaria incidence for interventions implemented during only one year was observed with three consecutive FSAT starting at the beginning of LTS and separated by time intervals of 7 to 60 days (Table 5.2), resulting in reductions of more than 40% and 30%, respectively after 1 and 5 years when using microscopy, and of more than 80% and 75%, respectively when using PCR. The impact of the number of consecutive FSAT at the start of LTS (one to three FSAT during only one year, separated by intervals of 30 days) on the predicted *P. falciparum* prevalence is shown in the Figure 5.4B. While three consecutive FSATs using PCR would have the largest effect on the predicted prevalence, one FSAT alone using PCR would result in a similar reduction compared to three consecutive FSATs using microscopy. In all simulations including PCR, annual *P. falciparum* incidence and prevalence would return slowly to the initial levels after 10 years, if FSAT interventions are implemented only during one year.

Figure 5.4C shows a progressive reduction of the predicted prevalence with annually repeated FSAT interventions starting at the beginning of LTS every year. All repeated interventions would reach the near-zero prevalence, however the rate of decrease largely depends on the number of monthly FSAT per year and the diagnosis test used for malaria detection. Indeed, while three-monthly FSATs using microscopy would require approximately 10 years to reach the zero level prevalence; this could be reached with three monthly FSATs using PCR for four consecutive years.

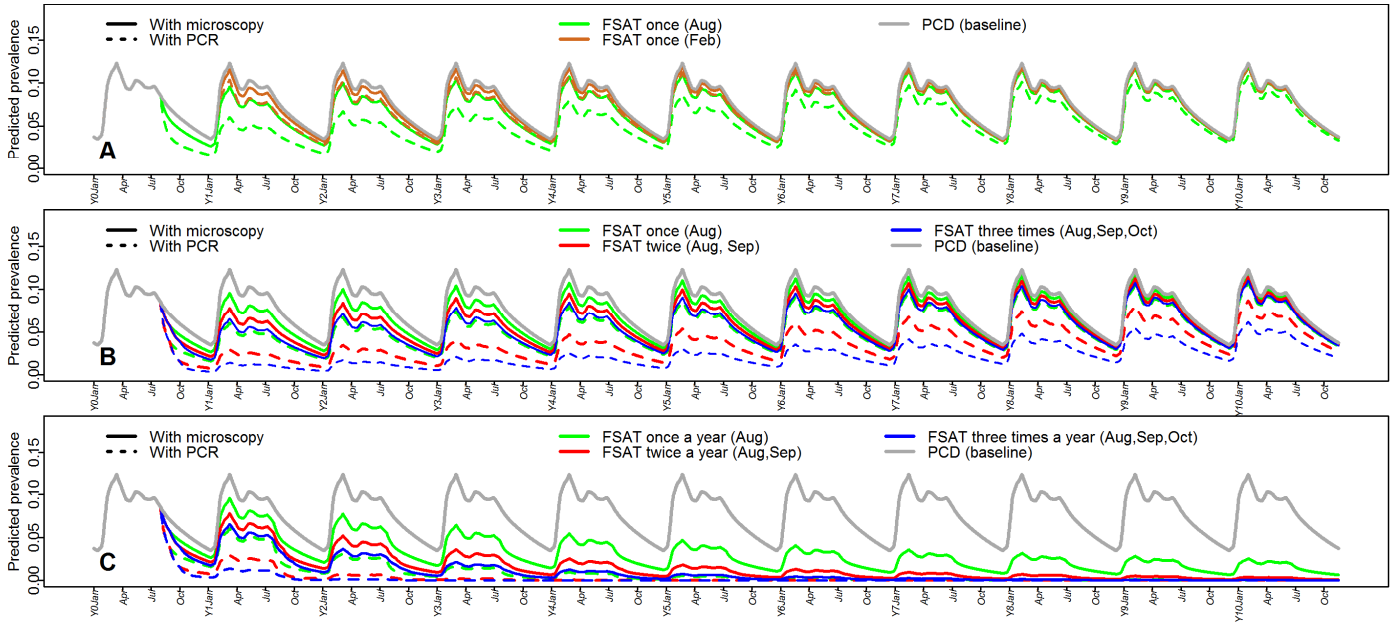


Figure 5.4. Model-predicted *P. falciparum* prevalence curves after the addition of different FSAT interventions to the baseline PCD. A) one FSAT at beginning of LTS only in year 0 (Y0) and in HTS only in year 1 (Y1), using microscopy or PCR; **B)** one to three successive FSAT starting at beginning of LTS only in Y0, with microscopy or PCR; **C)** one to three successive FSAT starting at beginning of LTS for consecutive ten years (from Y0 to Y10), with microscopy or PCR.

However, a decrease in the fraction of symptomatic infections from 0.65 to 0.5, or a decrease in the treatment effectiveness from 0.98 to 0.8, would substantially reduce the rate of progression to the elimination levels with FSATs using microscopy (Figure 5.5A), and consequently increase the time required to reach the elimination levels to more than 20 years. Furthermore, if a reduction of FSAT coverage from 100% to 80% is added to those scenarios, *P. falciparum* prevalence would start to increase again despite of the FSAT interventions. Conversely, if annually repeated FSAT interventions are implemented using PCR, the effects of similar changes (i.e. decrease in the fraction of symptomatic infections, in the treatment effectiveness, or in the FSAT coverage) on the *P. falciparum* prevalence would be smaller, hence delaying the time to reach zero prevalence levels only by one or two years (6 or 7 years after onset of interventions) (Figure 5.5B).

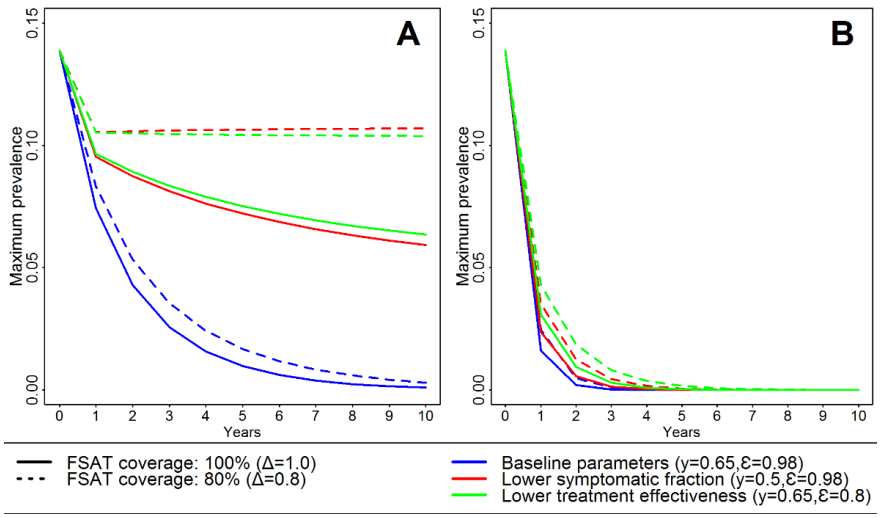


Figure 5.5 Long-term impact of changes in the fraction of symptomatic infections and the treatment effectiveness on the maximum predicted *P. falciparum* prevalence after the onset of annual FSAT interventions (three successive FSAT at beginning of LTS): A) FSAT interventions using microscopy for malaria screening; **B)** FSAT interventions using PCR for malaria screening.

5.5. Discussion

Using a relatively straightforward mathematical model, our analysis showed that targeting the asymptomatic parasite reservoir with FSAT interventions, in addition to routine PCD, would significantly reduce *P. falciparum* malaria in low transmission areas of the Peruvian Amazon. The strongest reduction was observed if three consecutive FSAT were implemented annually at the beginning of low transmission season, and if malaria diagnosis was done with a highly sensitive field PCR.

Modelling the effects of interventions that target the asymptomatic malaria reservoir requires a good knowledge of the burden of asymptomatic infections and their transmission dynamics. Cross-sectional surveys in the Peruvian Amazon reported that more than two thirds of malaria infections were asymptomatic [94,96]; however these studies did not take into account the incubation period for some of them, hence the potential development of symptoms at a later stage [111]. Therefore data from the cohort study conducting in peri-Iquitos communities including weekly monitoring of symptoms for one month of all infected individuals, enabled to overcome the limitations of previous survey results. Indeed, an important proportion of *P. falciparum* infections which were asymptomatic at the time of detection (either by microscopy or by retrospective PCR) developed malaria symptoms (reported and/or measured fever) after one week of follow-up [96], resulting in only 35% of asymptomatic infections among all confirmed cases in the Ninarumi community in 2004. Another study conducted in the Brazilian Amazon in 2004–2005 found similar results after combining PCD and ACD over a 14-month period, i.e. a total of 326 malaria infections were identified of which 96 (29.4%) were asymptomatic– a much lower proportion than reported in any of the cross-sectional surveys carried out during the same period [173].

The cohort study also allowed for an accurate measurement of seasonal variations in the incidence of symptomatic and asymptomatic *P. falciparum* infections in the area, and these seasonal dynamics were well reproduced by the baseline model. In addition, the

model predicted not only a 2–3 times higher prevalence of infections during the peak of HTS than during the LTS, but also temporal differences in the proportion of asymptomatic infections, which accounted for about 60% of all prevalent infections during the peak of HTS while they represented almost all infections in the middle of LTS. Moreover, the proportion of prevalent infections detectable by microscopy ("patent infections") varied also with season, i.e. from almost 50% (6.2% of a total prevalence of 12.3%) in the HTS to 20% (0.7% of a total prevalence of 3.4%) in the LTS. Those predictions suggest that in areas of low and seasonal transmission such as the Peruvian Amazon, non-detectable asymptomatic *P. falciparum* infections are able to persist during the LTS being potentially infectious to mosquitoes emerging after the start of the rains, as previously described in other areas with markedly seasonal transmission [174,175].

Since symptomatic *P. falciparum* infections are more likely to be identified and treated during PCD, and the duration of treated infections is considerably shorter than untreated infections; it is anticipated that potential changes in the fraction of symptomatic infections as well as in the effectiveness of case management, may significantly impact the malaria prevalence as shown by the sensitivity analysis. Indeed, simulations showed that a decrease in the fraction of symptomatic infections from 0.65 to 0.5 or a decrease in the treatment effectiveness from 0.98 to 0.8 resulted in similar effects as changes in entomological parameters (ratio female mosquitoes to humans from 75 to 90, human feeding rate by 20%) which are known as being important determinants for the vectorial capacity and the malaria transmission [176]. Asymptomatic infections are becoming increasingly common at low transmission levels [54,94,96,98,111,151,157-161], enlarging the human infectious reservoir which will enable onward transmission. On the other hand, treatment effectiveness can decrease due to the spread of drug-resistant strains of *P. falciparum* parasites and the lack of systems that assure the quality of both the antimalarial drugs and the case management procedures (e.g. poor prescribing practices by health workers, low patient adherence rates to treatment regimens) [46,177].

In contrast to the current strategy of the National Malaria Control Program consisting of adding FSAT with microscopy to PCD during HTS in prioritized localities of the Peruvian Amazon [92], our simulations showed that conducting a single FSAT at the beginning of LTS would not only result in a greater reduction in *P. falciparum* incidence and prevalence in the short term, but also decrease the progression rates of these indicators to the initial levels. Furthermore, the effect of FSAT would be maximal if three successive FSAT at time intervals of 7–60 days, would be implemented at the start of the LTS. Kern *et al.* found a similar impact on the *P. falciparum* transmission with a single FSAT with microscopy during the dry season using a seasonally-forced malaria model which incorporated periodically mosquito birth rates through a sinusoidal function and different levels of infectiousness for gametocyte carriers; however, while in areas of simulated low disease burden (entomological inoculation rates (EIR) < 10 infective bites/person/year) such as the Peruvian Amazon, the effect was sustained for over three years after the intervention, in areas of high endemicity (EIR > 200) the incidence rates of infection returned to their initial level in the subsequent year [178]. Moreover, the study also indicated that three FSAT scheduled at monthly intervals starting in the dry season had the greatest positive impact on transmission. Successive FSATs would not only allow for identifying infected individuals who were in a latent state at the time of the previous FSAT, but also would increase the coverage of the intervention in real-life situations by including individuals who were absent or not eligible (illness, pregnancy) during the previous FSAT. The relatively wide time interval (i.e. 7–60 days) for a subsequent intervention suggested by our model would facilitate the planning and logistics of mobile teams in charge of FSAT in the targeted communities.

Beside timing and frequency of a given intervention, the other important determinant of impact is the duration (number of years) the intervention needs to be applied to reach sustainable elimination levels. Our model suggested that three successive FSAT with microscopy for ten consecutive years, would result in a sustained declining *P. falciparum* prevalence and incidence and eventually reach elimination levels. Long-term effects of

FSAT has been previously described by Griffin *et al.* using a complex modelling approach (including immunity, super-infections, and heterogeneity in transmission intensity) fed with published transmission parameters and local epidemiological data of different endemic areas of Africa [179]. The results showed that in low-to-moderate transmission settings, 80% coverage of annual single round of FSAT using rapid diagnostic tests (RDTs) over 15 years, together with 80% coverage of insecticide treated nets (ITNs), would reduce the absolute parasite prevalence by 5–12%. On the other hand, in high transmission areas, the simulated impact of FSAT alone was marginal, the ultimate outcomes strongly depending on the initial transmission level, the frequency and duration of the intervention and the coverage of vector control (i.e. either ITN or indoor residual spraying) [179].

As a consequence of increased use of molecular tests for detecting submicroscopic malaria infections in both low and high-malaria transmission settings, as well as of recent studies reporting failures of FSAT to reduce transmission when using microscopy or RDTs [180], it has been suggested that PCR based strategies need to be deployed if such interventions are to be successful [181,182]. Our model simulations assessed the impact of FSAT interventions using an assumed field PCR with high sensitivity, under the assumption that any level of gametocyte density was potentially infectious [183]. Repeated FSAT interventions with PCR for several consecutive years would not only have a stronger impact on *P. falciparum* malaria transmission (halving time to reach elimination levels), but would also enable to sustain the impact despite eventual reductions in the proportion of symptomatic infections, treatment effectiveness and/or coverage of FSAT. Although these results support the importance of detecting and treating sub-microscopic infections at point of care for successful elimination strategies [46,184], implementing FSAT using PCR as routine strategy in prioritized localities of the Peruvian Amazon may not be feasible since current PCR tests are resource-intensive, time-consuming and expensive [113]. Recently, some studies have shown high sensitivity of non-conventional molecular tests based on loop-mediated isothermal amplification

(LAMP) with no requirement for specialist staff and expensive equipment [185]; these molecular tests need to be optimized quickly to be used in the field for species-specific identification of *Plasmodium* parasites.

Like with other mathematical models for malaria transmission, our predictions should be interpreted taking into account the underlying assumptions. Some assumptions were intended to simplify the complex malaria dynamics (e.g. constant mosquito and human populations, no individual differences in transmission parameters between subjects, similar levels of infectiousness for gametocyte carriers), while others attempted to fill the knowledge gap in the *P. falciparum* malaria transmission cycle (e.g. the development of mature gametocytes in all infected individuals, potential for transmission of any infectious individual independently of its gametocyte density in blood). Further research is still needed to define to what extend asymptomatic and submicroscopic malaria infections contribute to the infectious reservoir [186]; this information would considerably improve our model. Moreover, since model predictions may vary widely under the influence of uncertainties in many transmission parameters, a global sensitivity analysis may be required to accurately identify the parameters that have the biggest impact on the expected *P. falciparum* prevalence and incidence. In addition, after the development of a more complex model for *P. vivax* transmission using data of a recent cohort carried in the Peruvian Amazon [161], it will help to further fine-tune the model and define operational criteria for FSAT interventions to maximize the impact of the intervention on both species.

5.6. Conclusions

Our model suggested that FSAT combined to PCD enables to reach elimination levels in an area of low transmission of the Amazon Region, but the progression rates to those levels vary substantially according to the operational criteria used for the intervention (i.e., timing, number, coverage, and duration of FSAT) and the sensitivity of the

diagnostic test used for malaria screening. Despite its limitations, the modelling approach can contribute to design new field studies in the Peruvian Amazon to assess the effectiveness of FSAT and other strategies to further decrease malaria transmission. Even before easy-to-use molecular tests will be available at the point of care, as well as reliable evidence of the effectiveness of FSAT, the current strategy applied by the NMCP in Peru should be revised in the light of our results in order to be more cost effective. Programmatic FSAT interventions with microscopy (minimum of two successive FSAT) can be conducted at the beginning of the LTS in areas with known high risk of *P. falciparum* malaria transmission, trying to reach the maximum population coverage (avoiding areas with highly mobile populations). Alternatively to reach the areas of mobile populations, resources could be invested to implement a "smart surveillance system" based on reactive case detection guided by information from the PCD.

Authors' contributions

ARA, AE, ALLC, ARU, UDA, and NS conceived and designed the study; ARA, ALL, OB, HR, MS and DG collected the data; ARA, DB, EA, PL, GF, and NS analysed the data and developed the model; ARA, AE, ALLC, OB, and NS wrote the manuscript; and all authors gave the final approval of the version to be published.

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6. INCREASED MALARIA INCIDENCE IN SPATIAL CLUSTERS OF HIGH SEROPOSITIVITY IN A LOW ENDEMIC AREA OF THE PERUVIAN NORTHERN COAST: ONE-YEAR ANALYSIS AFTER A SEROLOGICAL SURVEY.

Authors: Angel Rosas-Aguirre, Gabriel Carrasco-Escobar, Mónica Melendez, Anna Rosanas-Urgell, Annette Erhart, Niko Speybroeck, Dionicia Gamboa, Edwar Pozo, Juan Contreras-Mancilla, Irene Soares, Herrera Sócrates, Alejandro Llanos-Cuentas

6.1. Abstract

Background: As transmission becomes increasingly focal in low transmission settings, an accurate identification of malaria hotspots is crucial to better design and deliver targeted interventions. The study evaluated if malaria hotspots detected using reported malaria incidence and survey serological data were associated with the *P. vivax* malaria incidence in the subsequent year in a low endemic area of the Peruvian northern coast.

Methods: After a full census and geo-referencing of all reported *P. vivax* episodes between June 2009 and May 2010 (year 1), a parasitological and serological survey was conducted (June 2010). Antibodies for specific IgG responses to *P. vivax* merozoite surface protein-1-19 (PvMSP-119) and apical membrane antigen-1 (PvAMA-1) were detected by ELISA. After the survey, an enhanced passive case detection (PCD) between June 2010 and May 2011 (year 2) allowed the detection of new *P. vivax* episodes in the study area. Spatial analysis of *P. vivax* incidence rates in year 1 as well as of *P. vivax* seropositivity at the moment of the survey allowed the retrospective identification of hotspots. Logistic regression was used to evaluate the association between hotspots and malaria incidence in the subsequent year.

Results: *P. vivax* malaria incidence decreased markedly from year 1 (416 episodes) to year 2 (32 episodes). Despite this reduction, logistic regression found that household (HH) location in hotspots (identified using either incidence rates in year 1 or seropositivity) were significantly associated with the occurrence of *P. vivax* episodes at HH in year 2 ($p<0.001$), and this association remained after the multivariate analysis adjusted for mean HH age, male HH proportion and HH distance to the drain.

Conclusions: Study findings confirmed that the spatial analysis using serological markers allowed for the accurate prediction of hotspots *P. vivax* malaria confirmed with subsequent PCD. These tools may support the risk stratification and prioritization of targeted interventions to better reduce transmission and move towards malaria elimination.

Keywords: Malaria, transmission, hot-spot, serology, low transmission

6.2. Background

Over the past century, the Americas have experienced a significant reduction in malaria cases and deaths, and countries such as Argentina, Belize, Costa Rica, Ecuador, El Salvador, Mexico, and Paraguay are close to eliminating malaria from their territories [28]. In Peru, the decline in malaria incidence over the past decade has modified the epidemiological profile of the country, mainly in the northwestern coast near the border with Ecuador where transmission is seasonal, only due to *P. vivax* and concentrated in a few localities [1,3,7].

Implementing control measures in low endemic areas with seasonal malaria transmission and increasing focal distribution poses a major challenge to national malaria control programmes [9]. Single communities or groups of households within communities that are at a substantially increased risk of malaria transmission, called hotspots, need to be accurately identified to better target the remaining parasite reservoirs [9,10].

The spatial analysis of cumulative symptomatic malaria infections detected by passive case detection is theoretically a good method for detecting hotspots [187,188] in areas where most malaria infections are symptomatic, detected by health facilities, and accurately traced for place of infection and/or residence. However, this is rarely the case in many low endemic areas of South America, where most infections remain asymptomatic [54,96,133,160]. Asexual parasite carriage detected using highly sensitive diagnosis tests as polymerase chain reaction (PCR) in cross-sectional surveys seems to be a more accurate indicator of transmission intensity [16,189], however its inability to catch seasonal variations and the low parasite carriage rates can make less clear the geographical stratification of the malaria risk.

The spatial identification of hotspots using serological measures is increasingly proposed for quantifying micro-geographical variations in malaria transmission based on epidemiological studies mostly conducted in *P. falciparum* endemic areas. Antibody

responses to malaria specific antigens are acquired in response to cumulative exposure and can be used to define small-scale variations in malaria exposure [10,20]. Because antibody responses are relatively long-lived, serological markers of malaria exposure are likely to be most suitable for detecting stable hotspots of malaria transmission [9,10], and most sensitive in areas of low endemicity with and without seasonal transmission patterns [57,59,115].

The present study aimed to evaluate the association between households (HHs) located in malaria hotspots - identified in June 2010 through spatial analysis using two different malariometric indicators (malaria incidence and seropositive rates)- and the reported *P. vivax* malaria incidence (between June 2010 and May 2011) in a low endemic area of the Peruvian northern coast.

6.3. Methods

Study area

Located in Piura department on the northwestern Peruvian coast, Bellavista (lat 4°53′24″S, 80°40′51″W) is one of the districts with the highest population density in the country with ~37,000 inhabitants living in an area of 3.09 km2. The main occupations are informal labourer, trader, moto-taxi driver and farmer [133]. In the district, warm and dry winters range from May to October, while hot and humid summers with moderate rains occur from December to April. The annual average temperature is around 24°C and the relative humidity above 70% [95].

Malaria transmission is currently exclusively by *P. vivax* [133]; the peak of cases occurs between February and May, the months in which the density of the main vector, *Anopheles albimanus*, increases [103,104]. After the intense droughts between 2004 and 2006 in the northern coast, Bellavista experienced a significant increase in the *P. vivax* malaria incidence reaching more than 1,500 annually reported cases in 2008 and 2009 (more than 50% of total cases of the Piura) (Figure 6.1). However, since then, *P. vivax*

incidence have decreased with only 659 and 160 reported cases in 2010 and 2011, respectively.

Two neighbourhoods were selected for the study based on their malaria endemicity and their proximity to an artificially created water drain situated at the eastern edge of the district, where water frequently accumulates after the rains thus providing favourable breeding habitats for *Anopheles* mosquitoes [103].

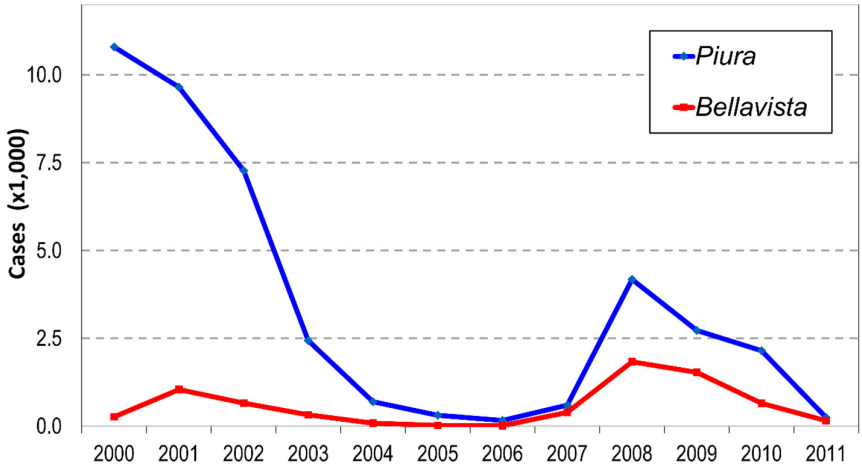


Figure 6.1. Annual *P. vivax* malaria incidence in Piura department and Bellavista district

Study design

A three-phase study was conducted. The first phase included a full census of the study population and the geo-referencing of the HHs of all reported *P. vivax* episodes in the study area between June 2009 and May 2010. The second phase involved a parasitological and serological survey intended to include all residents in the study area in June 2010. And, the third phase consisted of the passive case detection (PCD) of new *P. vivax* episodes at health facilities between June 2010 and May 2011, and the geo-referencing of the HHs of those episodes occurred in the study area.

Data collection

A full census of the study population was conducted two weeks prior to the survey, collecting data on socio-demographics and malaria history data. Each HH was identified with a unique code number and geo-referenced using a handheld global positioning system (GPS) device (Garmin's GPSMAP 60CSx, Garmin International Inc., USA). Presence/absence of malaria episodes in the 12 months prior the survey was confirmed in all censored individuals through the systematic revision of PCD records at the two nearby health facilities: Bellavista health post and Sullana Hospital. Moreover, a cross-checking of the census database (using names, age, gender and address) against the malaria surveillance database of the Sub-Regional Health Direction of Sullana (SRHDS) was performed to identify additional episodes registered in other health facilities.

The cross-sectional survey was done in June 2010. Each HH was visited and all available individuals were enrolled after providing written informed consent/assent. Whenever selected individuals were absent, the household was revisited within the next two days to maximize subject participation. Each participant had the axillary temperature taken, malaria symptoms were recorded, and a finger-prick blood sample collected for immediate microscopy (thick and thin blood smears) and on filter paper (Whatman grade 3, Whatman, Springfield Mill, USA) for later serological and molecular tests. Filter paper dried blood samples were individually stored at 4°C with desiccant until processed at Institute of Tropical Medicine Alexander von Humboldt, Lima (ITM-AvH). All individuals with a malaria infection detected by LM were treated according to the national guidelines.

After the survey, PCD activities at Bellavista health post and at Sullana Hospital were enhanced by the training of microscopists and other local malaria program staff as well as by the availability of new equipment (microscopes) and an expert microscopist at reference laboratory of SRHDS for the quality control of microscopic diagnosis. New detected individuals with *P. vivax* episodes between June 2010 and May 2011 were

carefully investigated and traced for place residence. A weekly cross-checking of the census database against the malaria surveillance database of SRHDS was performed to identify additional episodes registered in other health facilities. All identified individuals with *P. vivax* episodes living in the study area were visited and geo-referenced. According to national guidelines, a malaria episode detected by PCD occurs in an individual with fever (body temperature >37.5°C) and/or history of fever in the previous two weeks in a malaria risk area and a thick blood smear positive for *Plasmodium* [92]. Immediately after *P. vivax* malaria is confirmed, individuals receive directly observed therapy with chloroquine (25 mg/kg over 3 days) and primaquine (3.5 mg/kg over 7 days). The easy access to malaria diagnostic testing in health facilities as well as the high awareness of malaria and appropriate treatment-seeking behaviour in the study population facilitate the detection of most of malaria episodes in the study area.

Laboratory procedures

Microscopy

Thick and thin smears were stained for 10 min with a 10% Giemsa solution, and parasite density was computed after counting the number of asexual parasites for 200 white blood cells (WBC) in the thick smear and assuming a concentration of 6,000 WBCs/µl according to national guidelines [117]. Slides were read during the survey and then a day later by an expert microscopist at the reference laboratory in Iquitos. A slide was declared negative if no malaria parasite was found after examining 100 fields. Quality control was done blindly on all positive slides and 10% of randomly chosen negative slides by a senior technician at ITM-AvH. Any discordant results were reread by a second senior technician until agreement.

Species-specific polymerase chain reaction (ss-PCR)

Parasite DNA was extracted using the saponin lysis Chelex 100 method [118] and stored at 4°C for immediate use in PCR reactions or at -20°C for later use. All samples were first

analyzed by ssPCR following a protocol published elsewhere [119]. Briefly, this ssPCR consisted of a primary PCR with primers directed to specific Plasmodium sequences, and obtained amplicons were used for a second PCR using *P. falciparum* and *P. vivax* specific primers.

Serology

Antibodies for specific IgG responses to *P. vivax* merozoite surface protein-1(19-kDa C-terminal region, PvMSP-1_{19}) [122] and apical membrane antigen-1 (PvAMA-1) [190] were detected using an ELISA protocol published elsewhere [120]. Briefly, dried blood filter paper samples (5mm diameter disc/sample) were eluted overnight at 4°C in 2 ml of PBS-Blotto-Tween. Two hundred microliters of the eluate were added in duplicate to blocked-ELISA plates coated separately with each antigen. Pooled sera from five *P. vivax* infected patients, and from five control individuals without recent (negative PCR) and past history of malaria infection were diluted at 1:400 in PBS-Blotto, as positive and negative control respectively. Goat anti-human IgG (H+L) specific peroxidase (Merck Millipore, USA) diluted to 1:20,000 in PBS-Tween was used as conjugate and incubated for 1 h before development of the ELISA using 200µl ABTS substrate-chromogen solution. Optical density (OD) values at 405 nm (Lector Multi-Modal Synergy H1, BioTek, USA), were corrected by subtracting the mean OD of the antigen negative control wells from the mean OD of the corresponding antigen containing wells. Analyses with duplicate OD values that differed by more than 1.5-fold were rejected and rerun. To ensure a standardization of the sample results across ELISA plates, the percent positivity (PP) of each specimen was calculated using the OD of the positive control serum as 100%. Quality control was done blindly at ITM-AvH on 5% of randomly chosen samples. The criterion for positivity for each antigen was determined applying a mixture model to the PP data which assumed two inherent Gaussian distributions: a narrow distribution or sero-negatives, and a broader distribution of seropositives. Cut-off value was calculated as the mean plus 3 standard deviations of the narrow distribution [21]. Sero-positivity to *P. vivax*

was defined as an individual with a positive serology to any of the two antigens for *P. vivax*.

Data analysis

Spatial clusters analysis

Data were double entered, validated and cleaned in Excel (Microsoft Corp, USA). QGIS™ software v.2.0 [139] was used to map all census HHs and to classify them according to their *P. vivax* incidence rate in the 12 months prior the survey and to their proportion of *P. vivax* seropositivity in the survey. The annual parasite incidence (API) rate at the household level was calculated by dividing the annual number of reported *P. vivax* episodes in a HH during in the 12 months prior the survey by the total number of HH individuals. Using the API, HHs were classified into four categories: 0 episodes/individual, <0.25, 0.25-0.50, and >0.50. The proportion of seropositivity at the household level was calculated by dividing the number of individuals with seropositive to *P. vivax* by the total number of surveyed individuals in the household. Using the proportion of seropositivity, HHs were classified into four categories: 0 seropositives/individual, <0.25, 0.25-0.50, and >0.50.

SaTScan TM software v.9.3 (M Kulldorff and Information Management Services Inc, USA)[140] was used to detect clusters of significant high (hotspot) and low (coldspot) risk of *P. vivax* transmission in the study area using incidence (i.e. IPA) and serological (i.e. proportion of seropositivity) data at HH level. The main analyses were done without adjustment for covariates. A secondary analysis was only performed for the serological data including the proportion of individuals>10 years at household as covariate considering the reported significant higher seroprevalence of this age group compared to younger children in a previous study in the area [133]. The following settings were used in the analysis: purely spatial analysis [141]; Poisson probability model; latitude/longitude coordinates; report of most likely clusters with no geographical overlap of secondary clusters; and maximum spatial cluster size equal to 50% of total population.

SaTScan applies multiple circular windows, which are plastic in both position and size, across the study area. Each circle represents a possible cluster of high or low *P. vivax* transmission. Clusters were assessed based on 999 Monte Carlo simulations to determine the probability of the observed number of episodes/seropositives being due to chance relative to the expected number of episodes/seropositives. The null hypothesis of no clustering was rejected if any resulting p-value of assessed clusters was <0.05. The relative risk (RR) reported for each identified cluster was the estimated risk within the cluster divided by the estimated risk outside the cluster [140].

Risk factor analysis

The variable outcome was the presence/absence of *P. vivax* episodes at HH level between June 2010 and May 2011 (i.e. HH with/without episodes), while the independent variable was the location of the HH in clusters detected by SaTScan using *P. vivax* incidence or seropositivity (i.e. hotspots, coldspots, or none of them). Only results of age-covariate spatial analysis were considered for clusters of seropositivity. Uni- and multivariate (adjusted for mean HH age, male HH proportion, and HH distance to water drain) analyses were performed to determine the association between the independent variable and the variable outcome. All reported p-values are two-sided and were considered statistically significant if <0.05.

Ethical issues

Permission was received from health and local authorities after explaining the purpose and procedures of the study. Signed IC was obtained prior to participation and blood sampling by all adults and the parents of all participating children <18 years of age. In addition to their parent's consent, children more than seven years old provided signed informed assent prior to participation. Ethical clearance was obtained from the Ethics Review Board of the Universidad Peruana Cayetano Heredia, Lima, Peru (SIDISI code: 056736).

6.4. Results

A total of 745 HHs and 3,521 individuals were identified during the census (~10% of the total population of Bellavista district) (Table 6.1). The overall sex ratio was 0.94, and the age distribution showed that half of the population was <20 years old. A total 1,925 individuals (54.7%) living in 613 households (82.3%) were available and accepted to participate in the survey in June 2010. Despite very few *P. vivax* infections were confirmed by LM (4 individuals, 0.2%) and by PCR (16 individuals, 0.8%), one-fifth (418 individuals, 21.7%) of surveyed population were seropositive to at least one of the selected *P. vivax* antigens and this *P. vivax* seropositivity was significantly associated with past history of malaria. Indeed, individuals who presented confirmed (registered) *P. vivax* malaria episodes in the previous year were more likely to have positive serology than those who did not (OR: 4.7, 95% CI [3.5–6.2], p<0.001). Furthermore, individuals>10 years had higher odds of *P. vivax* seropositivity compared to children ≤10 years (OR: 3.2, 95% CI [2.4–4.3], p<0.001).

Table 6.1. Census and survey population

	Census		Survey	
	n	%	n	%
Total households (HH)	745	100.0	613	82.3
Total individuals	3521	100.0	1925	54.7
Individuals with positive microscopy	_	_	4	0.2
Individuals with positive PCR	_	_	16	0.8
Seropositive individuals	_	_	418	21.7

Analysis of PCD records and surveillance databases for the period June 2009 - May 2010 identified 416 registered *P. vivax* malaria episodes (37.1% of the total registered cases in Bellavista), which occurred in 370 individuals living in 272 HHs (36.5% of total censored HHs): 331 (89.5%) with unique episodes, 33 (8.9%) with two episodes, 5 (1.4%) with

three episodes, and one with four episodes. Between June 2010 and May 2011, the malaria incidence decreased markedly in all the district and PCD only detected 32 individuals with unique episodes of *P. vivax* malaria in the study area (28.1% of the total registered cases in Bellavista), which occurred in 29 HHs. Only 8 (25%) of these 32 individuals had also presented *P. vivax* episodes in the period June 2009 - May 2010.

No significant spatial clusters of infections were identified by SaTScan, given the small number of PCR-positive individuals in the survey. However, the analysis confirmed that one-year *P. vivax* malaria incidence rates before the survey as well as *P. vivax* seropositivity measured at the time of the survey were not randomly spatial distributed, detecting hotspots and coldspots respectively in the southeast and the northwest of the study area (Figures 6.2 and 6.3). The hotspot of malaria incidence was a 220 m radius cluster composed of 921 censored individuals in 214 HHs (RR=2.3, p<0.001), while the coldspot was a 180 m radius including 1305 individuals in 258 HHs (RR=0.3, p<0.001) (Table 6.2).

Table 6.2. Characteristics of identified spatial clusters

	Center coord (S,W)	Radius (meters)	Households (N=745)		Population (N=3521)		RR°	p-value
			n	%	n	%		
Clusters usingprevious year's incidence rate								
Coldspot	4.8827S, 80.6727W	180	258	34.6	1305	37.1	0.27	<0.001
Hotspot	4.8892S, 80.6683W	220	214	28.7	921	26.2	2.26	<0.001
Clusters using seropositivity (survey)								
Coldspot	4.8836S, 80.6732W	250	258	34.6	1331	37.8	0.53	<0.001
Hotspot	4.8893S, 80.6683W	65	56	7.5	235	6.7	2.13	0.017
Clusters using seropositivity adjusted for age* (survey)								
Coldspot	4.8842S, 80.6722W	210	255	34.2	1356	38.5	0.57	0.001
Hotspot	4.8900S, 80.6676W	190	171	23.0	721	20.5	1.73	0.003

* Analysis included the proportion of individuals aged>10 years as covariate; °RR=relative risk.

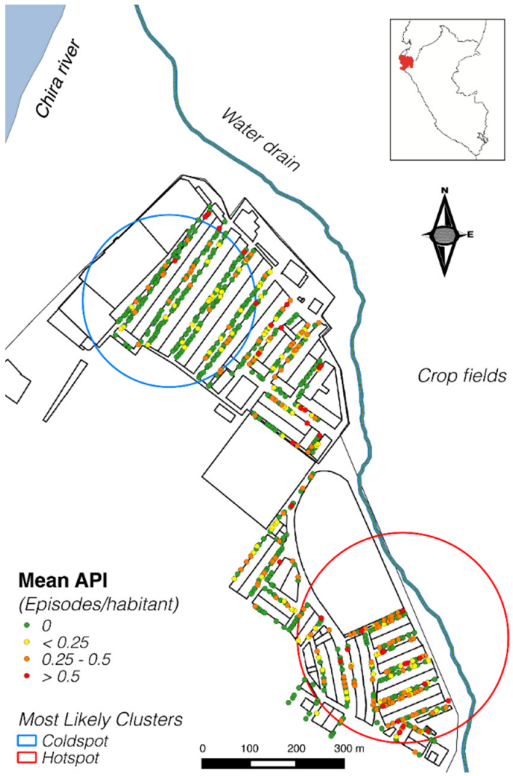


Figure 6.2. Significant spatial clusters of *P. vivax* incidence reported between June 2009 and May 2010 in the study area.

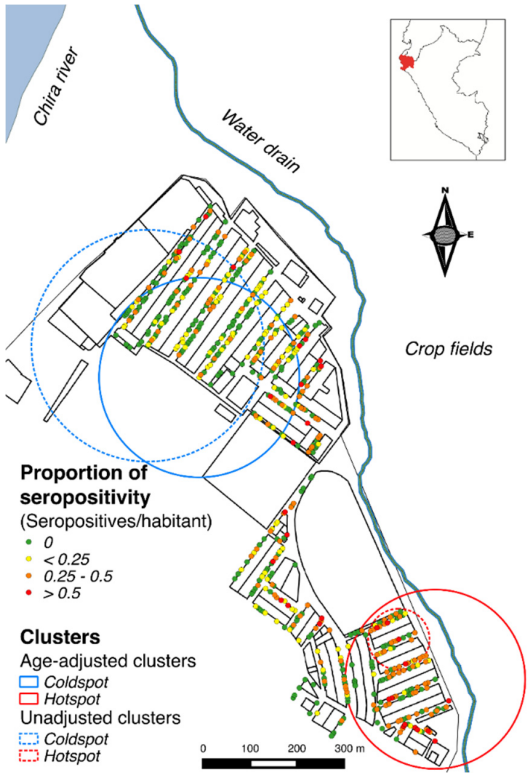


Figure 6.3. Significant spatial clusters of *P. vivax* seropositivity reported by the survey in June 2010 in the study area.

On the other hand, the hotspot of malaria seropositivity was a small cluster of a 65 m radius composed of 235 censored individuals in 56 HHs (RR=2.1, p=0.017), and the coldspot was a 250 m radius including 1331 individuals in 258 HHs (RR=0.5, p<0.001). The addition of the proportion of individuals >10 years at HH as covariate in the analysis increased three times the hotspot radius (190 m) including 721 individuals in 171 HHs (RR = 1.7, p=0.003), while the coldspot slightly changed its radius (210 m) and its included sub-population: 1356 individuals in 255 HHs (RR=0.6, p=0.001) (Table 6.2).

HH location in hotspots was significantly associated with the occurrence of *P. vivax* episodes at HH in the subsequent year (p<0.001), and this association remained after the multivariate analysis adjusted for mean HH age, male HH proportion and HH distance to the drain (Table 6.3). HHs included in the hotspot of malaria incidence were more likely to present *P. vivax* episodes in the subsequent year (AOR=4.3, 95% CI [1.1–17.2]) than HH included in coldspots. Similarly, HHs located in the hotspot of seropositivity had higher odds of *P. vivax* incidence in the following year compared to those located in the coldspot (AOR: 5.6, 95% CI [1.3–24.7]).

Table 6.3. Association between households (HHs) being identified in hotspots (using previous year's incidence rates or seropositivity) and malaria incidence in the subsequent year.

		HHs with 1-year reported incidence (after the survey)			Unadjusted		Adjusted	
		n	N	%	OR	95% CI	AOR	95% CI
Model 1								
Clusters using previous year's incidence rates	Coldspot	5	258	1.9	1		1	
	Other	9	273	3.3	1.7	[0.6, 5.2]	2.1	[0.6, 7.4]
	Hotspot	15	214	7	3.8*	[1.4, 10.7]	4.3*	[1.1, 17.2]
Model 2								
Clusters using seropositivy adjusted for age (survey)	Coldspot	5	255	2	1		1	
	Other	10	319	3.1	1.6	[0.5, 4.8]	1.8	[0.6, 5.7]
	Hotspot	14	171	8.2	4.5*	[1.6, 12.6]	5.6*	[1.3, 24.7]

† Multivariate analysis adjusted by mean HH age, male HH proportion, and HH distance to the drain;
* p<0.05.

6.5. Conclusions

In the study area of the northern coast, the spatial cluster of highest *P. vivax* seropositivity (adjusted for age) at the time of the survey not only coincided roughly with the spatial cluster of *P. vivax* incidencerate in the past year, but also it was able to predict accurately areas (hotspots) with the highest clinical malaria incidence detected by PCD in the subsequent year. This serological approach, once optimized and tested by geographical area, would represent a reliable and effective surveillance tool in low transmission areas to detect hotspots of high malaria risk (i.e., areas with high risk of having symptomatic, asymptomatic patent, and symptomatic sub-patent infections) in malaria endemic areas of Peru. The stability of malaria hotspots over time should also be monitored and considered by decision-makers to better design and deliver appropriate targeted interventions (e.g. focal screening and treatment (FSAT) or mass drug administration (MDA)).

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7. GENERAL DISCUSION

The first part of this thesis included two cross-sectional surveys that combined parasitological (i.e., parasite rates by light microscopy –LM– and by PCR) and serological (seroprevalence and seroconversion rates –SCR) metrics to characterize malaria transmission dynamics in low endemic areas of the Pacific northern coast **(Chapter 3)** and the Amazon region **(Chapter 4)** in Peru. Besides the serological evidence for high heterogeneity in malaria exposure, both to *Plasmodium falciparum* and *Plasmodium vivax,* at micro-geographical level and over time, PCR results confirmed that most of malaria infections in the study areas were asymptomatic and sub-microscopic, and therefore unlikely to be detected by the routine surveillance based on passive case detection (PCD). Considering those findings, a mathematical model was developed **(chapter 5) in** order to understand and predict the potential effect of adding focal screening and treatment (FSAT) interventions to the ongoing PCD on the incidence and prevalence of *P. falciparum* infections in an area of low transmission of the Amazon region. Although the model results suggested that combined interventions enabled to reach elimination levels, the progression rates to those levels varied substantially according to different operational criteria (timing, duration, diagnostic tool, etc.) used for the intervention.

As previously described in other malaria endemic areas in South America [54,94,96,98,111,160], cross-sectional surveys in both of our study sites consistently showed that a large proportion (>75%) of malaria infections were asymptomatic, despite differences in malaria prevalence and ecological characteristics. Though cross-sectional surveys might overestimate the proportion of asymptomatic carriers due to the potential later development of symptoms [96,173], previous longitudinal studies in Peru and Brazil have also confirmed, the high burden of asymptomatic carriers among all malaria infections, though at lower levels compared to survey data [173]. Indeed, a 6-month

cohort study in peri-Iquitos communities combining PCD, active case detection (ACD), and weekly monitoring of symptoms for one month of all malaria infected individuals, reported that asymptomatic infections accounted for about 40% of all microscopically confirmed cases during the study period [96]. Another study conducted in the Brazilian Amazon in 2004–2005 [173], including cross-sectional surveys, PCD and ACD over a 14-month period, found that among 93 asymptomatic infections confirmed by PCR during surveys, only 10 (10.7%) developed malaria symptoms over the subsequent two months of follow-up.

As transmission decreases, malaria surveillance becomes challenging due to the increasing proportion of asymptomatic cases that limit the performance of the routine PCD, and also because a significant proportion of these asymptomatic infections carry parasites at densities below the LM detection threshold [52,191,192]. The increasing use of molecular diagnostics in epidemiological studies allowed for a more accurate determination of the prevalence of infections [52,191,192], especially in low transmission settings where the asymptomatic reservoir is mainly composed of sub-microscopic infections [52,110,191,193,194]. This is exactly the case in our study areas, where the overall ratio LM/PCR prevalence of about 0.3 in both the northern coast and the Amazon region indicated that 70% of the total infections were not detected by LM.

The presence of known malaria mosquito vectors, i.e. *An. albimanus* in the northern coast and *An. darlingi* in the Amazon, together with the identification by LM of *P. falciparum* (only in the Amazon) and *P. vivax* (in both study areas) gametocyte carriers confirms potential for onward malaria transmission within our study areas. Considering that LM has a very low sensitivity for detecting sexual parasite stages, it is likely that a substantial proportion of individuals with asymptomatic and sub-patent infections would also carry gametocytes, thus contributing to a "hidden" human infectious reservoir that would maintain transmission as it remains largely undetected and therefore untreated [54,111,124,125]. Although further research is still needed to define to what extend sub-

patent infections contribute to malaria transmission, mosquito feeding experiments have shown an overall positive non-linear association between *P. falciparum* gametocyte density and mosquito infection rates with very low densities still able to infect mosquitoes [183,195,196]. Moreover, despite first assays using direct skin feeding suggesting that *P. vivax* transmission may be more efficient than that of *P. falciparum* [197], recent studies using membrane assays also found a weak positive association between *P. vivax* gametocyte density and infectiousness to mosquitoes [198,199]. Although the success rate of mosquito infections has been shown to be lower with sub-patent gametocytemia [196,200], it can be hypothesized that this low efficiency is somehow compensated by the number and duration of sub-patent infections.

Since serology provides information on cumulative exposure over time rather than a single current infection, it can usefully complement LM and PCR prevalence data allowing for a more accurate picture of the malaria transmission dynamics at community level [16,58]. This was illustrated in the northern coast **(C**hapter 3)** where the low *P. falciparum* seropositivity in young children and the single PCR detected *P. falciparum* infection during the 2010 survey, while correlating with the extremely low incidence of reported *P. falciparum* cases during the past five years, also suggested a residual transmission of *P. falciparum* infections in the study area. This may be due to asymptomatic and sub-patent *P. falciparum* infections that remain undetected unless actively screened with highly sensitive diagnostic tools such as PCR. Moreover, in the Amazon region (**Chapter 4)**, while available surveillance data suggested a predominance of *P.vivax* cases at district level, the cross sectional survey using PCR identified an unexpected higher prevalence of *P. falciparum* than *P. vivax* infection, and it also showed how the combination of serology, PCR, LM, together with contextual data allowed for a better understanding of the local malaria epidemiology. Indeed, in the community where the highest *P. falciparum* prevalence by PCR was found, the higher seropositivity levels and sero-conversion rates, for *P. falciparum* compared to those for *P. vivax* indicated that

exposure to *P. falciparum* was as high if not higher than that to *P. vivax* malaria. These serological findings and the identification of asymptomatic gametocyte carriers by LM suggested that *P. falciparum* transmission in the study site was more intense than previously thought.

In addition to the asymptomatic *P. falciparum* reservoir, other operational or social factors limiting the effectiveness of routine PCD to detect and treat symptomatic malaria infections (i.e., low treatment seeking behavior at community, poor quality of microscopy-based diagnosis, poor prescribing practices, low patient adherence to treatment regimens, etc.) can also contribute in maintaining *P. falciparum* malaria transmission [46]. Moreover, it should be mentioned that the Peruvian national malaria control program (NMCP) is currently not recommending the addition of a single dose primaquine treatment for uncomplicated *P. falciparum* malaria, thus losing one opportunity to further reduce malaria transmission [92].

Serological metrics are particularly suited to low endemic areas with seasonal transmission patterns [16] such as the Peruvian study areas, where the extremely low parasite rates at the time of the surveys did not allow for the identification of risk factors nor of malaria hotspots at micro-geographical level. Therefore, using serological markers (**Chapter 4)** we were able to show that exposure to *P. falciparum* in the Amazon region was independently associated with outdoor economical activities (e.g., farming, logging, and fishing) as well as with the place of residence confirming previously published studies from this area [93]. Although the place of residence as risk factor may be related with favorable ecological conditions for mosquito breeding or resting sites resulting in increased vector densities and human-vector contact rates [93,100,144], at smaller geographical scale (i.e. within the sites), the spatial heterogeneity in *P. falciparum* malaria exposure can also be explained by different human behaviour [132]. Indeed, in site A for example **(Chapter 4)**, the hotspot of *P. falciparum* exposure was the closest area to the river which floods seasonally after heavy rains, while in site B, this hotspot included a

majority individuals with outdoor occupations and with electricity in their houses therefore more likely to have evening outdoor activities.

Determining spatial heterogeneity in *P. vivax* malaria transmission was less clear in the Amazonian study sites **(Chapter 4)**. As expected, the site with the highest *P. vivax* prevalence was also the one with the highest *P. vivax* seroprevalence. Although the site of residence was the only independent risk factor for *P. vivax* exposure, the spatial analysis using serological data was not able to identify significant clusters of *P. vivax* exposure suggesting that for *P. vivax* transmission ecological factors would be less important than for *P. falciparum*. This could be due partly due to the chronic and relapsing nature of *P.vivax* infections which may hide the existing link between exposure to new infections and place of residence. Although high *P. vivax* recurrence rates (including relapses, recrudescence, and reinfections) have been reported in the Amazon region [161], it remains unclear how they influence antibody responses and consequently the identification of clusters of *P. vivax* exposure.

Conversely in the northern coast, a strong association between mosquito breeding sites (i.e., proximity to water drain) and *P. vivax* exposure [103,201] may explain why the spatial analysis using serological markers was more sensitive for identification of *P. vivax* malaria hotspots **(Chapter 6)**. Indeed, the spatial cluster of highest *P. vivax* seropositivity at the time of the survey was able to predict accurately hotspots with the highest clinical malaria incidence detected by PCD in the subsequent year. This serological approach, once optimized and tested by geographical area, would represent a reliable and effective surveillance tool in low transmission areas to detect hotspots of malaria (i.e., areas with high risk of having symptomatic, asymptomatic patent, and symptomatic sub-patent infections) in the remaining endemic areas of Peru. The stability of malaria hotspots over time should be closely monitored and also considered by decision-makers to better design and timely deliver appropriate targeted interventions (e.g. focal screening and treatment (FSAT) or mass drug administration (MDA)).

As previously reported in low transmission settings [21,22,57,202], SCRs calculated from age-adjusted seroprevelance data in single surveys allowed to detect historical changes in malaria transmission in our study areas **(Chapters 3 and 4)**. Most of those changes coincided with major changes in the malaria control strategies (or in the environmental conditions) and with changes in malaria incidence trends at district or departmental level. Although regional and local differences in the intensity of control efforts and transmission dynamics should be considered to understand differences in the history of exposure determined by age-seroprevalence models **(Chapter 4),** our results showed the high potential of this approach for assessing the impact of previous control efforts in Peru. As reported before, a strong advantage of SCRs is the ability to reconstruct the history of malaria exposure [16,59] which will be especially useful in countries such as Peru where malaria surveillance is still based mainly on PCD by LM. In addition, SCRs could be efficiently used in the common situation of unreliable or missing data from the health information system [59].

The uncovered asymptomatic and sub-patent reservoir is currently challenging malaria surveillance in Peru which is mainly based on PCD by LM [26]. Since the NMCP has recently introduced FSAT to overcome the inherent limitations of PCD to identify asymptomatic carriers [8], a modelling approach was developed to assess the potential impact of this strategy on the *P. falciparum* transmission in the Amazon region and to provide insights for the best criteria for its implementation **(Chapter 5)**. Our analysis suggested that targeting the asymptomatic *P. falciparum* reservoir with FSAT interventions using LM, in addition to routine PCD, had the potential to significantly reduce *P. falciparum* malaria transmission as long as 2-3 successive interventions at periodical intervals (i.e. 7-60 days) in low transmission season for consecutive years would be implemented. However, this positive impact would be reduced and even reversed if one or more of the following conditions are presented: poor coverage of FSAT interventions, PCD not efficiently detecting symptomatic infections, efficacy of the

antimalarial treatment reduced, poor prescription practices, patient adherence to the treatment reduced, and the burden of asymptomatic and sub-microscopic infections increasing. On the other hand, implementing FSAT interventions using a supposed field PCR under the same operational criteria used for FSAT with LM would not only have a stronger impact on *P. falciparum* transmission enabling to reach elimination levels, but would also be less affected by the conditions mentioned above.

Although these results support the importance of detecting and treating *P. falciparum* sub-microscopic infections for successful elimination strategies [46,184], implementing FSAT using PCR as routine strategy in well identified hotspots may not be feasible since current PCR tests are resource-intensive, time-consuming and expensive [113]. However, recent studies have shown high sensitivity of non-conventional molecular tests based on loop-mediated isothermal amplification (LAMP) and which do not require specialized staff or equipment [185]. Although, recent field studies carried out in in Zanzibar [203] and Colombia [204] have shown promising results with sensitivities above 97%, the LAMP assays still need further optimization in order to be used as true point of care tests.

CONCLUSIONS AND PERSPECTIVES

A key challenge faced by the Peruvian NMCP is the ability to efficiently target a human malaria infectious reservoir which remains largely undetected by the standard diagnostic tools. The use of PCR to detect current malaria infections and serology to identify past malaria exposure have repeatedly proved their added value in accurately understanding the local epidemiology, determining risk factors for malaria, describing spatial heterogeneity in transmission, and identifying recent changes in malaria transmission in the study areas of the northern coast and the Amazon region.

The uncovered asymptomatic and sub-patent reservoir calls for an immediate shift in the malaria control policy in Peru from detecting and controlling clinical malaria to targeting and eliminating all malaria infections (both asymptomatic and symptomatic infections). The high heterogeneity in malaria transmission in the northern coast and Amazon region creates opportunities for targeted interventions as focal screening and treatment (FSAT) or mass drug administration (MDA) with effective treatment combinations (including single-dose primaquine for *P. falciparum*), however, hotspots of malaria transmission need to be first accurately identified. Serological metrics have proven to be reliable tools to detect future malaria hotspots of clinical malaria cases in the northern coast, but they need to be evaluated in other ecological areas. Furthermore, serological tools should also be tested for their ability to predict clusters with high risk of asymptomatic and sub-patent infections.

Since the financial resources available to control malaria are currently limited, it is important for the NMCP to know what will be the contribution of new surveillance and control strategies aimed to target the asymptomatic and sub-patent reservoir, including the assessment of their cost-effectiveness. Trying always to keep the principle of parsimony, our current *P. falciparum* model can be further improved by including particularities of both the malaria dynamics and the targeted interventions, such as: the human mobility patterns (i.e, migration and visitation); the heterogeneity in transmission parameters between human sub-

populations by age (children, adults), occupation (farmers, loggers, fishers), and treatment behaviour practices (i.e., treatment seeking behaviour, treatment adherence, use of preventive malaria measures, etc.); the different levels of gametocyte infectiousness according to the age of the gametocyte carrier state; the variable duration of case detection interventions according to the size of the target population, the malaria diagnostic tool used, and the available human and logistic resources; the addition of a single dose primaquine to the treatment for uncomplicated *P. falciparum* malaria. New research lines should include the development of more complex models for *P. vivax* transmission using data of cohorts carried out in the Peruvian Amazon to better define the operational criteria for FSAT interventions to maximize the impact of the intervention on both species. In addition, models would also allow for evaluating the cost-effectiveness highly sensitive diagnostic tests such as loop-mediated isothermal amplification (LAMP) in active case detection interventions, as well as, to assess innovative malaria elimination strategies in countries where *P. falciparum* and *P. vivax* are co-endemic, such as the treatment of all individuals with confirmed infections by LAMP (treatment according to the identified species) plus the additional treatment of probable *P. vivax* hypnozoite carriers (e.g. *P. vivax* seropositive individuals). Considering MDA as a potential elimination strategy in well delimited areas, models can be adapted to simulate the impact of using a unique treatment based on ACT and primaquine (or tafenoquine when available) for treating both *P. falciparum* and *P. vivax* infections.

The significant reduction in malaria cases and deaths over the past decade in the Americas with many endemic countries close to eliminate malaria from their territories (i.e., Argentina, Belize, Costa Rica, Ecuador, El Salvador, Mexico, and Paraguay) has recently encouraged consultations in Peru between the research community and decision-makers on the relevance and feasibility of a national malaria elimination program. The good news is that malaria elimination in the country is not perceived as unachievable, but it will require in addition to political and financial commitment, the involvement of key stakeholders and experts at every level of government, as well as, integrated research activities to evaluate and implement sustainable interventions and tools in endemic regions.

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9. ANNEXES

Chapter 2

Text S2.1. R-code for basic malaria models

Chapter 3

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Chapter 4

Table S4.1. Survey coverage by site

Figure S4.1. Evolution of the annual malaria incidence in San Juan/Iquitos districts: 2002–2013.

Chapter 5

Figure S5.1. Schematic diagram of the human and vector model states.

Table S5.1. *P. falciparum* malaria prevalence and incidence rate in Ninarumi in 2004.

Text S5.1. Description of transmission parameters for baseline model.

Text S5.2. R-code for the baseline model.

Annexes chapter 2.

Text S2.1. R-code for basic malaria models

#1#Ross model

```
library(deSolve)

#Parameters
Nt <-500      #Total human population
Mt<- 15000    #Total mosquito population
m <- Mt/Nt    # m is equilibrium mosquito density per human.
a <- 0.2      # expected number of bites on humans per mosquito.
b <- 0.2      #transmission efficiency from infectious mosquito to susceptible human
c<- 0.5       #probability of infection of an uninfected mosquito by biting an infectious
human
g<- 0.2      #daily force of mosquito mortality
r<- 1/210     #daily recovery rate
tim<-365*1.5      #time in days

#Initial population number
Io<-1
So<-Nt-Io
Vo<-0
Uo<-Mt-Vo

#Function
mal1.dyn <- function(t,var,par) {
 # Rename the variables and parameters
 S<- var[1]
 I<- var[2]
 U<-var[3]
 V<- var[4]
 N<-S+I
 M<-U+V
 m <- par[1]
 a <- par[2]
 b <- par[3]
 c<- par[4]
 g<- par[5]
 r<- par[6]

 # Derivatives
 dS <- -m*a*b*V/M*S+r*I
 dI <- m*a*b*V/M*S-r*I
 dU <- -a*c*I/N*U+g*V
```

```
  dV <- a*c*I/N*U-g*V

  list(c(dS,dI,dU,dV))}  # Return the 5 values

#Preparing the numerical solution
par <- c(m,a,b,c,g,r)
init <- c(So,Io,Uo,Vo)  #Initial population
time <- seq(0,tim,by=0.1)

#Numerical solution
# lsoda(var,t,function,par)

mal1.sol <- lsoda(init,time,mal1.dyn,par)  # obtain a matrix with 5 columns (time, S,I,U,
V)

#Relabeling the variables
TIME <- mal1.sol[,1]
S1 <- mal1.sol[,2]
I1 <- mal1.sol[,3]
U1 <- mal1.sol[,4]
V1 <- mal1.sol[,5]
N1 <- S1+I1
M1 <- V1+U1

#Plots
windows()

par(mfrow=c(2,1), xaxs='i', yaxs='i')

plot(TIME,S1/Nt,type='l',main=paste('Ross model - Differential
equation'),xlab='time',ylab='Human population',col='blue',lwd=3,ylim=c(0,1))
lines(TIME,I1/Nt, type='l',col='red', lwd=3)
legend("topright", c("S", "I"), col=c("blue","red"),lty=c(1,1),inset=0.1)

plot(TIME,U1/Mt,type='l', main=paste('Ross model - Differential equation'),
xlab='time',ylab='Vector population',col='blue',lwd=3,ylim=c(0,1))
lines(TIME,V1/Mt, type='l',col='red', lwd=3)
legend("topright", c("U", "V"), col=c("blue","red"),lty=c(1,1),  inset=0.1)
```

#2#Mc Donald model##

```
library(deSolve)

#Parameters
Nt <-500      #Total human population
Mt<- 15000     #Total mosquito population
m <- Mt/Nt    # m is equilibrium mosquito density per human.
```

```
a <- 0.2      # expected number of bites on humans per mosquito.
b <- 0.2      #transmission efficiency from infectious mosquito to susceptible human
c<- 0.5       #probability of infection of an uninfected mosquito by biting an infectious
human
g<- 0.2      #daily force of mosquito mortality
r<- 1/210     #daily recovery rate
n<- 10        #mosquito incubation period
tim<-365*1.5      #time in days

#Initial population number
Io<-1
So<-Nt-Io
Vo<-0
Lo<-0
Uo<-Mt-Vo-Lo

#Function
mal1.dyn <- function(t,var,par) {

 #Setting the lag for mosquito incubation period
 lagM <- t - n
 if (lagM <= 0)
   varlagM <- c(So,Io,Uo,Lo,Vo)  #"n" past values of compartments before time 0
 else
   varlagM <- lagvalue(lagM)

 # Rename the variables and parameters
 S<- var[1]
 I<- var[2]
 U<-var[3]
 L<-var[4]
 V<- var[5]
 N<-S+I
 M<-U+L+V
 m <- par[1]
 a <- par[2]
 b <- par[3]
 c<- par[4]
 g<- par[5]
 r<- par[6]
 n<-par[7]
 I_lagM<-varlagM[2]
 U_lagM<-varlagM[3]

 # Derivatives
 dS <- -m*a*b*(V/M)*S+r*I
 dI <- m*a*b*(V/M)*S-r*I
 dU <- -a*c*(I/N)*U+g*(V+L)
```

```
  dL <- a*c*(I/N)*U - a*c*(I_lagM/N)*U_lagM*exp(-g*n)-g*L
  dV <- a*c*(I_lagM/N)*U_lagM*exp(-g*n)-g*V

  list(c(dS,dI,dU,dL,dV))}  # Return the 6 values

#Preparing the numerical solution
par <- c(m,a,b,c,g,r,n)
init <- c(So,Io,Uo,Lo,Vo)  #Initial population
time <- seq(0,tim,by=0.1)

#Numerical solution
# lsoda(var,t,function,par)
mal1.sol <- dede(init,time,mal1.dyn,par)  # obtain a matrix with 6 columns (time,
S,I,U,L,V)

#Relabeling the variables
TIME <- mal1.sol[,1]
S2 <- mal1.sol[,2]
I2 <- mal1.sol[,3]
U2 <- mal1.sol[,4]
L2 <- mal1.sol[,5]
V2 <- mal1.sol[,6]
N2 <- S2+I2
M2 <- V2+L2+U2

#Plots
windows()
par(mfrow=c(2,1), xaxs='i', yaxs='i')
plot(TIME,S2/Nt,type='l',main=paste('Mac Donald model - Differential
equation'),xlab='time',ylab='Human population',col='blue',lwd=3,ylim=c(0,1))
lines(TIME,I2/Nt, type='l',col='red', lwd=3)
legend("topright", c("S", "I"), col=c("blue","red"),lty=c(1,1),inset=0.1)

plot(TIME,U2/Mt,type='l', main=paste('Mac Donald model - Differential equation'),
xlab='time',ylab='Vector population',col='blue',lwd=3,ylim=c(0,1))
lines(TIME,L2/Mt, type='l',col='orange', lwd=3)
lines(TIME,V2/Mt, type='l',col='red', lwd=3)
legend("topright", c("U","L","V"), col=c("blue","orange","red"),lty=c(1,1,1),  inset=0.1)
```

#3#Anderson & May model#

```
library(deSolve)

#Parameters
Nt <-500      #Total human population
Mt<- 15000     #Total mosquito population
m <- Mt/Nt    # m is equilibrium mosquito density per human.
a <- 0.2      # expected number of bites on humans per mosquito.
```

```
b <- 0.2      #transmission efficiency from infectious mosquito to susceptible human
c<- 0.5       #probability of infection of an uninfected mosquito by biting an infectious
human
g<- 0.2       #daily force of mosquito mortality
r<- 1/210     #daily recovery rate
n<- 10        #mosquito incubation period
h<-0.00005    #daily force of human mortality
l<- 21        #human incubation period

tim<-365*1.5      #time in days

#Initial population number
Io<-1
Eo<-0
So<-Nt-Io-Eo
Vo<-0
Lo<-0
Uo<-Mt-Vo-Lo

#Function
mal1.dyn <- function(t,var,par) {

 #Setting the lag for mosquito latent period
 lagM <- t - n
 if (lagM <= 0)
   varlagM <- c(So,Eo,Io,Uo,Lo,Vo)  #"n" past values of compartments before time 0 #
Put Io-Io to have the same curve of the paper
 else
   varlagM <- lagvalue(lagM)

 #Setting the lag for human latent period
 lagH <- t - l
 if (lagH <= 0)
   varlagH <- c(So,Eo,Io,Uo,Lo,Vo)  #"l" past values of compartments before time 0
 else
   varlagH <- lagvalue(lagH)

 # Rename the variables and parameters
 S<- var[1]
 E<- var[2]
 I<- var[3]

 U<-var[4]
 L<-var[5]
 V<- var[6]
 N<-S+E+I
 M<-U+L+V
 m <- par[1]
```

```
 a <- par[2]
 b <- par[3]
 c<- par[4]
 g<- par[5]
 r<- par[6]
 n<-par[7]
 h<-par[8]
 l<-par[9]

 S_lagM<-varlagM[1]
 E_lagM<-varlagM[2]
 I_lagM<-varlagM[3]
 U_lagM<-varlagM[4]
 L_lagM<-varlagM[5]
 V_lagM<-varlagM[6]

 S_lagH<-varlagH[1]
 E_lagH<-varlagH[2]
 I_lagH<-varlagH[3]
 U_lagH<-varlagH[4]
 L_lagH<-varlagH[5]
 V_lagH<-varlagH[6]

 # Derivatives
 dS <- -m*a*b*(V/M)*S+r*I+r*E-h*S+h*(S+I+E)
 dE <-  m*a*b*(V/M)*S-m*a*b*(V_lagH/M)*S_lagH*exp(-l*(r+h))-r*E-h*E
 dI <-  m*a*b*(V_lagH/M)*S_lagH*exp(-l*(r+h))-r*I-h*I

 dU <- -a*c*(I/N)*U+g*(V+L)
 dL <- a*c*(I/N)*U - a*c*(I_lagM/N)*U_lagM*exp(-g*n)-g*L
 dV <- a*c*(I_lagM/N)*U_lagM*exp(-g*n)-g*V

 list(c(dS,dE,dI,dU,dL,dV))}  # Return the 7 values

#Preparing the numerical solution
par <- c(m,a,b,c,g,r,n,h,l)
init <- c(So,Eo,Io,Uo,Lo,Vo)  #Initial population
time <- seq(0,tim,by=0.1)

#Numerical solution
# lsoda(var,t,function,par)
mal1.sol <- dede(init,time,mal1.dyn,par)  # obtain a matrix with 7 columns (time,
S,E,I,U,L,V)

#Relabeling the variables
TIME <- mal1.sol[,1]
S3 <- mal1.sol[,2]
E3 <- mal1.sol[,3]
```

```
I3 <- mal1.sol[,4]
U3 <- mal1.sol[,5]
L3 <- mal1.sol[,6]
V3 <- mal1.sol[,7]
N3 <- S3+E3+I3
M3 <- V3+L3+U3

#Plots
windows()
par(mfrow=c(2,1), xaxs='i', yaxs='i')
plot(TIME,S3/Nt,type='l',main=paste('Anderson & May model - Differential
equation'),xlab='time',ylab='Human population',col='blue',lwd=3,ylim=c(0,1))
lines(TIME,E3/Nt, type='l',col='orange', lwd=3)
lines(TIME,I3/Nt, type='l',col='red', lwd=3)
legend("topright", c("S","E", "I"), col=c("blue","orange","red"),lty=c(1,1,1),inset=0.01,
cex=0.5)

plot(TIME,U3/Mt,type='l', main=paste('Anderson & May model - Differential
equation'), xlab='time',ylab='Vector population',col='blue',lwd=3,ylim=c(0,1))
lines(TIME,L3/Mt, type='l',col='orange', lwd=3)
lines(TIME,V3/Mt, type='l',col='red', lwd=3)
legend("topright", c("U","L","V"), col=c("blue","orange","red"),lty=c(1,1,1),
inset=0.01, cex=0.5)


#####COMPARISON

windows()
plot(TIME,I1/Nt,type='l',xlab='time',ylab='Prevalence (I or
V)',col='blue',lwd=3,ylim=c(0,1))
lines(TIME,I2/Nt, type='l',col='green', lwd=3)
lines(TIME,I3/Nt, type='l',col='red', lwd=3)
lines(TIME,V1/Mt, type='l',col='blue', lwd=3, lty=3)
lines(TIME,V2/Mt, type='l',col='green', lwd=3, lty=3)
lines(TIME,V3/Mt, type='l',col='red', lwd=3, lty=3)
legend("right", c("Ross","Mac Donald","Anderson & May"),
col=c("blue","green","red"),lty=c(1,1,1),lwd=c(3,3,3),,inset=0.01, cex=1.1)
```

Annexes chapter 3.

Table S3.1. Prevalence of *P. vivax* infections by age-groups and occupation

		Prevalence		n	N
		%	95% CI		
Age groups (years)*	0.5-5	0.89	[0.34,2.36]	4	447
	6-10	0.60	[0.15,2.36]	2	335
	11-14	1.30	[0.49,3.43]	4	307
	15-19	1.19	[0.38,3.60]	3	253
	20-29	1.17	[0.49,2.77]	5	429
	30-39	1.09	[0.45,2.58]	5	460
	40-49	0.40	[0.06,2.81]	1	249
	≥50	0.53	[0.07,3.71]	1	187
	Total	**0.94**	**[0.63,1.40]**	**25**	**2667**
Occupation as farmer°*	No	0.95	[0.54,1.66]	12	1269
	Yes	0.00		0	56
	Total	**0.91**	**[0.52,1.59]**	**12**	**1325**
Occupation as moto-taxi driver°*	No	0.87	[0.48,1.57]	11	1260
	Yes	1.54	[0.22,10.18]	1	65
	Total	**0.91**	**[0.52,1.59]**	**12**	**1325**

* No differences between groups (p > 0.05); ° in the past 12 months in individual ≥ 20 years;

Table S3.2. *P. falciparum* seropositivity by occupation as moto-taxi driver

		Prevalence		n	N
		%	95% CI		
Occupation as moto-taxi driver	No	10.96	[9.32,12.85]	138	1259
	Yes	21.54	[13.22,33.09]	14	65
	Total	**11.48**	**[9.84,13.35]**	**152**	**1324**

* Significant difference between groups (OR=2.2, p<0.008); ° in the past 12 months in individual ≥ 20 years;

Annexes chapter 4.

Table S4.1. Survey coverage by site and age group

Site	Age	Census	Enrolled	%
Site A	< 6 m	41	–	–
	6 m - 6.9 y	146	109	74.7
	7-14.9 y	149	42	28.2
	15-30 y	188	47	25.0
	> 30 y	131	40	30.5
	Total	**655**	**238**	**36.3**
Site B	< 6 m	31	–	–
	6 m - 6.9 y	105	74	70.5
	7-14.9 y	60	19	31.7
	15-30 y	58	17	29.3
	> 30 y	123	43	35.0
	Total	**377**	**153**	**40.6**
Site C	< 6 m	45	–	–
	6 m - 6.9 y	169	121	71.6
	7-14.9 y	124	40	32.3
	15-30 y	125	44	35.2
	> 30 y	154	55	35.7
	Total	**617**	**260**	**42.1**
Total	< 6 m	117		
	6 m - 6.9 y	420	304	72.4
	7-14.9 y	333	101	30.3
	15-30 y	371	108	29.1
	> 30 y	408	138	33.8
	Total	**1649**	**651**	**39.5**

Table S4.2. Uni- and multivariate risk factor analysis for individuals included in *P. falciparum*-exposure cluster in site B (N=152)

	Univariate					*Multivariate*		
	%	**n/N**	**OR**		**[95% CI]**	**AOR**		**[95% CI]**
Age groups (years)								
< 7	39.5	27/73	1			-		
7-14.9	23.9	5/19	0.5		[0.2;1.5]			
15-30	41.9	7/17	1.1		[0.4;2.7]			
> 30	47.8	18/43	1.4		[0.6;3.4]			
Gender								
Female	31.7	28/76	0.5		[0.2;1.2]	-		
Male	46.5	29/76	1					
Outdoor occupation								
No	34.3	45/125	1			1		
Yes	60.0	12/27	2.9	*	[1.1;7.8]	4.4	*	[1.6;11.8]
Predominant material in roof								
Palm leaf, straw	35.5	29/88	0.7		[0.3;2.0]	-		
Tin roof	43.6	28/64	1					
Electricity available								
No	22.1	15/62	1			1		
Yes	48.2	42/90	3.3	*	[1.2;9.1]	4.5	*	[1.6;12.9]
Malaria episode in the past 12 months								
No	34.2	44/127	1					
Yes	59.7	13/25	2.9	º	[1.0;8.4]	-		

*p<0.05; º p<0.1

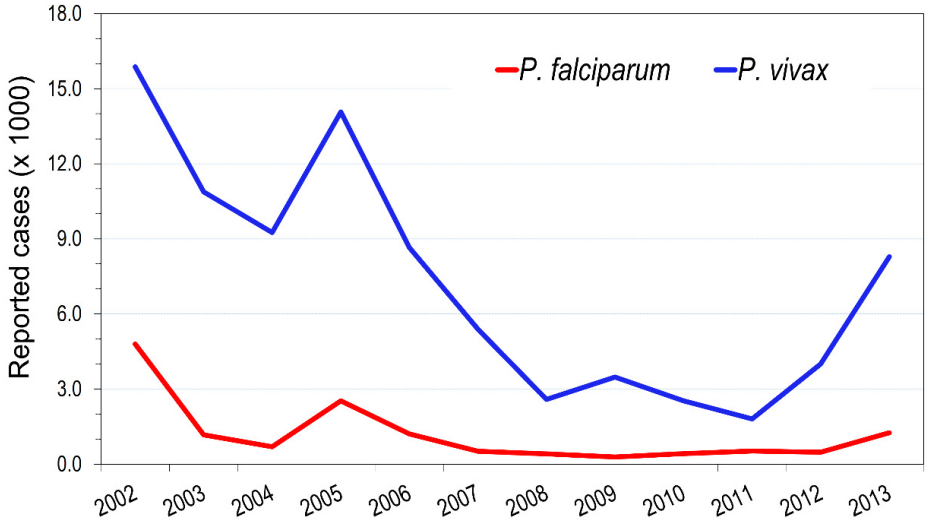


Figure S1. Evolution of the annual malaria incidence in San Juan/Iquitos districts: 2002–2013.

Annexes chapter 5.

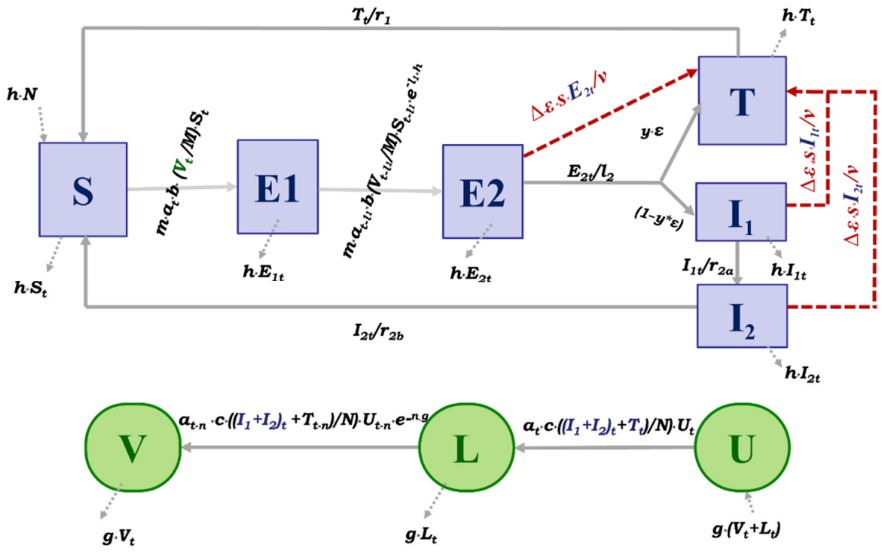


Figure S5.1. Schematic diagram of the human and vector model states including all transmission parameters. The total human population (N) is divided into 4 compartments: susceptible (S), infected latent (E), infectious treated (T) and infectious untreated (*I*) individuals. The latent period has two sub-states: latent which have not yet developed asexual blood-stage parasites (E_1) and latent with asexual blood-stage parasites (E_2). Before becoming recovered, individuals in I compartment need to pass through two sub-states: those who have not yet (I_1) and those who have spontaneously cleared the asexual blood stage parasites (I_2). The total mosquito population (M) is divided into three compartments: uninfected (U), infected latent (L) and infectious (V) mosquitoes. The addition of FSAT to PCD allows for the detection and treatment of individuals in E_2, I_1 and I_2 compartments.

Table S5.1. *P. falciparum* malaria prevalence and incidence rate in Ninarumi in 2004.

		n	N	%	95% CI
Prevalence by microscopy	February 2004	28	432	**6.5**	[5.6; 7.4]
	August 2004	3	269	**1.1**	[0.3; 2.0]
Annual incidence	Symptomatic infections	91	-	**65.4**	-
	Asymptomatic microscopically confirmed infections	29	-	**20.9**	-
	Asymptomatic sub-microscopic infections	19	-	**13.7**	-
	Total	139	-	**100.0**	-
Annual *P. falciparum* parasitological index	278 *P. falciparum* cases per 1000 inhabitants				

Text S5.1. Description of transmission parameters for baseline model.

The susceptibility of an individual to infection (*b*) was assumed to be 0.05 based on estimates of two malariometric indices in Ninarumi: the annual *P. falciparum* force of infection in 2004 (*Pf-FOI* = 0.28 *P. falciparum* infections/person/year) and the annual *P. falciparum* entomological inoculation rate (*Pf-EIR* = 5.57 infective bites/person/year). The annual Pf-EIR was calculated by multiplying the annual-average *An. darlingi* human biting rate (Ad-HBR) by the *P. falciparum* sporozoite rate in *An. darlingi* mosquitoes (*Pf-SR*) and the number of days in a year (365). The annual Ad-HBR was estimated at 15.25 *An.darlingi* bites/person/night from mosquito collections undertaken in Ninarumi along 2003 [1]. Pf-SR was conservatively assumed to be 0.001 (0.1%), as observed in two entomological surveys carried out in 2008 in another area near Iquitos in the Peruvian Amazon [2].

The average human latent period (*l*) was set to 21 days based on previous reports about the biology of *P. falciparum* transmission stages [3]. The probability for an individual to develop symptoms upon infection and being detected by PCD was assumed to be 65% (y=0.65) based on results of the monitoring of symptoms in asymptomatic infections as part of the cohort study in Ninarumi [1]. ACT treatment effectiveness was assumed to be 98% (ε=0.98) according to an efficacy and effectiveness study of MQ-AS combination therapy for *P. falciparum* malaria in the Peruvian Amazon [4]. Daily recovery rate without treatment (r_2) for *P. falciparum* was set to 1/200 based on case data compiled by Macdonald from several previous studies [5]. Daily recovery rate with ACT (r_1) was conservatively assumed to be 1/14 according to antimalarial trials which measured gametocyte clearance from blood after treatment with MQ-AS [6-8] .

Susceptibility of *An. darlingi* was conservatively assumed to be 0.41 for *P. falciparum* based on published data for membrane-fed *An. darlingi* caught in Belize [9]. Considering an average annual temperature of 28°C for the Loreto region [10], the latent period for mosquito (*n*) (i.e. the sporogonic period) was estimated at 11 days for *P. falciparum* based on data from various experimental studies compiled and plotted against ambient temperature by

Macdonald [11]. While daily human mortality rate (*h*) was set to 0.00005 considering an expected lifespan of 50 years at the median age of the population [12]; daily mosquito mortality rate (*g*) was assumed to be 0.16, after its estimation from the average parous rate [13] of *An. darlingi* in the Upper-Maroni forest region of French Guiana [14].

Basic malaria models have usually assumed arbitrary values between 1 and 100 for the mosquito density *(m)* (15), while the human feeding rate *(a)* is estimated by dividing the human blood index (proportion of mosquito-blood meals obtained from humans) by the duration of gonotrophic cycle in time units [16]. Due to large variations in values for both parameters, these were estimated by fitting the parameterized model to the observed monthly incidence of symptomatic *P. falciparum* infections in 2004 in Ninarumi. Setting a constant mosquito density *(m)* to 75, allowed the model to estimate monthly values of the human feeding rate *(a)* between 0.035 to 0.32 bites on humans per *An. darlingi* per day; a range considered realistic considering previously reported data on human blood indices (between 0.15 and 0.72 [17, 18]), and gonotrophic cycle periods (between 2.19 and 4.4 days [19, 20]) for *An. darlingi* in the Brazilian Amazon. Therefore, the human feeding rate *(a)* was the only time-dependent parameter in the model which allowed for the assessment of the seasonal variations of malaria transmission.

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Text S5.2. R-code for the baseline model.

```
library(deSolve)

year<-20
tim<-360*year #time in days
time <- seq(0,tim,by=1)
es <- 1  # number of scenarios.  It is only one scenario: the baseline model.

#Parameters

m <- 75       #mosquito density
Nt <-500      #total human population
Mt<- Nt*m  #total mosquito population
b<- 0.05      #susceptibility of an individual to infection after being bitten by an infectious mosquito
c<- 0.41      #mosquito susceptibility to infection after biting an infectious individual
e<-0.98       #treatment effectiveness
y<-0.65      #fraction of symptomatic infections
r1<-14        #duration of infectious period in treated humans with ACT (in days)
r2<-200       #duration of infectious period in untreated humans in days (r2= r2a+r2b)
r2a<-80       #duration of presence of blood asexual parasites in untreated humans in days
r2b<-120      #duration of infectious period in untreated humans after clearance of blood asexual parasites in days

rr1<-1/r1; rr2<-1/r2; rr2a<-1/r2a; rr2b<-1/r2b

n<- 11        #duration of mosquito latent period in days
l<- 21        #duration of human latent period for P.falciparum in days (l= l1+l2+ne)
l1<-10        #duration from sporozoite inoculation to development of blood asexual parasites in days
l2<-10        #duration from asexual blood stage infection to development of gametocytes in days
rl2<-1/l2     #daily rate from appearance of blood asexual parasites to development of patent blood infection
ne<-1         #time spent in days (only one day) in a fictitious compartment called "New". "New" allows calculating daily incidence
rnew<-1/ne  #rate to enter/leave the fictitious compartment
h<- 1/(70*360)   #daily human mortality rate
g<-0.16           #daily mosquito mortality rate
v<-1           #duration of FSAT in days
vv<-1/v
cov<-1        #coverage of intervention
sm1<-0.5      #sensitivity of microscopy for detecting asexual parasites
sp1<-0.95     #sensitivity of PCR for detecting asexual parasites
sm2<-0.1      #sensitivity of microscopy for gametocytes
sp2<-0.8      #sensitivity of PCR for detecting gametocytes
```

```
# monthly values of the human feeding rate (expected number of bites on humans per
mosquito)
a<rep(c(rep(0.32,30),rep(0.19,30),rep(0.04732,30),rep(0.12,30),rep(0.07,30),rep(0.095,30),
rep(0.035,30), rep(0.035,30),rep(0.035,30),
rep(0.035,30),rep(0.035,30),rep(0.035,30)),year)
a.t<-c(0,a)
A.t<-function(i) a.t[i]

# Scenario: Only passive case detection (PCD)
# Timing for the intervention
t1<-array(0,c(es,length(time)))
t1[1,]<-c(0,rep(0,360*year))

#Initial population number
To<-4
I1o<-9
I2o<-7
NEWo<-0
E1o<-0
E2o<-0
So<-Nt-I1o-I2o-E1o-E2o-To-NEWo
Vo<-0.001*Mt
Lo<-0
Uo<-Mt-Vo-Lo

#For storage
TIME<- array(0,c(es,length(time)))
Sx<- array(0,c(es,length(time)))
E1x<- array(0,c(es,length(time)))
NEWx<-array(0,c(es,length(time)))
E2x<- array(0,c(es,length(time)))
Tx<- array(0,c(es,length(time)))
I1x<- array(0,c(es,length(time)))
I2x<- array(0,c(es,length(time)))
Ux<- array(0,c(es,length(time)))
Lx<- array(0,c(es,length(time)))
Vx<- array(0,c(es,length(time)))
Nx<- array(0,c(es,length(time)))
Mx<- array(0,c(es,length(time)))

ITm<-array(0,c(es,((length(time)-1)/30)))

#Loop for scenarios
for(j in 1:es)
{
 t1.t<-function(i) t1[j,][i]
```

```
#Function
mal1.dyn <- function(t,var,par) {

  #Setting the lag for mosquito latent period
  lagM <- t - n
  if (lagM <= 0)
    varlagM <- c(0,0,0,0,0,0,0,0,0,0)  #"n" past values of compartments before time 0
  else
    varlagM <- lagvalue(lagM)

  #Setting the lag for human latent period1
  lagH <- t - l1
  if (lagH <= 0)
    varlagH <- c(0,0,0,0,0,0,0,0,0,0)  #"l1" past values of compartments before time 0
  else
    varlagH <- lagvalue(lagH)

  #Setting the time dependent gonotrophic cycle for a mosquito
  a <- A.t(t+1)

  #Setting the lag for gonotrophic cycle for a mosquito
  if (lagM <= 0)
    a_lagM <- 0
  else
    a_lagM <- A.t(t+1-n)

  #Setting the lag for human latent period1
  if (lagH <= 0)
    a_lagH <- 0
  else
    a_lagH <- A.t(t+1-l1)

  # Rename the variables and parameters
  S<- var[1]
  E1<- var[2]
  NEW<- var[3]
  E2<- var[4]
  I1<- var[5]
  I2<- var[6]
  T<- var[7]
  U<-var[8]
  L<-var[9]
  V<- var[10]

  N<-S+E1+NEW+E2+NEW+I1+I2+T
  M<-U+L+V

  m <- par[1]
```

```
b <- par[2]
c<- par[3]
rr1<- par[4]
rr2a<- par[5]
rr2b<- par[6]
n<-par[7]
h<-par[8]
l1<-par[9]
l2<-par[10]
y<-par[11]
sm1<-par[12]
sp1<-par[13]
sm2<-par[14]
sp2<-par[15]
vv<-par[16]
g<-par[17]
e<-par[18]
cov<-par[19]
rnew<-par[20]
rl2<-par[21]

#Time dependent timing for the intervention
t1 <- t1.t(t+1)

# Rename Lags for mosquito and human latent period
S_lagM<-varlagM[1]
E1_lagM<-varlagM[2]
NEW_lagM<-varlagM[3]
E2_lagM<-varlagM[4]
I1_lagM<-varlagM[5]
I2_lagM<-varlagM[6]
T_lagM<-varlagM[7]
U_lagM<-varlagM[8]
L_lagM<-varlagM[9]
V_lagM<-varlagM[10]

S_lagH<-varlagH[1]
E1_lagH<-varlagH[2]
NEW_lagH<-varlagH[3]
E2_lagH<-varlagH[4]

I1_lagH<-varlagH[5]
I2_lagH<-varlagH[6]
T_lagH<-varlagH[7]
U_lagH<-varlagH[8]
L_lagH<-varlagH[9]
V_lagH<-varlagH[10]
```

```
  # Derivatives
  dS <- -m*a*b*(V/M)*S+rr1*T+rr2b*I2-h*S+h*(S+E2+E1+I1+I2+T)
  dE1 <-  m*a*b*(V/M)*S-m*(a_lagH)*b*(V_lagH/M)*S_lagH*exp(-l1*h)-h*E1
  dNEW <- m*(a_lagH)*b*(V_lagH/M)*S_lagH*exp(-l1*h)-NEW*rnew
  dE2 <-  NEW*rnew-rl2*E2-h*E2-(sm1+sp1)*t1*cov*vv*e*E2
  dI1 <-  rl2*E2*(1-y*e)-rr2a*I1-h*I1-(sm1+sp1)*t1*cov*vv*e*I1
  dI2 <-  I1*rr2a-rr2b*I2-h*I2-(sm2+sp2)*t1*cov*vv*e*I2
  dT <-   rl2*E2*(y*e)-rr1*T-
h*T+(sm1+sp1)*t1*cov*vv*e*E2+(sm1+sp1)*t1*cov*vv*e*I1+(sm2+sp2)*t1*cov*vv*e*
I2

  dU <- -a*c*((I1+I2+T)/N)*U+g*(V+L)
  dL <- a*c*((I1+I2+T)/N)*U-
(a_lagM)*c*((I1_lagM+I2_lagM+T_lagM)/N)*U_lagM*exp(-g*n)-g*L
  dV <- (a_lagM)*c*((I1_lagM+I2_lagM+T_lagM)/N)*U_lagM*exp(-g*n)-g*V

  list(c(dS,dE1,dNEW,dE2,dI1,dI2,dT,dU,dL,dV))}  # Return the 10 values

#Preparing the numerical solution
par <- c(m,b,c,rr1,rr2a,rr2b,n,h,l1,l2,y,sm1,sp1,sm2,sp2,vv,g,e,cov,rnew,rl2)
init <- c(So,E1o,NEWo,E2o,I1o,I2o,To,Uo,Lo,Vo)  #Initial population
time <- seq(0,tim,by=1)

#Numerical solution
mal1.sol <- dede(init,time,mal1.dyn,par)

#Relabeling the variables
TIME[j,]<- mal1.sol[,1]
Sx[j,]<- mal1.sol[,2]
E1x[j,] <- mal1.sol[,3]
NEWx[j,]<-mal1.sol[,4]
E2x[j,] <- mal1.sol[,5]
I1x[j,]<- mal1.sol[,6]
I2x[j,]<- mal1.sol[,7]
Tx[j,]<- mal1.sol[,8]
Ux[j,]<- mal1.sol[,9]
Lx[j,]<- mal1.sol[,10]
Vx[j,]<- mal1.sol[,11]
Nx[j,]<- Sx[j,]+E1x[j,]+E2x[j,]+NEWx[j,]+I1x[j,]+I2x[j,]+Tx[j,]
Mx[j,]<- Vx[j,]+Lx[j,]+Ux[j,]

#Monthly incidence calculation
for (x2 in 1:((length(time)-1)/30))
{
 ITm[j,x2]<-sum(mal1.sol[2:(length(time)),4][(30*(x2-1)+1):(30*x2)])
}
}
```

```
#Plots
# 1. Prevalence of compartments
windows()
par(mfrow=c(2,1), xaxs='i', yaxs='i')
plot(Sx[1,]/Nt,type='l',xlab='time',main=paste('Prevalence'), ylab='Human
population',col='blue',lwd=3,ylim=c(0,1))
lines((E1x[1,]+E1x[1,])/Nt, type='l',col='yellow', lwd=3)
lines((I1x[1,]+I2x[1,])/Nt, type='l',col='red', lwd=3)
lines(Tx[1,]/Nt, type='l',col='green', lwd=3)
legend("right", c("S","E","I","T"),
col=c("blue","yellow","red","green"),lty=c(1,1,1,1),inset=0.01, cex=0.5)

plot(Ux[1,]/Mt,type='l', xlab='time',ylab='Vector
population',col='green',lwd=3,ylim=c(0,1))
lines(Lx[1,]/Mt, type='l',col='purple', lwd=3)
lines(Vx[1,]/Mt, type='l',col='orange', lwd=3)
legend("right", c("U","L","V"), col=c("green","purple","orange"),lty=c(1,1,1),  inset=0.01,
cex=0.5)


###Figure 2...exported to TIFF file

##2.  Prevalence and incidence by P. falciparum only 3 years
tiff(file = "PCDBaselineMAR1.tiff", width = 360*6, height = 360*6, units = "px", pointsize
=6, res = 360)
par(mfrow=c(2,1))
par(mar=c(4,4.5,1,1))


# Figure 2A Incidence
observed<-rep(c(7,32,15,7,11,8,6,1,1,1,1,1),3)  # Observed monthly incidence in Ninarumi

ax1<-seq(1,3*12,3)
ay1<-c("Y1 Jan","Apr","Jul","Oct","Y2 Jan","Apr","Jul","Oct","Y3
Jan","Apr","Jul","Oct")
leg<-c("Model-predicted overall incidence             ","Model-predicted symptomatic
incidence            ",
      "Observed symptomatic incidence")

INC<-ITm[1,(12*10+1):(12*(year-7))]

par(mar=c(4,4.5,1,1))
plot(INC*y, type="l",lty=1,lwd=2, col="blue", xaxt="n", xlab="", ylab="P.falciparum
monthly incidence", ylim=c(0,max(INC*1.23)),cex=2)
text(1, max(INC*1.2),"A", cex=2, col="black",  font=2)
lines(ITm[1,(12*10+1):(12*(year-7))],type="l", lty=1, lwd=2, col="dark gray")
lines(observed, type="l", lty=2, lwd=2, col="black")
```

```
legend(3.5,max(INC*1.24), leg, inset=0.004, cex = 1.1,lty = c(1,1,3), lwd=2, col=c("dark
gray","blue","black"), horiz=F, y.intersp=1, box.col="white",ncol=2)
axis(1, at=ax1,labels=ay1, las=2, cex.axis=0.8, tck=-.02)

# Figure 2B  Prevalence

ax1<-seq(1,3*360,13*7)
ay1<-c("Y1 Jan","Apr","Jul","Oct","Y2 Jan","Apr","Jul","Oct","Y3
Jan","Apr","Jul","Oct")

leg1<-c("Observed prevalence by microscopy","Model-predicted overall prevalence
","Model-predicted prevalence by microscopy")

leg2<-c("Model-predicted asymptomatic prevalence", "Model-predicted asymptomatic sub-
microscopic  prevalence",
      "Model-predicted symptomatic prevalence")

cu<-(360*10+1):(360*(year-7))

prevPCD<-(E2x[1,cu]+I1x[1,cu]+I2x[1,cu]+Tx[1,cu])/Nt

eo<-c(rep(NA,360),rep(NA,60),6.5/100,rep(NA,29+180),1.1/100,
rep(NA,89),rep(NA,360)) #Observed prevalence in Ninarumi

plot(prevPCD,type='l',xaxt="n", xlab="", lty=1, ylab='P.falciparum   prevalence',col='dark
gray',lwd=2,ylim=c(0,1.25*max(prevPCD)), cex=2)

text(0,1.23*max(prevPCD),"B", cex=2, col="black",  font=2)
lines((E2x[1,cu]*0.5+I1x[1,cu]*0.5+I2x[1,cu]*0.1+Tx[1,cu])/Nt, type='l',col='dark gray',
lty=2, lwd=2) #Microscopy prevalence
lines((E2x[1,cu]*0.35+I1x[1,cu]+I2x[1,cu])/Nt, type='l',col='red', lty=1, lwd=1.5)        #
Asymptomatic   prevalence
lines((E2x[1,cu]*0.35*0.5+I1x[1,cu]*0.5+I2x[1,cu]*0.9)/Nt, type='l',col='red', lty=2,
lwd=1.5) # Asymptomatic sub-microscopic  prevalence
lines((E2x[1,cu]*0.65+Tx[1,cu])/Nt, type='l',col='blue', lty=1, lwd=1.5) #Symptomatic
prevalence
#lines(NEWx[1,cu]/Nt, type='l',col='black', lwd=3.5)
points(1:(360*3),eo, pch ="*", bg = "black",cex=2)

legend(100,1.29*max(prevPCD), leg1, col=c("black","dark gray","dark
gray"),lty=c(NA,1,3), pch=c("*",NA,NA),
     inset=0.004, lwd=c(NA,2,2), cex=1.1, box.col="white",y.intersp=1, ncol=1)
legend(360*1.5,1.29*max(prevPCD), leg2, col=c("red","red","blue"),lty=c(1,3,1),
     inset=0.004, lwd=c(2,2,2), cex=1.1, box.col="white",y.intersp=1, ncol=1)
axis(1, at=ax1,labels=ay1, las=2, cex.axis=0.8, tck=-.02)
text(c(460,675),c(0.065,0.01),c(0.065,0.01),cex = 1)

dev.off()
```

SCIENTIFIC CURRICULUM VITAE



Angel Rosas-Aguirre is a medical doctor with substantial work experience managing malaria control projects in South America. Because of the importance of the evidence-based decision making, his professional career has always been continuously interwoven with teaching and research activities. His current research aims to understand malaria transmission dynamics in Peruvian endemic areas and to generate evidence for improving case detection and treatment strategies for the malaria control and elimination.

Positions and Employment

1999 -2001	Research Assistant, Universidad Peruana Cayetano Heredia (UPCH) – Faculty of Public Health and Administration (FPHA). Health Management Research Unit.
2000 – 2001	Medical assistant/ Medical Epidemiologist, Ministry of Health, Regional Direction of Pasco, Villa Rica Hospital.
2002 - 2006	Malaria Advisor, VIGIA Project "Addressing the Threats of Emerging and Re-emerging Infectious Diseases", Ministry of Health/ United States Agency for International Development (MOH/USAID), Lima-Peru.
2007	Malaria Advisor for "Diagnosis and design of Entomological Surveillance and Vector Control Systems in Peru" consulting, Ministry of Health, Lima-Peru.
2006 - 2011	Multi-Country Project Technical Coordinator, Global Fund Multi-Country Malaria Project "Malaria control on the cross border areas of the Andean Region: A community based approach"-PAMAFRO/ Organismo Andino de Salud- Convenio Hipolito Unanue (ORAS-CONHU), Lima-Peru.
2009 - 2011	Tutor, Diploma in Health and Local Development, Panamerican Health Organization (PAHO), Washington (US)/Lima (Peru).

2011	Academic coordinator, International Diploma in malaria control, UPCH/MCDI/Marathon Oil, Lima-Peru.
2011 - 2012	Coordinator, Course Control of malaria and other vector transmitted diseases, Master in Control of Tropical Infectious Diseases,UPCH Lima-Peru.
2012 - 2013	Principal Consultant/Advisor "Assessment of the diagnosis and treatment in areas of low malaria transmission in Peru", Systems for Improved Access to Pharmaceuticals and Services (SIAPS)- Management Sciences for Health (MSH), Washington DC.
2006 - now	Associated Researcher, Malaria Research Group, Institute of Tropical Medicine Alexander von Humboldt, UPCH, Lima-Peru.

Honors

2000	Sixth (6th) of 121 students at Medical School. Universidad Peruana Cayetano Heredia. Lima-Peru, 2000.
2006	Grant for research project from Macro International Inc./USAID – Peruvian National Institute of Statistical and Informatics
2007	Third (3rd) place in Induquimica Prize Award 2007. "Validity of Parascreen® as malaria rapid diagnostic test". X Meeting of Peruvian Society of Tropical and Infectious Diseases (PSTID).
2007	Fifth (5th) place in Induquimica Prize Award 2007. "Associated factors to homes with malaria during the last year in communities of Loreto Region-Peru". X Meeting of PSTID.
2007	Travel grant for Regional Meeting of Health and Local Development. PAHO.
2009	Travel grant for Vivax Malaria Research III 2009 and Beyond Conference. USF Health, Gates Foundation, Welcome Trust.
2009	Scholarship for attending Diploma in "Quantitative Risk Analysis" Belgian Technical Cooperation (BTC). Institute of Tropical Medicine (ITM), Antwerp, Belgium. Sep-Dec 2009.
2009	Malaria Champion of the Americas" award to PAMAFRO project from PAHO.
2011	Scholarship for PhD studies. BTC. ITM Antwerp - Université catholique de Louvain, Antwerp-Brussels, Belgium. January 2011 – June 2015.
2013	Scholarship for attending "Mathematical Models for Infectious Disease Dynamics" course. The Welcome Trust. Cambridge, UK. February 2013.
2013	Scholarship for "Science of Eradication: Malaria leadership development course" participation. ISGlobal/Barcelona Institute for Global Health/ Swiss Tropical & Public Health Institute/ Harvard School of Public Health Barcelona, Spain. May 2013.

Contribution to Science

1. His publications are mainly intended to understand the malaria transmission in Peruvian endemic areas and to generate evidence for the design and implementation of malaria control strategies, particularly: preventive and malaria case detection and treatment strategies in the perspective of low transmission. Many study findings have been used for the design of new research studies and for adjusting malaria control strategies at regional and national level. He served as the primary investigator or co-investigator in all of these studies.

a. Rosas-Aguirre Á, Gamboa D, Rodriguez H, Llanos-Zavalaga F, Aguirre K, Llanos-Cuentas A. [Use of standardized blood smear slide sets for competency assessment in the malaria microscopic diagnosis in the Peruvian Amazon]. Rev Peru Med Exp Salud Publica. 2010 Oct-Dec;27(4):540-7.
b. Rosas-Aguirre A, Guzmán-Guzmán M, Moreno-Gutierrez D, Rodriguez-Ferrucci H, Vargas-Pacherrez D, Acuña-González Y. [Long-lasting insecticide - treated bednet ownership, retention and usage one year after their distribution in Loreto, Peru]. Rev Peru Med Exp Salud Publica. 2011 Jun;28(2):228-36
c. Arevalo-Herrera M, Quiñones ML, Guerra C, Céspedes N, Giron S, Ahumada M, Piñeros JG, Padilla N, Terrientes Z, Rosas A, Padilla JC, Escalante AA, Beier JC, Herrera S. Malaria in selected non-Amazonian countries of Latin America. Acta Trop. 2012 Mar;121(3):303-14.
d. Grietens KP, Muela Ribera J, Soto V, Tenorio A, Hoibak S, Aguirre AR, Toomer E, Rodriguez H, Llanos Cuentas A, D'Alessandro U, Gamboa D, Erhart A. Traditional nets interfere with the uptake of long-lasting insecticidal nets in the Peruvian Amazon: the relevance of net preference for achieving high coverage and use. PLoS One. 2013;8(1):e50294.
e. Rosas-Aguirre A, Llanos-Cuentas A, Speybroeck N, Cook J, Contreras-Mancilla J, Soto V, Gamboa D, Pozo E, Ponce OJ, Pereira MO, Soares IS, Theisen M, D'Alessandro U, Erhart A. Assessing malaria transmission in a low endemicity area of north-western Peru. Malar J. 2013 Sep 22;12:339.
f. Delgado-Ratto C, Soto-Calle VE, Van den Eede P, Gamboa D, Rosas A, Abatih EN, Rodriguez Ferrucci H, Llanos-Cuentas A, Van Geertruyden JP, Erhart A, D'Alessandro U. Population structure and spatio-temporal transmission dynamics of *Plasmodium vivax* after radical cure treatment in a rural village of the Peruvian Amazon. Malar J. 2014 Jan 6;13:8.
g. Rosas-Aguirre A, Ponce OJ, Carrasco-Escobar G, Speybroeck N, Contreras-Mancilla J, Gamboa D, Pozo E, Herrera S, Llanos-Cuentas A. *Plasmodium vivax* malaria at households: spatial clustering and risk factors in a low endemicity urban area of the northwestern Peruvian coast. Malar J. 2015 Apr 24;14:176.
h. Rosas-Aguirre A, Erhart A, Llanos-Cuentas A, Branch O, Berkvens D, Abatih E, Lambert P, Frasso G, Rodriguez H, Gamboa D, Sihuincha M, Rosanas-Urgell A, D'Alessandro U, Speybroeck N. Modelling the potential of focal screening and treatment as elimination strategy for *Plasmodium falciparum* malaria in the Peruvian Amazon Region. Parasit Vectors. 2015 May 7;8:261.

2. In addition to the contributions related to malaria case detection interventions described above, he participated in scientific and operative studies aimed to generate evidence to support the value of community-based malaria diagnosis using malaria rapid diagnostic tests (RDTs). Studies assessed the performance of the diagnostic test for use with local parasite strains (including the first report of low sensitivity of one HRP2 based-RDT to detect P. falciparum in Peru), documented the delays caused by microscopy and the time saved by RDT use, and demonstrated that promotores (volunteers community agents with limited training) could use the tests safely and effectively. Then, a cost-effectiveness study strongly suggested that RDT use would generate savings for Ministry of Health by greatly reducing complicated cases and reducing overtreatment in Amazonian communities with limited access to health facilities.

a. Llanos F, Huayta E, Mendoza D, Rosas A, Contreras C, et al. [Knowledge and perceptions of health workers about the malaria rapid diagnostic test Parasight in a malaria endemic area of Peru]. Revista Medica Herediana. 2000; 11(4):115-21.
b. Casapía M, Vásquez L, Rosas A, Pinedo-Ríos N, Cabezas C, et al. [Improvements in timely malaria diagnosis and appropriate therapy with the use of rapid tests by health promoters in Peruvian Amazon Region]. Revista peruana de medicina experimental y salud pública. 2008; 25(4):540-47
c. Rosas Aguirre AM, Llanos Zavalaga LF, Trelles de Belaunde M. [Cost-effectiveness ratio of using rapid tests for malaria diagnosis in the Peruvian Amazon]. Rev Panam Salud Publica. 2009 May;25(5):377-88.
d. Bendezu J, Rosas A, Grande T, Rodriguez H, Llanos-Cuentas A, Escobedo J, Gamboa D. Field evaluation of a rapid diagnostic test (Parascreen) for malaria diagnosis in the Peruvian Amazon. Malar J. 2010 Jun 7;9:154.
e. Mendoza NM, Rosas AM, Burgos JD. [Evaluation of rapid diagnostic tests for malaria in Colombia as an integral part of the disease control strategy]. Biomedica. 2011 Mar;31(1):55-69.

3. However the interest in research whose results can be directly applied for improving health policies and programs was not only limited to the malaria control. He has also participated in evaluations that have allowed the adjustment of important public social and health programs in Peru, such as: maternal and child care program, and food and nutrition program.

a. Rojas C, Montes C, Segura L, Rosas A, Llanos-Zavalaga F, et al. [Approach to the effect of a School Breakfast Program on the intellectual performance of children in elementary and primary schools of Peru]. Revista peruana de medicina experimental y salud pública. 2003; 20(1):31-38.
b. Mispireta M, Rosas A, Velásquez J, Lescano A, Claudio L. [Nutrition transition in Peru, 1991-2005]. Revista peruana de medicina experimental y salud pública. 2007; 24(2):129-35.
c. Velásquez Hurtado JE, Kusunoki Fuero L, Paredes Quiliche TG, Hurtado La Rosa R, Rosas Aguirre ÁM, Vigo Valdez WE. [Neonatal mortality, analysis of surveillance

registers, and neonatal medical histories of 2011 in Huanuco and Ucayali, Peru]. Rev Peru Med Exp Salud Publica. 2014 Apr;31(2):228-36.

d. Velásquez Hurtado JE, Solís Alcedo L, Vigo Valdez WE, Rosas Aguirre AM, Giusti Hundskopf P, Alfaro Fernandez P, Cabrera Arredondo H. [Evaluating maternal child care practices in extreme poverty areas in Peru, 2012]. Rev Peru Med Exp Salud Publica. 2014 Apr;31(2):243-53.

Complete List of Published Work in:

http://www.ncbi.nlm.nih.gov/sites/myncbi/1NgQ8_C1fMSAn/bibliography/48324733/public/?sort=date&direction=ascending

Ongoing Research Support

Project:	Latin American Center for Malaria Research and Control. ICERM
Role:	Co-investigator
Period:	2010-2017
Agency:	NIH – USA
Objective:	To generate knowledge and provide technical and scientific support to governments to strengthen malaria control measures and to facilitate malaria elimination programs in the region
Project:	Research Circle "Towards the elimination of malaria in Peru"
Role:	Principal investigator in two of the four project studies
Period:	2014-2018
Agency:	Peruvian National Council for Science and Technology (CONCYTEC)
Objective:	Generate solid scientific evidence about strategies of detection and treatment of asymptomatic malaria infections, in order to support the decision-making in the malaria control and to facilitate the way to its elimination in the Peruvian Amazon.
Project:	Strengthening the diagnosis, treatment and surveillance strategies for the malaria control and elimination in the Peruvian Amazon
Role:	Local principal investigator
Period:	2014-2019
Agency:	L'Académie Belge de Recherche et d'Enseignement supérieur-Commission de la Coopération au Développement (ARES-CCD)
Objective:	To strengthen local capacity for malaria diagnosis, treatment, surveillance and research in the Peruvian Amazon Region.