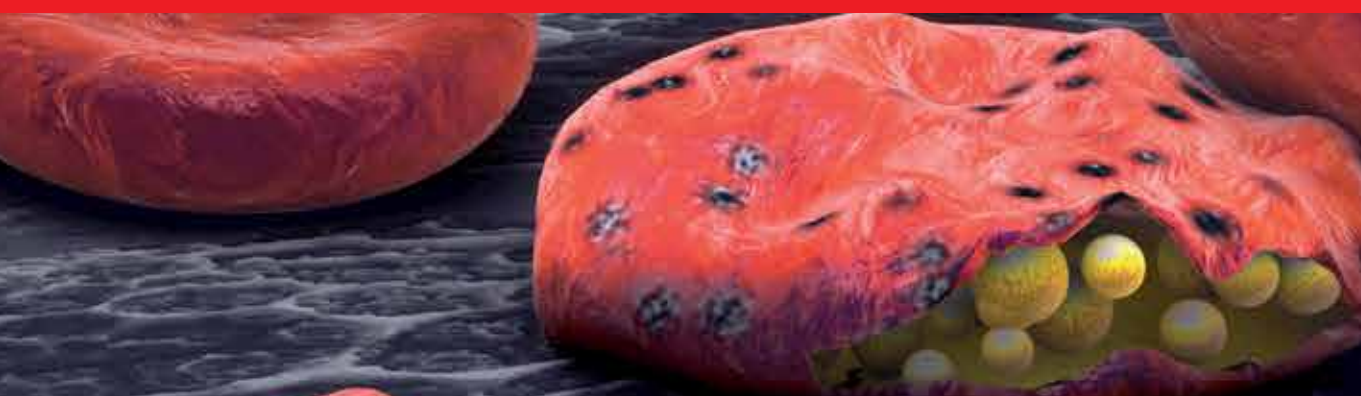
IntechOpen

Towards Malaria Elimination

A Leap Forward

Edited by Sylvie Manguin and Vas Dev



TOWARDS MALARIA ELIMINATION - A LEAP FORWARD

Edited by **Sylvie Manguin** and **Vas Dev**

Towards Malaria Elimination - A Leap Forward
http://dx.doi.org/10.5772/intechopen.69750
Edited by Sylvie Manguin and Vas Dev

Contributors

John Beier, Andre Wilke, Giovanni Benelli, Kinley Wangdi, Archie Clements, Charles Wondji, Jacob Riveron, Magellan Tchouakui, Leon Mugenzi, Benjamin Menze, Mu-Chun Chiang, Evangelia - Theophano Piperaki, Jan Conn, Maria Anice Mureb Sallum, Aung Pyae Phyo, Francois Nosten, Umberto D'Alessandro, Arantxa Roca-Feltrer, Jeffrey Hii, Indra Vythilingam, Chris Drakeley, Bronner Goncalves, Hannah Slater, Lucy Okell, Laurent Renia, Yun Shan Goh, Katian Peng, Marjorie Mauduit, Georges Snounou, Puji Budi Setia Asih, Kevin Baird, Din Syafruddin, Gerry Killeen, Liwang Cui, Yaming Cao, Jaranit Kaewkungwal, Amnat Khamsiriwatchara, Saranath Lawpoolsri, Than Naing Soe, Myat Phone Kyaw, Jetsumon Sattabongkot, Colin Ohrt, Thang Duc Ngo, Thieu Quang Nguyen, Ogobara Keguerem Doumbo, Shuisen Zhou, Shaosen Zhang, Li Zhang, Jun Feng, Jianhai Yin, Xinyu Feng, Zhigui Xia, Roger Frutos, Sylvie Manguin, Vas Dev, Sunil Dhiman, Vijay Veer

First published in London, United Kingdom, 2018 by IntechOpen
eBook (PDF) Published by IntechOpen, 2019
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, The Shard, 25th floor, 32 London Bridge Street
London, SE19SG – United Kingdom
Printed in Croatia

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Additional hard and PDF copies can be obtained from orders@intechopen.com

Towards Malaria Elimination - A Leap Forward
Edited by Sylvie Manguin and Vas Dev

p. cm.

Print ISBN 978-1-78923-550-0

Online ISBN 978-1-78923-551-7

eBook (PDF) ISBN 978-1-83881-413-7

Meet the editors

Sylvie Manguin is a full Research Professor at the UMR-HSM, Institute of Research for Development (IRD), based at the University of Montpellier, France. She is a leading medical entomologist, academician and researcher whose main interest concerns mosquitoes and vector-borne diseases such as malaria and dengue. With strong collaborations in the South, she has developed research studies on *Anopheles* mosquitoes from three continents (Asia, Africa and Americas) in the fields of molecular species identification, population genetics, phylogenetics, vectorial capacities, spatial surveillance, midgut microbiota biodiversity, salivary immunological markers and vector control approaches. She is teaching medical entomology at the University of Montpellier (UM, France), Kasetsart University (KU, Thailand) and Gadjah Mada University (UGM, Indonesia). She is the author of more than 90 indexed publications, 6 book chapters and 3 books including "*Anopheles Mosquitoes: New Insights into Malaria Vectors*" for which she is the editor (InTechOpen Access) and "*Biodiversity of Malaria in the World*" (John Libbey Ed.), respectively, published in 2013 and 2008. She is also the secretary-general of the International Federation of Tropical Medicine (IFTM), http://www.iftm-hp.org/board.html, a member of the editorial boards of the Malaria Journal and Acta Tropica, and serves as reviewer in several international institutions and in more than 20 scientific journals. In 2018, she has been appointed by the National Agency for Sanitary Security of Food, Environment and Work (ANSES, France) as an expert in the working group on 'Vectors'.

Vas Dev has superannuated from the National Institute of Malaria Research, New Delhi - a premier Indian research organization for malaria research, and served as Officer-in-Charge of one of its Field Stations based in Assam, north-eastern region of India, for over 25 years. He was actively involved in operational research for evaluation of newer technologies for vector control, routine surveillance of antimalarial medicines for treatment, diagnostics and human resource development. His primary research interests are in vector biology with special reference to epidemiology and control of malaria and other vector-borne diseases. His research efforts have culminated in number of technologies, viz., long-lasting insecticidal nets for vector control, artemisinin-based combination therapy for treatment of drug-resistant malaria, rapid diagnostic test kits that all have been incorporated for benefit of state healthcare services of north-eastern states, major public and private sectors and defence establishments alike resulting in substantial disease transmission reduction. He has over 150 research publications in peer-reviewed journals and is a serving member of the editorial board of many scientific periodicals. Dr. Dev is recipient of several coveted fellowships, awards and distinctions in his field of research, and is currently an active member of learned societies.

Contents

Reminiscing Professor Ogobara K. Doumbo
(1956-2018)

Professor Doumbo, a renowned scientist in Tropical Medicine and Parasitic Diseases, more specifically malaria, died on June 9th, 2018 of complications of surgery. 'Ogo', as we used to call him, was a talented, inspiring, kind and brilliant man from the famous Dogon region of Mali, West Africa. He graduated with degree in Medicine and served as surgeon in remote and poor areas of Mali. In 1992, he founded the Malaria Research and Training Center (MRCT) in Bamako (the capital city of Mali) where he built a strong network of collaborations especially with scientists from the USA and France. Ogo was a researcher 'par excellence' bestowed with several national and international awards and fellowships of prestigious academies. In May 2017, he accepted with enthusiasm our invitation to participate in this book for contributing a chapter entitled "*Malaria transmission-blocking vaccines: Present status and future perspectives*" keeping us abreast with the latest in this discipline of research. It is with an immense grief and sorrow that we learned about his demise and wish to express our deepest condolences to bereaved family and his close associates. In fond memories of this departed soul, Prof Sylvie Manguin and Dr Vas Dev dedicate this book to Professor Ogobara Doumbo for his great acumen for research and services in sharing knowledge and expertise, which would continue to inspire future generations of scientists in service to mankind.

Foreword

E (for elimination) = MC2 (for malaria control squared)

'Before the role of anophelines in the spread of malaria was known, efforts to control the disease were sporadic, infrequent and insignificant' [1].

While malaria control is at crossroads [2], the book edited by Prof. Sylvie Manguin and Dr. Vas Dev is an important landmark in the current process of global malaria elimination. It is the first book that highlights so accurately the current situation and trends of malaria in Southeast Asia (SE Asia) and South America. It should be kept in mind that in some ways, the current malaria situation in SE Asia foreshadows what could be the situation in Africa South of the Sahara (SS), within the foreseeable future, given the major threat of spread of artemisinin resistance and consequent increase of cases. Then, a long-term scenario will possibly be the decrease of transmission (and immunity), outdoor transmission, localized vectors, malaria in adults, etc. Therefore, the research and proposals developed in SE Asia are of paramount importance to circumvent wrong track and implement efficient adapted Malaria Control Programs in Africa SS countries.

While comparing a former global geographic distribution of malaria in the 1960s (Figure 1) and the recently published CDC map (Figure 2), the following points clearly emerged:

Malaria disappeared from Europe (as thoroughly presented in a specific chapter of this book) and all countries of the northern hemisphere.

Malaria almost disappeared or is in control/elimination phase in several countries of SE Asia and South America despite great concerns (artemisinin-resistance, outdoor transmission, insecticide resistance, political instability, poverty, etc.), as clearly demonstrated in several chapters of this book, and malaria was recently eliminated from all north African countries after decades of various control interventions including environmental management.

Malaria is still present and vivid in almost all countries of Africa SS.

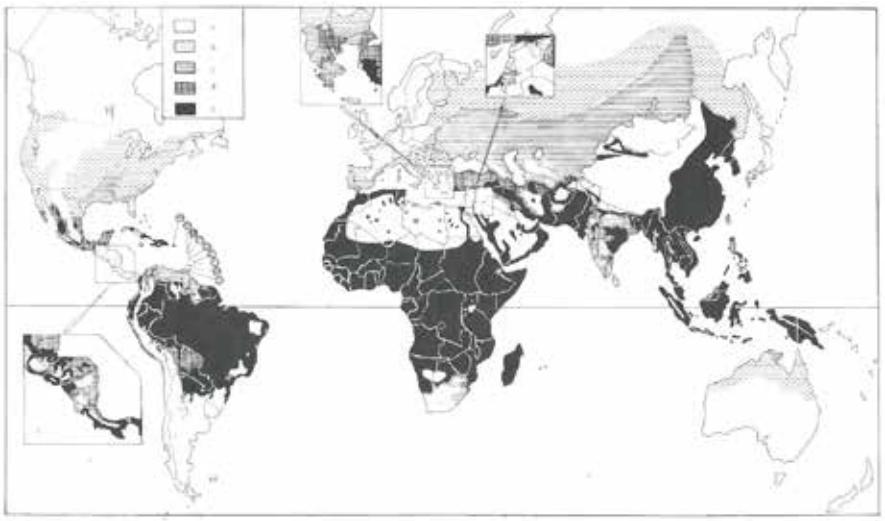


Fig. 35.13 Global geographic structure of malaria (Lysenko et al 1968): (a) originally malaria-free; (b) malaria disappeared by 1950 without specific programme; (c) malaria eradicated by specific programme after 1950; (d) areas in consolidation phase of malaria eradication; (e) areas in attack phase or without eradication programme. Redrawn by L P Arsenyeva, M A Shakhova and E P Sokolova

Figure 1 Global geographic structure of malaria in the late 1960s: (a) originally malaria-free, (b) malaria disappeared in 1950 without specific programme, (c) malaria eradicated by specific programme after 1950, (d) areas in consolidation phase of malaria eradication and (e) areas in attack phase or without eradication programme (Redrawn by L.P. Arsenyeva, M.A. Shakhova and E.P. Sokolova [3]).

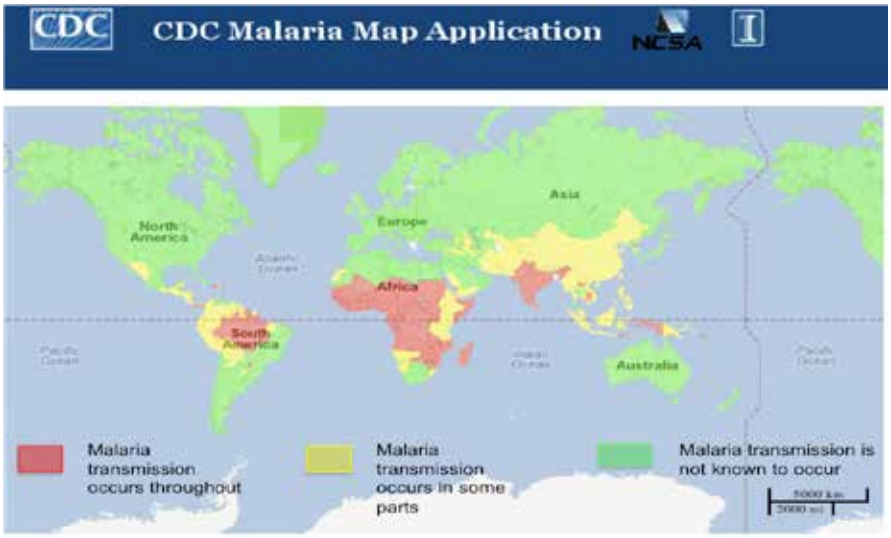


Figure 2. CDC Malaria distribution map (CDC, 2018).

According to the last WHO Malaria Report [2], 18 countries reached malaria elimination since 2000 including Algeria (2016), Argentina (2013), Armenia (2011), Azerbaijan (2015), Egypt (2000), Georgia (2012), Iraq (2011), Kazakhstan (2004), Kyrgyzstan (2013), Morocco (2007), Oman (2004), Paraguay (2014), Sri Lanka (2015), Syrian Arab Republic (2007), Turkey (2012), Turkmenistan (2009), the United Arab Emirates (2000) and Uzbekistan (2013).

Not a single country of Africa SS is in this list, although concentrating some 90% of the burden of malaria in terms of morbidity and mortality and with a transmission of great intensity.

Therefore, the success and issues of malaria elimination in the 'green countries' of the map (Figure 2) must be used as lessons.

Malaria control in Africa SS is confronted with three main issues: biological, financial and structural, and social.

Biological: in Africa SS, the most efficient vectors such as *An. gambiae s.l.* and *An. funestus*, along with several other local vector species, are present, allowing permanent and high-intensity transmission with great ecological and socio-economic biodiversity. The pyrethroid resistance, added to previously well-described insecticide resistance, is of major concern, while scaling up of LLIN is in process, which induced the striking reduction of malaria observed this past decade. In addition, the most predominant *Plasmodium* species, *P. falciparum*, the deadliest one, is already resistant to commonly available drugs posing great concern that artemisinin-resistant strains, present in the Mekong Basin, arrive and spread all over Africa SS, like what happened with the amino-4-quinoleins drugs.

Financial and structural: due to the well-known issues of Peripheral Health Centers (PHCs), which often includes lack of data reliability [4, 5], their coverage and actual efficiency to diagnose and correctly treat malaria [6]. PHCs are doing passive case detection (PCD) only, and it is clear that there is a need to know what happens at the community level, and for that, active case detection (ACD) must be done. It is possible to combine PCD and ACD in developing mobile teams going and surveying populations on a regular basis, doing 'on-the-spot' some diagnoses using rapid detection tests (RDT) and giving first-line adequate treatment. According to a recent study of the Malaria Atlas Project dealing with population coverage of artemisinin-based combination therapy (ACT) based on survey data of 2003 and 2015, the coverage in ACT for children under 5 years old increased, but in 2015, only 19.5% of these children were likely to receive treatment. The likelihood of treatment was lower in poorer, rural populations. Indeed, the Africa Malaria Report [7] underlined that 'poor people are at increased risk of becoming infected with malaria and getting infected more frequently'. Child mortality rates are known to be higher in poorer households, and malaria is responsible for a substantial proportion of these deaths.

Social: a special accent must clearly be given to education, as well as wide campaign of information, communication and involvement of the communities, along with mass distribution of long-lasting impregnated nets (LLIN) with follow-up to avoid as much as possible their often-noticed misusage.

Nevertheless, since 2000, due to the implementation of considerable increased support and concerted integrated campaign, malaria in Africa SS

halved. It was estimated that 663 million of clinical cases have been averted, and the result is largely attributable to the use of insecticide-treated nets (ITN) (68%), while ACT is responsible for 22% and insecticide residual spraying (IRS) for 10%. Thus, vector control contributed to some three-fourths of malaria cases averted!

From the experience and issues of malaria control in SE Asia and South America, as they are well described in this book, it clearly appeared that malaria control in Africa SS must be based upon:

Research and development (R&D) of innovative tools including new drugs, novel insecticides, new vector control approaches [8], operational vaccines, improved housing, wide use of immunological markers to better evaluate the efficacy of the control measures [9] and intensified surveillance using spatial technologies. For example, it was recently demonstrated that modern houses with metal roof and finished walls are associated with >9% reduction in the odds of malaria in children of Africa SS when compared to traditional thatched houses [10]. Malaria elimination will need the implementation of integrated control management with better use of tools already available (LLIN, IRS and ACT) and new ones in development (as described in this book).

Special emphasis on education (remote learning system) and information-communication, thanks to recently developed media, smartphones, widely available even at the village level.

Strengthening health structures at peripheral, median and central levels to get accurate data on the current situation and their evolution with malaria control activities, along with improved diagnosis and malaria treatment but also of other infectious diseases so often not diagnosed and not rightly cured in due time.

Actual full involvement of all, from communities to the central level, national, international and non-governmental organizations (NGO).

Secure sustainable support at medium and long-term basis.

Actually, as it was recently well underlined, 'we must remain vigilant otherwise the striking improvement observed this last decade could be a bright interval, if financial, logistic and training support are not maintained as they have to be' [11]. Also, in the last Malaria Report [2], the WHO rings the alarm bell recognizing that 'after an unprecedented period of success in global malaria control, progress has stalled' in 2016 with even an increase (of 5 millions) of malaria cases over 2015 and deaths reaching 445,000 cases, a similar number with the previous year.

Nevertheless, this book *Towards Malaria Elimination*: *A Leap Forward* is an excellent encouragement for malaria programme managers, revealing the malaria situations and the effectiveness of the methods implemented in SE Asia and South America, thus paving the way to what needs to be imple-

mented in Africa SS for a progressive but real elimination of malaria on this continent, as it has been possible to obtain elsewhere.

Pierre Carnevale, PhD, DRCE, Full Research Professor
Formerly with the Institute of Research for Development (IRD)
Portiragnes, France
Correspondence to: pjcarnevale2001@yahoo.fr

References

[1] Boyd MF: Historical Review. Malariology, MF Boyd ed. The WB Saunders Co, Philadelphia, Pa. 1949; 3-25.
[2] WHO: World Malaria Report. World Health Organization, Geneva, Switzerland. 2017;196.
[3] Lysenko A, Semashko I, Fonareva K: The structure of modern world of malaria (map). Manual of microbiology, clinical aspects and epidemiology of infectious diseases, Moscow. 1968.
[4] Besnard P, Foumane V, Le Mire J, Foucher J-F, Chilombo M, Fortes F, et al.: Surnotification du paludisme, l'exemple des consultations dans les Centres de Santé de Lobito, Angola. Sci Med Afr. 2009;1:53-59.
[5] Manguin S, Foumane V, Besnard P, Fortes F, Carnevale P: Malaria overdiagnosis and subsequent overconsumption of antimalarial drugs in Angola: Consequences and effects on human health. Acta Trop. 2017;171:58-63.
[6] Plucinski M, Ferreira M, Ferreira C, Burns J, Gaparayi P, João L, et al.: Evaluating malaria case management at public health facilities in two provinces in Angola. Malar J. 2017;16:186.
[7] WHO-Unicef: The Africa Malaria Report 2003. In: WHO-Unicef, editor. 2003. p. 120.
[8] Duchet C, Allan R, Carnevale P: Vector Control: Some New Paradigms and Approaches in *Anopheles* mosquitoes - New Insights into Malaria Vectors, Edited by S. Manguin, Intech Open Access. 2013; Chapter 22:705-753.
[9] Brosseau L, Drame P, Besnard P, Toto J, Foumane V, Le Mire J, et al.: Human antibody response to *Anopheles* saliva for comparing the efficacy of three malaria vector control methods in Balombo, Angola. PLoS One. 2012;7:e44189.
[10] Tusting L, Bottomley C, Gibson H, Kleinschmidt I, Tatem A, Lindsay S, et al.: Housing Improvements and Malaria Risk in Sub-Saharan Africa: A Multi-Country Analysis of Survey Data. PLoS Med. 2017; 14(2):e1002234.
[11] Gentilini M, Caumes E, Danis M, Richard-Lenoble D, Bégué P, Touze J, et al.: Paludisme. Médecine Tropicale, 6th Edition, Médecine Sciences Publications, 2012; Chapter 10:191-231.

Preface

Malaria, even though preventable and curable, continues to inflict insurmountable morbidity particularly in resource-poor countries affecting equitable socio-economic development. Over the past few decades, a lot of new information has been generated on disease distribution and determinants in understanding parasite biology and vector bionomics aided by molecular tools [1,2], but what ails mankind is the proper utilization of study results and equity in healthcare services in place and time, as well as lack of unified intercountry strategies to contain the disease spread. *Plasmodium* parasites and their vectors are continuously evolving in the changing disease epidemiology landscape challenging the human endeavours to conquer malaria. Human population with the expanding growth of "after population" movements are also increasingly functioning as 'vectors' of the disease. Human malaria parasites are getting multi-resistant to available armamentarium and so do mosquito vector species to insecticides establishing outdoor transmission hard to control, evading exposure. However, at the turn of century, the advent of new intervention tools including Noble prize–winning discovery of artemisinin by Tu Youyou for treatment of malaria, combined with large-scale implementation of insecticide-treated netting materials for vector containment, has once again renewed the optimism of malaria elimination globally. With large-scale funding from international agencies/philanthropists for 'universal coverage' of these intervention tools, malaria map is shrinking with more than 35 countries certified to be malaria-free and another 21 countries that are likely to reach zero indigenous transmission (categorized by WHO as E-2020) set to be declared malaria-free by 2020 [3,4]. Many more countries are moving forward from control to elimination, and given the continuing declining trends of malaria transmission, WHO has given a clarion call for 'Malaria-Free World by 2030' [5].

Nevertheless, the path to malaria elimination seems to be an uphill task given the constraints and myriad of challenges. After a decade of decrease of malaria cases and deaths in all regions of the world due to roll-out of core interventions, including wide-scale use of long-lasting insecticidal nets and improved treatments, WHO expressed the concern that malaria control is stalling and could reverse the gains due to insufficient funding and deficiency of political will [3]. However, a number of bodies and alliances have come forward, viz., Malaria Eradication Scientific Alliance (MESA), Malaria Eradication Research Agenda (malERA), Multilateral Initiative on Malaria (MIM), Asia Pacific Leaders Malaria Alliance (APLMA), Asia-Pacific Malaria Elimination Network (APMEN) and Regional Malaria Elimination Initiative (RMEI), involved at different scales, for shared experiences and to promote coordinated actions for decisive attack to 'kill malaria'. In addition, after decades of the use of routine vector control tools based on insecticides, scientists are now deploying new innovative approaches that are environment-friendly to be used in integrated control management that would include chemotherapy, vector control, manipulation of environmental and ecological characteristics, and vaccination. This book is one little step forward to bring together experiences of 67 malaria experts from 5 continents to present updated information

on disease epidemiology and control at the national/regional level, highlighting the constraints, challenges, accomplishments and prospects in achieving the cherished goal of elimination. It is a compilation of 17 chapters, and what evolved from these contributions is that among array of issues specific to the region, most share common denominators such as (i) emerging multidrug-resistant malaria and pandemic risk, (ii) cross-border malaria, (iii) asymptomatic sea of parasite reservoir, (iv) surfacing of *Plasmodium vivax* in the disappearing phase, (v) insecticide resistance in *Anopheles* vectors and outdoor malaria transmission and (vi) emergence of the fifth human malaria parasite, *P. knowlesi*, making inroads in Southeast Asia; all of which call for sustained research to develop more robust and innovative tools in addressing these issues, which continue to thwart the elimination efforts.

The disease remains geographically entrenched in low-socioeconomic population groups living in poverty and/or regions under political instability across tropical countries little aware of disease prevention and cure or with no access to medical care due to chaotic economic situation. Southeast Asia is only second to African countries (South of Sahara), which contribute 90% of cases; together, they account for 97% of the malaria global burden. It is the Southeast Region, considered the epicentre of emerging drug-resistant strains, for which we believe that larger share of global investments in this part of the world would yield rich dividend in preventing next pandemic in Africa. Currently, the funding gap is too wide (less than half of what is required) between demand and supply in strengthening healthcare services ensuring equity in access to prevention and treatment achieving 'universal coverage' [6]. Even more important are the information, education and communication activities for keeping informed the stakeholders and the beneficiaries alike for enhanced compliance and concerted action against the age-old scourge. No one should get sick of malaria and die in this age of information dissemination and high technologies, and everyone has the right to access for prevention and treatment. In keeping pace with the malaria elimination efforts, it is the opportune time for national governments and international donors alike for firm and sustained commitment to increased allocation of resources for 'universal health coverage (UHC)' of core interventions ensuring that everyone, everywhere, can access essential quality health services without facing financial hardship (Dr. Tedros Adhanom Ghebreyesus, World Health Day 2018). Together, we can beat malaria and create the enabling environment to overcome the dogma of '*business as usual'* rather making it '*business unusual'* accelerating towards malaria elimination [6]. We strongly believe that 'malaria elimination' will be a big leap forward of this millennium in service to the mankind.

It is projected that this document would inspire, motivate and guide the programme officials, policy-/decision-makers and stakeholders towards this objective and invigorate fraternity of research scientists and students to address emerging issues and invent newer intervention tools that are community-based and sustainable in making 'malaria history'.

We are deeply indebted to our contributors spread across continents for sharing updated information in understanding the biology of malaria parasite and its vectors, and presenting newer and promising interventions in the context of malaria elimination. We are particularly grateful to Prof. Pierre Carnevale, who developed in 1983 the innovative concept of insecticide-treated nets that greatly improved malaria control efforts, for kindly accepting to write the foreword of this book. We do sincerely hope that the next decade would be an exciting and promising one in transforming this knowledge to spearheading technologies to end malaria transmission for good. We are particularly grateful to Ms. Romina Skomersic, the Author Service Manager at InTechOpen, and her team for their unstinted support, crea-

tivity and professionalism in making this project a reality. We also wish to thank Ms. Ana Pantar, Senior Commissioning Editor, for her help and extended cooperation all throughout the publication process. Finally, we do wish to acknowledge the initiative, sponsorship and concept of the IntechOpen publishers (www.intechopen.com) to share knowledge for open science and open minds for wider public access.

Sylvie Manguin, PhD
Institute of Research for Development (IRD), HSM, CNRS
University Montpellier
Montpellier, France

Vas Dev, PhD
Formerly with the National Institute of Malaria Research (NIMR)
New Delhi, India

References

[1] Anopheles mosquitoes – New insights into malaria vectors. Edited by Sylvie Manguin. Published by InTech Open Access (www.intechopen.com, http://dx.doi.org/10.5772/3392). Croatia, 2013, 813 pp.

[2] Malaria – Biology in the Era of Eradication. A subject collection from Cold Spring Harbor Perspectives in Medicine. Edited by Dyann F. Wirth and Pedro L. Alonso. Cold Spring Harbor Laboratory Press, New York, USA, 2017, 312 pp.

[3] WHO. World Malaria Report 2017. World Health Organization, Geneva, Switzerland, 2017, 196 pp. Available at http://www.who.int/malaria/world_malaria_report_2017.

[4] Newby G, Bennett A, Larson E, Cotter C, Shretta R, Phillips AA, Feachem RG. The path to eradication: a progress report on the malaria-eliminating countries. Lancet, 2016;387: 1775-1784.

[5] WHO. Global Technical Strategy 2016-2030. Global Malaria Programme, World Health Organization, Geneva, Switzerland, 30 pp. Available at http://www.who.int/malaria/areas/global_technical_strategy/en/.

[6] Singh KP, Travis P. Universal health coverage in the World Health Organization South-East Asia Region: how can we make it "business unusual"? WHO South-East Asia Journal of Public Health, 2018;7: 1-4.

Introduction

Chapter 1

Malaria Elimination: Challenges and Opportunities

Umberto D'Alessandro

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.77092

Abstract

In 2016, 91 countries reported a total of 216 million cases of malaria, an increase of 5 million cases over the previous year, and the estimated malaria deaths worldwide were 445,000 like in 2015. This suggests that despite a substantial reduction in the malaria burden observed since 2010, largely attributed to the scale-up of effective control measures (vector control interventions, efficacious antimalarial treatment), the rate of decline of both clinical cases and malaria deaths has stalled since 2014 and in some regions even reversed. Achieving universal access to standard control interventions, such as case management, implementation of vector control methods, seasonal malaria chemoprevention, and intermittent preventive treatment for pregnant women, remains a priority. It is essential to contain emerging drug resistance in malarial parasite and insecticide resistance in mosquito vector species. Additional new interventions to accelerate interruption of transmission are in crucial need for their rapid integration within the standard control activities. These integrated control approaches must be implemented at community level with the active involvement of the local populations to reach high coverage. Finally, political and financial supports should be maintained and even doubled to reach the 2030 targets of the WHO global technical strategy for malaria.

Keywords: malaria elimination, mass drug administration, drug resistance, insecticide resistance

1. Introduction

In 2016, 91 countries reported a total of 216 million cases of malaria, an increase of 5 million cases over the previous year. The estimated number of malaria deaths worldwide was 445,000, about the same number reported in 2015 [1]. This suggests that, despite a substantial reduction in the malaria burden observed since 2010, largely attributed to the scale-up of effective control measures, including vector control interventions and treatment with

efficacious antimalarial medicines, the rate of decline of both clinical cases and malaria deaths has stalled since 2014 and in some regions (the Americas mainly and marginally in the Southeast Asia, Western Pacific, and African regions) even reversed [1]. The World Health Organization (WHO) has estimated that to meet the 2030 targets of global malaria strategy, a minimum investment of US$ 6.5 billion per year by 2020 is required [2]. In 2016, such investment was US$ 2.7 billion, less than half of that required amount, and since 2014 in many high-burden countries, investments in malaria control have declined [1]. The call for malaria eradication launched at the Malaria Forum in October 2007 by the Bill & Melinda Gates Foundation and then supported by the WHO, Roll Back Malaria (RBM) Partnership, and many other organizations and institutions seems to be at crossroads [3].

2. Components of malaria elimination strategy

The WHO currently considers malaria elimination at the national level as a continuum rather than the achievement of milestones for specific phases [2]. It is structured in 4 components (A–D), each of them to be implemented according to the malaria transmission intensity. Component "A" consists of enhancing and optimizing vector control and case management, which includes universal access to malaria preventions, diagnosis, and treatment for at-risk populations, and once elimination has been achieved, "focalized" vector control programs rather than scaling back these activities; component "B" aims at increasing the sensitivity and specificity of surveillance to detect, characterize, and monitor all cases (individual and in foci), namely, to transform malaria surveillance into a core intervention; component "C" aims at accelerating transmission reduction in which new interventions such as mass drug administration (MDA) or new vaccines are included; and component "D" is implemented when transmission intensity is low to very low, which includes the search for the few remaining infections and any foci of ongoing transmission, clearing them with appropriate treatment and possibly additional vector control activities [2].

3. Resistance of *Plasmodium falciparum* to anti-malaria drugs

Resistance to first-line treatments for *Plasmodium falciparum* malaria and to the insecticides used for *Anopheles* vector control is threatening malaria elimination efforts [4]. Artemisinin and its derivatives provide the fastest parasite clearance among available antimalarial drugs and have been combined with an antimalarial drug of a different class in order to (i) enhance complete cure rates, (ii) shorten the duration of therapy for artemisinin monotherapies, and (iii) delay the selection and spread of resistant parasites [5, 6]. Artemisinin-based combination treatments (ACTs) are currently recommended for the management of uncomplicated malaria cases. In 2007, the first cases of delayed parasite clearance, suggesting artemisinin resistance, were observed at the Thailand-Cambodia border [7, 8]. Artemisinin resistance has now been reported in 5 countries of the Greater Mekong Subregion (GMS), which includes Cambodia, Myanmar, Laos, Thailand, and Vietnam, and delayed parasite clearance has been linked to

point mutations in the propeller region of a *P. falciparum* protein gene on chromosome 13 (K13) [9]. Artemisinin resistance may have spread to or emerged in Bangladesh [10] and has extended across much of Myanmar with a high prevalence of *P. falciparum* parasites carrying K13-propeller mutations reported next to the north-western border of India [11]. Resistance may have also emerged in South America, including Guyana, Suriname, French Guiana, and bordering areas of Brazil and Venezuela, [12, 13] that shares several characteristics with the GMS, increasing the risk of selecting resistant parasites. These include higher *P. falciparum* transmission than the rest of the Amazon Basin, highly mobile populations, availability and widespread use of several antimalarial drugs of questionable quality, including artemisinin monotherapies, and poor access and use of formal malaria diagnostic and treatment facilities [14]. Besides artemisinin resistance, the prevalence of molecular markers correlated to resistance to the partner drugs has increased. For example, changes in the prevalence of *pfcrt* and *pfmdr1* alleles have been observed in many areas where ACTs including amodiaquine or lumefantrine have been intensively used [4]. However, outside the GMS, recommended ACTs' efficacy remains acceptable (4). In Southeast Asia, the intensive use of dihydroartemisinin-piperaquine (DP) has resulted in selection of parasites with multiple resistance mechanisms, and in Cambodia high levels of treatment failure to DP are now observed [15]. Resistance to piperaquine (clinical and *in vitro*) may be associated to *plasmepsins* 2–3, but other markers could be involved [4].

4. Resistance of *Anopheles* mosquito vectors to insecticides

Resistance of malaria vectors to the 4 insecticide classes (pyrethroids, organochlorines, organophosphates, and carbamates) used for vector control interventions threatens malaria prevention and control efforts. Of the 76 malaria endemic countries that reported standard monitoring data from 2010 to 2016, resistance was detected in 61 countries to at least one insecticide in one malaria vector from one collection site, and 50 countries had resistance to 2 or more insecticides [1]. Resistance to pyrethroids, insecticides used in all long-lasting insecticidal nets (LLINs), is widespread though its impact on LLIN effectiveness is unclear [16]. There was no association between malaria disease burden and the level of resistance in a WHO-coordinated study implemented in 5 countries (Sudan, Kenya, India, Cameroon, and Benin) [1]. However, given the complexity in measuring the impact of insecticide resistance, it is not possible to equate lack of evidence of impact with evidence for no impact [16].

5. Asymptomatic malaria infections and mass drug administration (MDA)

One of the major problems to achieve malaria elimination is represented by the hidden parasite reservoir in the human host. Microscopy (and rapid diagnostic tests (RDTs)) underestimates by about half the prevalence of *Plasmodium* infection, and this difference is greatest in low-transmission settings—many asymptomatic infections can persist for significant periods of

time. The presence of *P. falciparum* gametocytes is positively associated with the absence of clinical symptoms and low asexual parasite densities; mosquitoes can become infected with gametocyte densities as low as 5 gametocytes/μl and theoretically as low as one gametocyte/μl—children with undetectable gametocytaemia by molecular methods were still observed to be infectious to mosquitoes [17]. To accelerate achieving malaria elimination, the human reservoir of infection needs to be tackled with new approaches. There is a growing interest in MDA of at-risk populations or in malaria hot-spot areas with an effective antimalarial to reduce the parasite reservoir in human host [18]. MDA aims to provide full post-treatment courses to the whole population to clear asymptomatic infections and provide posttreatment prophylaxis to prevent reinfection. The use of MDA is recommended in areas approaching interruption of transmission, with good access to treatment, effective vector control, and surveillance systems, ensuring a minimal risk of reintroduction of infection [19]. MDAs have been conducted using a variety of drug regimens at different dosages, timings, and frequency. There is evidence of substantial but short-lived reduction in *P. falciparum* parasite carriage [20]. In Zambia, a cluster-randomized control trial implemented in a population of 330,000 individuals, distributed in 56,000 households, compared MDA with DP (2 rounds), at the household level (DP to all members of household with at least a RDT-positive individual) and standard control measures (case management, LLIN, indoor residual spraying (IRS), and intermittent preventive treatment during pregnancy). MDA decreased significantly malaria prevalence and incidence in low (malaria prevalence <10%) but not in high (malaria prevalence ≥10%) transmission areas [21]. With the growing awareness of heterogeneity and clustering in transmission, MDA approaches have been modified by systematic (mass screening and treatment) or focused (focal screening and treatment) screening and treatment of populations in defined geographical areas. Reactive case detection, i.e., screening and treating positive contacts in response to a clinical event, has been tested and implemented in some countries [22–25]. However, its impact has been variable as it is affected by the sensitivity of the diagnostic tool and the radius of intervention around a clinical case [26–29].

The antimalarial treatment administered during MDA campaigns could be complemented by single low-dose of primaquine, an 8-aminoquinoline that is able to clear mature *P. falciparum* gametocytes [30], and/or ivermectin, a systemic endectocidal drug that can be administered safely to both humans and animals but proven toxic to *Anopheles* mosquitoes when they take a blood meal from a host that has recently received the drug [31, 32]. Primaquine may cause a dose-dependent hemolysis, mainly in individuals with deficiency of the enzyme glucose 6-phosphate dehydrogenase (G6PD) in red blood cells [33], and this has slowed down its implementation. Nevertheless, a single low-dose of primaquine can significantly reduce gametocyte carriage in both symptomatic [33] and asymptomatic [34] individuals and reduces onward transmission from man to vector [35]. Ivermectin can be safely administered with an ACT [36, 37] and has been used widely against parasitic diseases in humans, with record of more than 2 billion doses in MDA campaigns against onchocerciasis and lymphatic filariasis. In Burkina Faso, Liberia, and Senegal, one round of MDA with ivermectin at the standard dose of 150 μg/kg decreased substantially *An. gambiae* survival for 6 days and reduced the proportion of sporozoite-positive (infectious) mosquitoes for 2 weeks [38]. However, evidence of ivermectin as an additional tool to decrease malaria transmission is limited and needs to be further quantified, possibly by a cluster randomized

trial in a country with high coverage of standard control interventions and substantial residual malaria transmission.

6. Conclusions

In conclusion, achieving universal access to standard control interventions, namely, case management, LLIN, IRS, seasonal malaria chemoprevention, and intermittent preventive treatment for pregnant women, remains a priority. It is essential to contain emerging drug resistance in malarial parasite and insecticide resistance in mosquito vector species. There is a dire need of additional new interventions to accelerate interruption of transmission. These should be evaluated and rapidly integrated within the standard control activities. Most of these should be implemented at the community level, and it will be important to actively involve the local populations to reach high coverage. Finally, political and financial supports should be maintained and even increased; current financial support is less than half of that estimated to reach the 2030 targets of the WHO global technical strategy for malaria [1].

Author details

Umberto D'Alessandro

Address all correspondence to: udalessandro@mrc.gm

Medical Research Council Unit, The Gambia and London School of Hygiene and Tropical Medicine, Fajara, The Gambia

References

[1] WHO. World malaria report. Geneva: World health organization; 2017. Licence: CC BY-NC-SA 3.0 IGO. http://apps.who.int/iris/bitstream/handle/10665/259492/9789241565523-eng.pdf;jsessionid=DBAAA4A8F412049581DEC3652C2EE8E5?sequence=1; 12 December 2017

[2] WHO. Global Malaria Programme. A framework for malaria elimination. Geneva: World Health Organization; 2017. http://apps.who.int/iris/bitstream/handle/10665/254761/9789241511988-eng.pdf?sequence=1; 12 December 2017

[3] Alonso PL, Brown G, Arevalo-Herrera M, Binka F, Chitnis C, et al. A research agenda to underpin malaria eradication. PLoS Medicine. 2011;**8**:e1000406. DOI: 10.1371/journal.pmed.1000406

[4] The malERA Refresh Consultative Panel on Insecticide and Drug Resistance. An updated research agenda for insecticide and drug resistance in malaria elimination and eradication. PLoS Medicine. 2017;**14**:e1002450. DOI: 10.1371/journal.pmed.1002450

[5] White NJ, Olliaro PL. Strategies for the prevention of antimalarial drug resistance: Rationale for combination chemotherapy for malaria. Parasitology Today. 1996;**12**:399-401

[6] White NJ. Preventing antimalarial drug resistance through combinations. Drug Resistance Updates. 1998;**1**:3-9

[7] Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM. Artemisinin resistance in Cambodia 1 (ARC1) study consortium. Evidence of artemisinin-resistant malaria in Western Cambodia. The New England Journal of Medicine. 2008;**359**:2619-2620

[8] Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, Lwin KM, Ariey F, Hanpithakpong W, Lee SJ, Ringwald P, Silamut K, Imwong M, Chotivanich K, Lim P, Herdman T, An SS, Yeung S, Singhasivanon P, Day NP, Lindegardh N, Socheat D, White NJ. Artemisinin resistance in *Plasmodium falciparum* malaria. The New England Journal of Medicine. 2009;**361**:455-467

[9] Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, Khim N, Kim S, Duru V, Bouchier C, Ma L, Lim P, Leang R, Duong S, Sreng S, Suon S, Chuor CM, Bout DM, Ménard S, Rogers WO, Genton B, Fandeur T, Miotto O, Ringwald P, Le Bras J, Berry A, Barale JC, Fairhurst RM, Benoit-Vical F, Mercereau-Puijalon O, Ménard D. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. Nature. 2014;**505**:50-55

[10] Takala-Harrison S, Laufer MK. Antimalarial drug resistance in Africa: Key lessons for the future. Annals of the New York Academy of Sciences. 2015;**1342**:62-67

[11] Tun KM, Imwong M, Lwin KM, Win AA, Hlaing TM, Hlaing T, Lin K, Kyaw MP, Plewes K, Faiz MA, Dhorda M, Cheah PY, Pukrittayakamee S, Ashley EA, Anderson TJ, Nair S, McDew-White M, Flegg JA, Grist EP, Guerin P, Maude RJ, Smithuis F, Dondorp AM, Day NP, Nosten F, White NJ, Woodrow CJ. Spread of artemisinin-resistant *Plasmodium falciparum* in Myanmar: A cross-sectional survey of the K13 molecular marker. The Lancet Infectious Diseases. 2015;**15**:415-421

[12] Vreden SG, Jitan JK, Bansie RD, Adhin MR. Evidence of an increased incidence of day 3 parasitaemia in Suriname: An indicator of the emerging resistance of *Plasmodium falciparum* to artemether. Memórias do Instituto Oswaldo Cruz. 2013;**108**:968-973

[13] Chenet SM, Akinyi Okoth S, Huber CS, Chandrabose J, Lucchi NW, Talundzic E, Krishnalall K, Ceron N, Musset L, Macedo de Oliveira A, Venkatesan M, Rahman R, Barnwell JW, Udhayakumar V. Independent emergence of the *Plasmodium falciparum* Kelch propeller domain mutant allele C580Y in Guyana. The Journal of Infectious Diseases. 2016;**213**:1472-1475

[14] Achan J, Mwesigwa J, Edwin CP, D'Alessandro U. Malaria medicines to address drug resistance and support malaria elimination efforts. Expert Review of Clinical Pharmacology. 2017;**11**:61-70. DOI: 10.1080/17512433.2018.1387773

[15] Amaratunga C, Lim P, Suon S, Sreng S, Mao S, Sopha C, Sam B, Dek D, Try V, Amato R, Blessborn D, Song L, Tullo GS, Fay MP, Anderson JM, Tarning J, Fairhurst RM. Dihydroartemisinin-piperaquine resistance in *Plasmodium falciparum* malaria in Cambodia: A multisite prospective cohort study. The Lancet Infectious Diseases. 2016;**16**:357-365. DOI: 10.1016/S1473-3099(15)00487-9. PMID: 26774243

[16] Ranson H, Lissenden N. Insecticide resistance in African *Anopheles* mosquitoes: A worsening situation that needs urgent action to maintain malaria control. Trends Parasitol. 2015;**32**:187-196. DOI: 10.1016/j.pt.2015.11.010. PMID: 26826784

[17] Lindblade KA, Steinhardt L, Samuels A, Kachur SP, Slutsker L. The silent threat: Asymp tomatic parasitemia and malaria transmission. Expert Review of Anti-Infective Therapy. 2013;**11**:623-639

[18] Bousema T, Drakeley C. Epidemiology and infectivity of *Plasmodium falciparum* and *Plasmodium vivax* gametocytes in relation to malaria control and elimination. Clinical Microbiology Reviews. 2011;**24**:377-410

[19] WHO. Global Malaria Programme: The role of mass drug administration, mass screening and treatment, and focal screening and treatment for malaria. Geneva: World Health Organization; 2015. http://www.who.int/malaria/publications/atoz/role-of-mda-for-malaria.pdf?ua=1; 12 December 2017

[20] Poirot E, Skarbinski J, Sinclair D, Kachur SP, Slutsker L, Hwang J. Mass drug administration for malaria. Cochrane Database of Systematic Reviews. 2013;**12**:CD008846

[21] Eisele TP, Bennett A, Silumbe K, Finn TP, Chalwe V, Kamuliwo M, Hamainza B, Moonga H, Kooma E, Chizema Kawesha E, Yukich J, J1 K, Porter T, Conner RO, Earle D, Steketee RW, Miller JM. Short-term impact of mass drug administration with dihydroartemisinin plus piperaquine on malaria in Southern Province Zambia: A cluster-randomized controlled trial. The Journal of Infectious Diseases. 2016;**214**:1831-1839

[22] Kern SE, Tiono AB, Makanga M, Gbadoé AD, Premji Z, Gaye O, Sagara I, Ubben D, Cousin M, Oladiran F, Sander O, Ogutu B. Community screening and treatment of asymptomatic carriers of *Plasmodium falciparum* with artemether-lumefantrine to reduce malaria disease burden: A modelling and simulation analysis. Malaria Journal. 2011;**10**:210

[23] Stresman GH, Kamanga A, Moono P, Hamapumbu H, Mharakurwa S, Kobayashi T, Moss WJ, Shiff C. A method of active case detection to target reservoirs of asymptomatic malaria and gametocyte carriers in a rural area in Southern Province, Zambia. Malaria Journal. 2010;**9**:265

[24] Sturrock HJ, Novotny JM, Kunene S, Dlamini S, Zulu Z, Cohen JM, Hsiang MS, Greenhouse B, Gosling RD. Reactive case detection for malaria elimination: Real-life experience from an ongoing program in Swaziland. PLoS One. 2013;**8**:e63830

[25] Littrell M, Sow GD, Ngom A, Ba M, Mboup BM, Dieye Y, Mutombo B, Earle D, Steketee RW. Case investigation and reactive case detection for malaria elimination in northern Senegal. Malaria Journal. 2013;**12**:331

[26] Hustedt J, Canavati SE, Rang C, Ashton RA, Khim N, Berne L, Kim S, Sovannaroth S, Ly P, Ménard D, Cox J, Meek S, Roca-Feltrer A. Reactive case-detection of malaria in Pailin Province, Western Cambodia: Lessons from a year-long evaluation in a pre-elimination setting. Malaria Journal. 2016;**15**:132

[27] Searle KM, Hamapumbu H, Lubinda J, Shields TM, Pinchoff J, Kobayashi T, Stevenson JC, Bridges DJ, Larsen DA, Thuma PE, Moss WJ. Evaluation of the operational challenges in implementing reactive screen-and-treat and implications of reactive case detection strategies for malaria elimination in a region of low transmission in Southern Zambia. Malaria Journal. 2016;**15**:412

[28] Smith Gueye C, Sanders KC, Galappaththy GN, Rundi C, Tobgay T, Sovannaroth S, Gao Q, Surya A, Thakur GD, Baquilod M, Lee WJ, Bobogare A, Deniyage SL, Satimai W, Taleo G, Hung NM, Cotter C, Hsiang MS, Vestergaard LS, Gosling RD. Active case detection for malaria elimination: A survey among Asia Pacific countries. Malaria Journal. 2013;**12**:358

[29] Tiono AB, Ouedraogo A, Ogutu B, Diarra A, Coulibaly S, Gansane A, Sirima SB, O'Neil G, Mukhopadhyay A, Hamed K. A controlled, parallel, cluster-randomized trial of community-wide screening and treatment of asymptomatic carriers of *Plasmodium falciparum* in Burkina Faso. Malaria Journal. 2013;**12**:79

[30] WHO Policy Brief on Single-Dose Primaquine as Gametocytocide in *Plasmodium falciparum* malaria; 2015. http://www.who.int/malaria/publications/atoz/who_htm_gmp_2015.1.pdf?ua=1; 12 December 2017

[31] Foy BD, Kobylinski KC, da Silva IM, Rasgon JL, Sylla M. Endectocides for malaria control. Trends in Parasitology. 2011;**27**:423-428

[32] Reddy MR, Overgaard HJ, Abaga S, Reddy VP, Caccone A, Kiszewski AE, Slotman MA. Outdoor host seeking behaviour of *Anopheles gambiae* mosquitoes following initiation of malaria vector control on Bioko Island, Equatorial Guinea. Malaria Journal. 2011;**10**:184. DOI: 10.1186/1475-2875-10-184

[33] Eziefula AC, Pett H, Grignard L, Opus S, Kiggundu M, Kamya MR, Yeung S, Staedke SG, Bousema T, Drakeley C. Glucose-6-phosphate dehydrogenase status and risk of hemolysis in *Plasmodium falciparum*-infected African children receiving single-dose primaquine. Antimicrobial Agents and Chemotherapy. 2014;**58**:4971-4973

[34] Okebe J, Bousema T, Affara M, Di Tanna GL, Dabira E, Gaye A, Sanya-Isijola F, Badji H, Correa S, Nwakanma D, Van Geertruyden JP, Drakeley C, D'Alessandro U. The gametocytocidal efficacy of different single doses of primaquine with dihydroartemisinin-piperaquine in asymptomatic parasite carriers in the Gambia: A randomized controlled trial. eBioMedicine. 2016;**13**:348-355

[35] Dicko A, Brown JM, Diawara H, Baber I, Mahamar A, Soumare HM, Sanogo K, Koita F, Keita S, Traore SF, Chen I, Poirot E, Hwang J, McCulloch C, Lanke K, Pett H, Niemi M, Nosten F, Bousema T, Gosling R. Primaquine to reduce transmission of *Plasmodium falciparum* malaria in Mali: A single-blind, dose-ranging, adaptive randomised phase 2 trial. The Lancet Infectious Diseases. 2016;**16**:674-684. DOI: 10.1016/S1473-3099(15)00479-X

[36] Smit MR, Ochomo E, Aljayyoussi G, Kwambai T, Abong'o B, Bayoh N, Gimnig J, Samuels A, Desai M, Phillips-Howard PA, Kariuki S, Wang D, Ward S, Ter Kuile FO. Efficacy and safety of high-dose ivermectin for reducing malaria transmission: Protocol for a double-blind, randomized, placebo-controlled, dose-finding trial in Western Kenya. JMIR Research Protocols. 2016;**5**:e213

[37] Ouédraogo AL, Bastiaens GJ, Tiono AB, Guelbéogo WM, Kobylinski KC, Ouédraogo A, Barry A, Bougouma EC, Nebie I, Ouattara MS, Lanke KH, Fleckenstein L, Sauerwein RW, Slater HC, Churcher TS, Sirima SB, Drakeley C, Bousema T. Efficacy and safety of the mosquitocidal drug ivermectin to prevent malaria transmission after treatment: A double-blind, randomized, clinical trial. Clinical Infectious Diseases. 2015;**60**:357-365

[38] Alout H, Krajacich BJ, Meyers JI, Grubaugh ND, Brackney DE, Kobylinski KC, Diclaro JW 2nd, Bolay FK, Fakoli LS, Diabaté A, Dabiré RK, Bougma RW, Foy BD. Evaluation of ivermectin mass drug administration for malaria transmission control across different West African environments. Malaria Journal. 2014;**13**:417

Section 2

Major Challenges

Chapter 2

The Artemisinin Resistance in Southeast Asia: An Imminent Global Threat to Malaria Elimination

Aung Pyae Phyo and François Nosten

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76519

Abstract

Malaria remains a leading cause of mortality and morbidity in many low- and middle-income countries. Artemisinin combination therapies (ACTs) have contributed to the substantial decline in the worldwide malaria burden, renewing the optimism that malaria elimination is achievable in some regions of the world. However, this prospect is threatened by the emergence of artemisinin resistance in *Plasmodium falciparum* leading to clinical failure of ACTs in Southeast Asia. Historically, drug resistance in *P. falciparum* has emerged in SEA and spread to Africa. Today, resistance to ACTs could reverse all the achievements of control and elimination efforts globally. With no new drug available, *P. falciparum* malaria must be eliminated from the Greater Mekong before it becomes untreatable.

Keywords: falciparum malaria, artemisinin, ACT, resistance, malaria elimination, Southeast Asia

1. Introduction

The emergence of artemisinin-resistant falciparum malaria along the Thai-Cambodian border follows a familiar pattern. History shows that chloroquine resistance had arisen from this region in the 1950s (**Table 1**) and leads to the failure of the Global *Malaria Eradication* Programme [1, 2] Resistance to artemisinin with concomitant emergence of partner drug resistance is now causing high artemisinin combination therapy (ACT) treatment failure rates in Cambodia, Vietnam, Thailand, Laos and Myanmar (**Table 1**). The prospect of untreatable malaria has once again loomed and threatened the effective malaria control and elimination efforts.

Antimalarial drug	Year of first deployment	Place of first deployment	Year of resistance emerged	Place of emergence of resistance
Quinine	1630 [34]	South America [34]	1910*	Brazil [28, 29]
Chloroquine	1945	Global Malaria Eradication Campaign [127]	1957	Colombia, Cambodia-Thailand border [41, 128–130]
Amodiaquine	1948	Americas [131, 132]	1961	Colombia [56, 57]
Atovaquone	1996	Thailand [73]	1996	Thailand [72, 73, 75]
Proguanil	1948	Various African countries [133]	1949	Aden Protectorate, Yemen [134]
Sulfa + antifols°	1967	Thailand [135]	1967	Thailand [135]
Mefloquine	1967	Vietnam [136]	1982	Thailand [7, 8, 43]
Piperaquine	1978	China [137]	1985	China [138]
Artemisinin	1979	China [139]	2008	Cambodia [6]
Mefloquine-artesunate	1994	Thailand [140]	2002α	Cambodia [141]
Artemether-lumefantrine	1994	China [142]	2006α	Cambodia [143, 144]
Dihydroartemisinin-piperaquine	2001	Cambodia [145]	2013α	Cambodia [86, 146, 147]

*There is no high-grade resistance to quinine.

αTherapeutic efficacy <90% (cut-off threshold of WHO to switch the ACT policy).

°Sulfa + antifols: Sulfadoxine + antifolates.

Table 1. Different antimalarial drugs and years/places of deployment and emergence of resistance [references in bracket].

2. Background

Resistance in *Plasmodium falciparum* has already developed to all antimalarial drug classes deployed for treatment. Paradoxically, the number of antimalarials available or in development has remained small. For most of the twentieth century, chloroquine was the main drug used to treat or prevent malaria. The discovery of chloroquine after World War II, and the widespread use of DDT for vector control, had triggered hope that malaria eradication was possible [3]. Unfortunately, chloroquine resistance did emerge and spread to the African continent within two decades annihilating the prospect of malaria eradication [4]. Although several countries did achieve malaria elimination (in Europe and the Americas), others saw a dramatic resurgence of the disease [3]. Over the following period, *P. falciparum* developed resistance to all antimalarial drugs, including sulfadoxine, pyrimethamine, mefloquine, atovaquone, artemisinin derivatives and piperaquine [5–8]. The most accurate and up-to-date data repository of the clinical trials on the efficacy of antimalarials, and the temporal and geographical spread of resistance is accessible at the Worldwide Antimalarial Resistance Network (WWARN: www.wwarn.org).

In 2007, the Bill and Melinda Gates Foundation announced that it was investing millions of dollars to revitalise the efforts of malaria elimination [9]. Ten years later, this seems to be an achievable goal since the global malaria burden has diminished (**Figure 1**), an encouraging

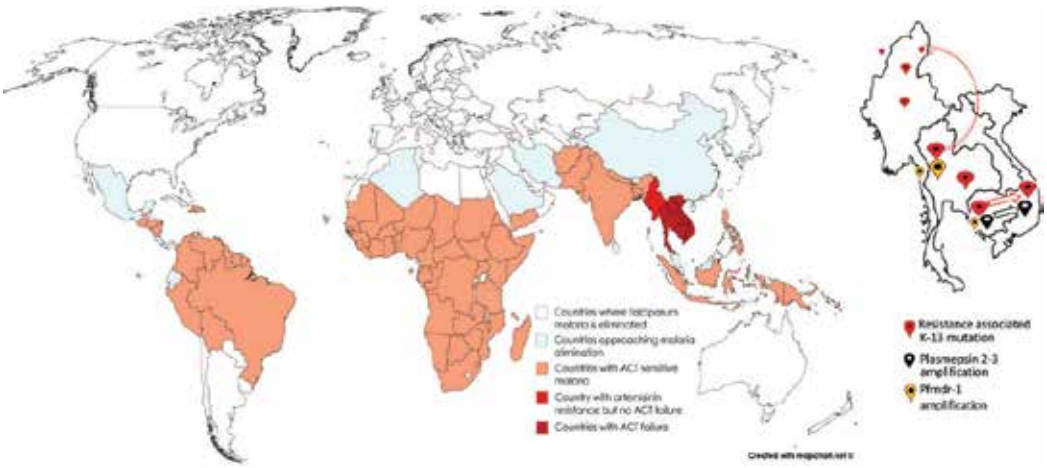


Figure 1. World atlas showing the countries with different stages of malaria endemicity [10] and status of drug resistance [121]. Right side: Prevalence (small pin: <10%, medium pin: 10–50%, large pin: >50% prevalence) of *Pfmdr-1* CNV [25, 122], Plasmepsin 2–3 CNV [26, 87], K-13 mutation [101, 123, 124] and possible spread [125, 126].

result attributed to the widespread deployment of long-lasting impregnated nets (LLINs), the ACTs and increased availability of malaria diagnostic tests [10]. However, the failure of the ACTs, the extension of vector resistance to the insecticides and the recent increase in the number of malaria cases are clear reminders that malaria is a formidable foe. Without new strategies, the same causes will lead to the same consequences [10] .

3. Mechanisms and emergence of antimalarial drug resistance

Causal stimuli of antimalarial resistance consist of spontaneous mutations in the parasite genome, antimalarial pharmacokinetics and the magnitude of parasite gene pool, which is proportionate to transmission intensity.

Primarily, as an innate survival strategy of microorganisms, mutation(s) occur *de novo*, independent of drug pressure. However, the parasite's genome replication rate, mutation rate per base-pair per parasite generation and the total number of parasites at any given time are the principal determinants in spontaneous mutation [11, 12]. These spontaneous mutations can be either minor scale modification, such as insertion, deletion or variation in a nucleotide (frameshift mutation or single-nucleotide polymorphism), or bulky transfiguration of large chromosomal regions (gene amplification/deletion/copy number variations). For some drugs, a single genetic event may be all that is required. A single point mutation in the parasite genome is sufficient to confer resistance (e.g. atovaquone), while for other drugs, multiple unlinked events (epistatic modulation) may be necessary (e.g. triple mutant in pyrimethamine [13, 14], Kelch-10, Kelch-13 and background mutations [15–17] in artemisinin resistance).

Spontaneous mutations, in the particular genes encoding the drug target, cause the reduction in drug accumulation or efflux (chloroquine, amodiaquine, quinine, mefloquine, halofantrine

resistance) or reduced affinity of the drug target (pyrimethamine, cycloguanil, sulphonamide, atovaquone resistance), which finally enables the parasite to withstand the antimalarial treatment. Afterwards, the drug pressure facilitates the resistant parasites to propagate by eliminating the susceptible parasites, which are usually more fit and would outcompete the resistant ones in the absence of the drug. Eventually resistance becomes established and can persist or be reintroduced. In the absence of drug pressure, the resistant parasites have no longer any survival advantage and can be overtaken by wild-type (sensitive) parasites [18, 19]. But as soon as the abandoned drug is reintroduced, the resistant isolates regain their survival advantage and expand rendering the drug inefficient within a short time [20].

Large-scale and/or long-term distribution of several tons of medicated salt took place in many countries and was an important factor implicated in the emergence of both chloroquine and sulfadoxine/pyrimethamine (SP) resistance and accelerating their spread [21–23]. In WHO supported programs, the doses of antimalarial received by each individual were highly variable, and constant exposure to sub-parasiticidal (or even parasiticidal) drug concentrations might have eliminated the highly and moderately sensitive parasites, providing a selective advantage for less sensitive counterparts. Thus, the speed of selection of mutant parasites depends principally on the pharmacokinetics of the drug (slowly eliminated drugs with a long tail of sub-parasiticidal concentrations generally select faster) and the magnitude of drug use within a population (the higher the drug pressure per parasite, the faster the selection).

With ACTs, the newly emerged drug-resistant parasite has to overcome the parasiticidal action of the partner drug as well as the host immunity. At this point, with compromised efficacy of partner drug, along with declining immunity of the population, resistance to ACT combination is inevitable [24]. This is the reason why artemisinin resistance has led to the clinical failure of mefloquine-artesunate and DHA-piperaquine combinations [25, 26].

The reason why antimalarial resistance always emerged in the same region of the world (SEA and specifically in Western Cambodia) is currently unknown. Some contributing factors have been proposed such as the low level of acquired immunity, the weak and seasonal transmission, the availability of antimalarial drugs, usage of monotherapies, sub-standard or counterfeit drugs, porous borders. The answer will probably be given by studies of the parasite population genetics, and recent work has shown the existence of "founding populations" favourable to the emergence of resistant parasites [17].

The emergence of drug resistance to various antimalarial compounds is mentioned by chronology in **Table 1** (antimalarial drugs and years/places of deployment and emergence of resistance).

3.1. Quinine resistance

Quinine, initially as cinchona bark, was first used as a fever medicine and officially introduced into the London Pharmacopoeia in 1677 [27]. The earliest resistance to quinine was reported in 1910 [28, 29]. Like chloroquine, quinine has been shown to accumulate in the parasite's digestive vacuole inhibiting the haem detoxification process. Quinine resistance also seems to be associated with reduced drug uptake by the parasite. There is a weak association between quinine resistance and *Pfmdr-1* amplification or *Pfmdr-1* SNP as well as Pf Na-H exchanger (*Pfnhe-1*) and *Pfcrt* [30, 31]; hence, it is probable that multiple genes are influencing susceptibility and probably in a strain-dependent manner. There were only a few *in vitro* data in Asia

[32], South America [33] and Africa [34] showing diverse range of sensitivities. However, the review paper of over 400 clinical trials showed that the failure rates for quinine (the only compound besides artemisinins, derived from nature) reported over the past 30 years remain steady and high grade clinical resistance to quinine is very rare [35].

3.2. Chloroquine resistance

Chloroquine, considered as one of the most successful medications ever deployed, saving several millions of lives, was developed in 1934 [2, 36] and replaced quinine for shorter regimen with better adherence. Single nucleotide polymorphisms in *Pfcrt* gene encoding for a transporter, chloroquine (CHQ) resistance transporter in the food vacuole causing the efflux of CHQ [37, 38], and acidification of the food vacuole [39] are significantly associated to CHQ resistance *in vitro* and are sensitive markers for therapeutic failure. Phylogenetic analysis revealed that a single lineage of CHQ-resistant *Pfcrt* alleles, that is, *CVIET/S* (K76T and mutations in three other amino acids, at positions 72, 74, 75 and 76) [40], which had emerged on the Thai-Cambodia border in 1957 [41], spread to India and Middle East countries between 1977 and 1987, reached West Africa in 1987 and propagated throughout the African continent leading to the death of millions of children [2, 38, 42, 43].

3.3. Antifolate resistance

After the emergence of chloroquine resistance, sulfadoxine-pyrimethamine (SP) combination was deployed by the Thai Malaria Control Program as the first-line regimen for falciparum malaria in 1973. Afterwards, SP was extensively used throughout the country and was also available as an over-the-counter fever remedy in local dispensaries. Attributed to a number of reasons, including unrestricted usage, distribution of pyrimethamine medicated salt [23], superfluous drug pressure (prophylactic as well as presumptive use for fever) and poor compliance especially in migrant mobile population, the resistance to SP combination had emerged around 1980 in the Thai-Cambodian border [5, 44]. Then, in the early 1980s, even with an increased dose (i.e. three tablets of SP, instead of two tablets flat dosing), a cure rate of only 30–40% was achieved [44].

Point mutations at codons 51, 59, 108 and 164 in the *dhfr* gene [45, 46] confer resistance to pyrimethamine; double or triple mutant resistant strains generated from sequential point mutations, based upon the common S108 N allele, are associated with 100-fold rise of *in vitro* sensitivity to pyrimethamine compared to wild-type [47]. Similarly, sulfadoxine resistance is associated with *DHPS* mutations at codons 436, 437, 581, 613 and 540 [48, 49]. Pyrimethamine resistant double mutant alleles (S108 N plus one more mutation at position 51 or 59) with low-level resistance of *dhfr* have multiple independent origins [50, 51]; by contrast, there were only a few or perhaps a single founding mutant lineage for the triple (N51I + C59R + S108 N) mutant *dhfr* allele, which originated from Southeast Asia (SEA) and spread to Africa [13, 14].

3.4. Amodiaquine resistance

Amodiaquine is structurally related to chloroquine but these amino-4-quinolines have different resistance patterns. Amodiaquine is effective against chloroquine-resistant isolates. However, parasites carrying the *CVIET* allele on the *Pfcrt* gene, as well as 86Y and 1246Y

polymorphisms on the *Pfmdr-1* gene, are resistant to amodiaquine [52–55]. The earliest report of resistance was documented since 1961 [56, 57], and widespread resistance to amodiaquine monotherapy was seen in 1980s [58].

3.5. Mefloquine resistance

Mefloquine was first produced in 1969 by the US Army Antimalarial Drug Development Program, primarily for the chemoprophylaxis in the military. The early therapeutic efficacy trial of mefloquine in Thailand showed 100% efficacy in 1976 [59] and in combination with SP where 97% efficacy was proven in a large-scale trial during 1983–1985 [60, 61]. Then, in 1991, mefloquine monotherapy was used as the first line regimen for *P. falciparum* malaria in Thailand [62]. Even with the stringent regulatory measures in Thailand, the therapeutic efficacy of mefloquine fell hastily especially in the border areas [7, 63]: because of the difficulties in restricting all access to the drug which was available across neighbouring porous borders. Then, in 1992, the cure rate of mefloquine monotherapy had fallen to 49% with 16% of high-grade failures in children [7, 63].

Resistance to mefloquine was proven to be mediated by *Pfmdr-1* gene amplification. *Pfmdr-1* is the gene encoding a transporter pump, P-glycoprotein homologue 1 (*Pgh1*), localised at the surface of the digestive vacuole of parasite (**Figure 2**). It confers drug resistance through both gene copy number variation (CNV) and point mutation (at nucleotide level). Altering the gene copy number provides a modest way to change gene expression without affecting the

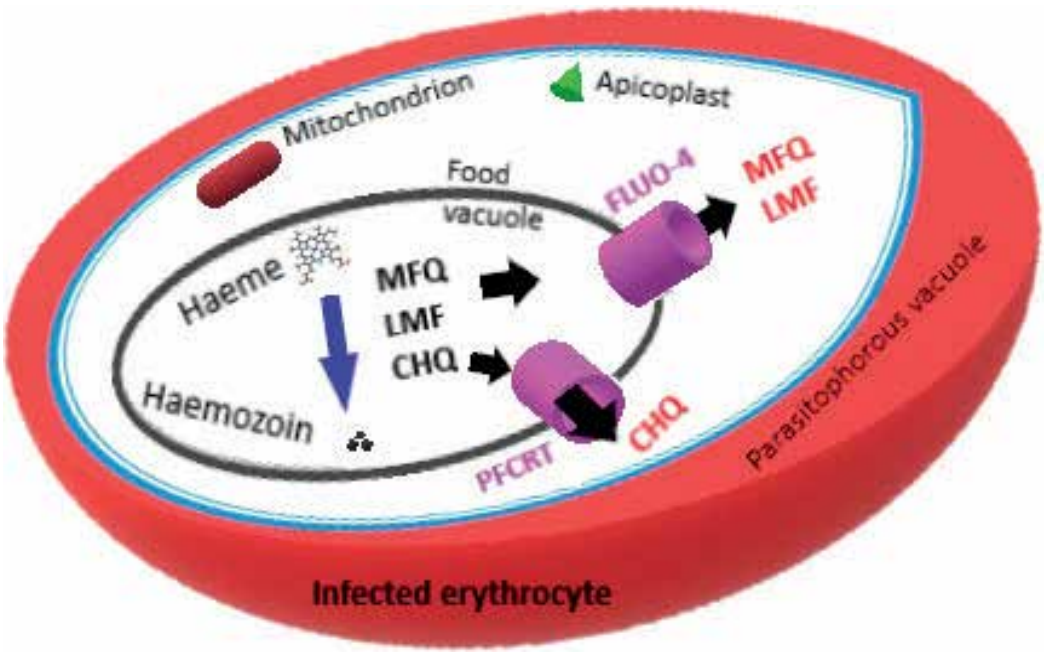


Figure 2. *Pfmdr-1* gene and mechanism of Pgh-1 pump. MFQ – mefloquine, LMF – lumefantrine, CHQ – chloroquine and RBC – red blood cell.

nucleotide sequence [64]. Increased *Pfmdr-1* copy number is a significant independent risk factor for recrudescence in patients treated with mefloquine containing therapy [65–67] as well as *in vitro* mefloquine resistance [68]. *Pfmdr-1* gene amplification can be selected *in vitro* by exposing the parasites to stepwise increasing concentrations of mefloquine [69]. Reciprocally, reducing the copy number from isolates with multiple copies resulted in increased *in vitro* sensitivity of isolates to mefloquine, lumefantrine, halofantrine, quinine and artemisinin due to reduced transcription and encoding of *Pgh-1* pump [70]. This is also true for the clinical efficacy since the rise and fall of amplified *Pfmdr-1* prevalence is temporally associated with the deployment of mefloquine in Cambodia [65, 71]. Along the Thailand-Myanmar border, patients infected with parasites having both *Pfmdr-1* multiple copy number and K-13 mutation were 14 times more likely to get recrudescence compared to the patients infected with wild-type infections [25].

3.6. Atovaquone resistance

Atovaquone was trialled as a monotherapy as well as in combination with proguanil between 1990 and 1996 in Thailand, and the therapeutic efficacy of atovaquone-proguanil was proven to be superior to mefloquine monotherapy, chloroquine, amodiaquine monotherapy and SP [72, 73]. A single point mutation (codon 268 in the *cyt-b* gene) in the ubiquinol oxidation region of cytochrome b confers atovaquone resistance *in vivo* [74, 75]. Generally, resistance conferred by a single point mutation can be rapidly acquired both *in vivo* and *in vitro*, and once the mutation is acquired, resistance becomes complete. Thus, not very long after deployment, atovaquone-resistant parasites could be selected *in vitro* after 5 weeks of continuous culture [76, 77]. In addition, atovaquone-resistant parasites were also resistant to the synergistic effects of proguanil [78], suggesting that once atovaquone resistance arises, the atovaquone-proguanil combination (Malarone) will be ineffective since cycloguanil (proguanil) resistance is already established in most malaria endemic areas.

3.7. Pyronaridine resistance

Pyronaridine is a quinoline derivative compound with similar molecular structure as chloroquine and amodiaquine. There was a strong correlation between *in vitro* sensitivity of pyronaridine and that of amodiaquine and halofantrine [79]. *Ex vivo* data indicated that there is an association between reduced susceptibility to pyronaridine and K76 T polymorphism in *Pfcrt* gene. However, there are scanty data on clinical trials and no confirmed report of molecular marker of pyronaridine resistance has been documented. Pyronaridine-artesunate combination had been granted a positive scientific opinion by the European Medicines Agency, removing all restrictions on repeat dosing with a condition to use only in areas of high resistance and low transmission, and has been included in WHO's list of prequalified medicines [80]. However, day-42 cure rate of <90% in Western Cambodia has challenged the expediency of the pyronaridine-artesunate combination in ACT resistance setting [81].

3.8. Piperaquine resistance

Piperaquine (PPQ) has no cross resistance with chloroquine, and susceptibility is not associated with mutations on the *Pfcrt* gene [82, 83]. PPQ resistance is inversely correlated with

mefloquine resistance *in vitro* and hence with *Pfmdr-1* copy number amplification [84–86]. Later findings have shown that the amplification of *Plasmepsin-2* gene (probably *Plasmepsin-3* as well) on chromosome 14 is significantly associated with piperaquine resistance *in vitro* as well as *in vivo* [26, 87]. Worryingly, a recent study in Cambodia has demonstrated the presence of parasite isolates with amplification of both *Pfmdr-1* and *plasmepsin-2* genes [20]. This finding indicates that the parasite has successfully adapted to acquire concomitant mutations related to resistance to these two different antimalarial partner drugs [20].

3.9. Artemisinin resistance

Artemisinins are thought to be inhibitor of *P. falciparum* phosphatidylinositol-3-kinase (*PfPI3K*), which phosphorylates phosphatidylinositol to produce phosphatidylinositol 3-phosphate involved in cell survival pathways. Hence, inhibition of *PfPI3K* activity causes a reduction in PI3P level, which subsequently leads to parasite death. After the introduction of artemisinins in the 1990s, the unanimous opinion by the experts was that resistance was unlikely to emerge because of inherent pharmacokinetic-dynamic property of the molecule. However, artemisinins were not everlasting drugs and the artemisinin resistance did emerge in 2008 [6].

There are two main proposed pathways for artemisinin resistance with the involvement of Kelch (*K-13*) mutations, that is, a cell survival signalling pathway with *PfPI3K* and an unfolded protein response pathway (*UPR*) [88].

In Kelch (propeller) mutant alleles, the mutations may alter the topology of the Kelch protein probably by modification of surface charges that disrupt interactions with other enzymes such as *PfPI3K* [89]. This leads to a reduced amount of ubiquitination, as well as degradation of *PfPI3K* associated with increased levels of both the enzyme *PfPI3K* and the substrate *PI3P* [90, 91]. The *PI3P* facilitating the host remodelling is present in the apicoplast and food vacuole and contributes to the cell survival pathways either through redox, transcriptional or DNA repair [90–94]. All of which have been implicated in artemisinin resistance [90, 95–98].

Possible mechanisms proposed by transcriptomic study [99] is through upregulation of genes involved in the UPR pathway (especially two putative chaperonin complexes, *Plasmodium* reactive oxidative stress complex/PROSC and TCP-1 ring complex/TRiC) which enhances the capacity of parasites to quickly repair or degrade proteins or other cellular components. (The UPR pathway is usually damaged by brief artemisinin exposures in patients, but these genes are upregulated in artemisinin resistant parasites) and/or downregulation of genes involved in DNA replication, which is associated with developmental arrest and dormancy [100].

The role of Kelch non-propeller mutation (before the amino acid position 441) is still unclear. Some SNPs like E252Q emerged earlier along the Thai-Myanmar border and associated with reduced efficacy of ACT [25] but are being taken over by the propeller SNPs particularly C580Y [101]. All these findings indicate that artemisinin resistance is likely to be multi-locus and that other genetic changes, such as P623T polymorphism in *Kelch-10* gene [15] and background mutations (arps10-apicoplast ribosomal protein S10, *Pfmdr-2*, ferredoxin, *Pfcrt* [17], etc.), are providing compensatory fitness for K-13 mutant parasites or perhaps conferring partner drug resistance.

4. Resistance facilitates the transmission potential

For the newly selected resistant parasites to be propagated, the recrudescent infection is essential [102]. The threshold for successful transmission of malaria is around six viable gametocytes in one blood meal [103]. Post-treatment gametocytaemia is a composite of ongoing gametocytogenesis despite treatment (especially with ineffective drug) and the release of sequestered gametocytes, which is enhanced by drug-induced stress [104]. If the malaria infection is treated with partially effective drugs, post-treatment gametocytaemia is more likely. This was clearly shown for drugs such as CHQ and SP [105] as evidenced in patients with slower parasite clearance after artesunate treatment [106]. Moreover, mutant isolates were also related to pre- and post-treatment gametocytaemia [107–110] and hence possess transmission advantage (**Figure 3**).

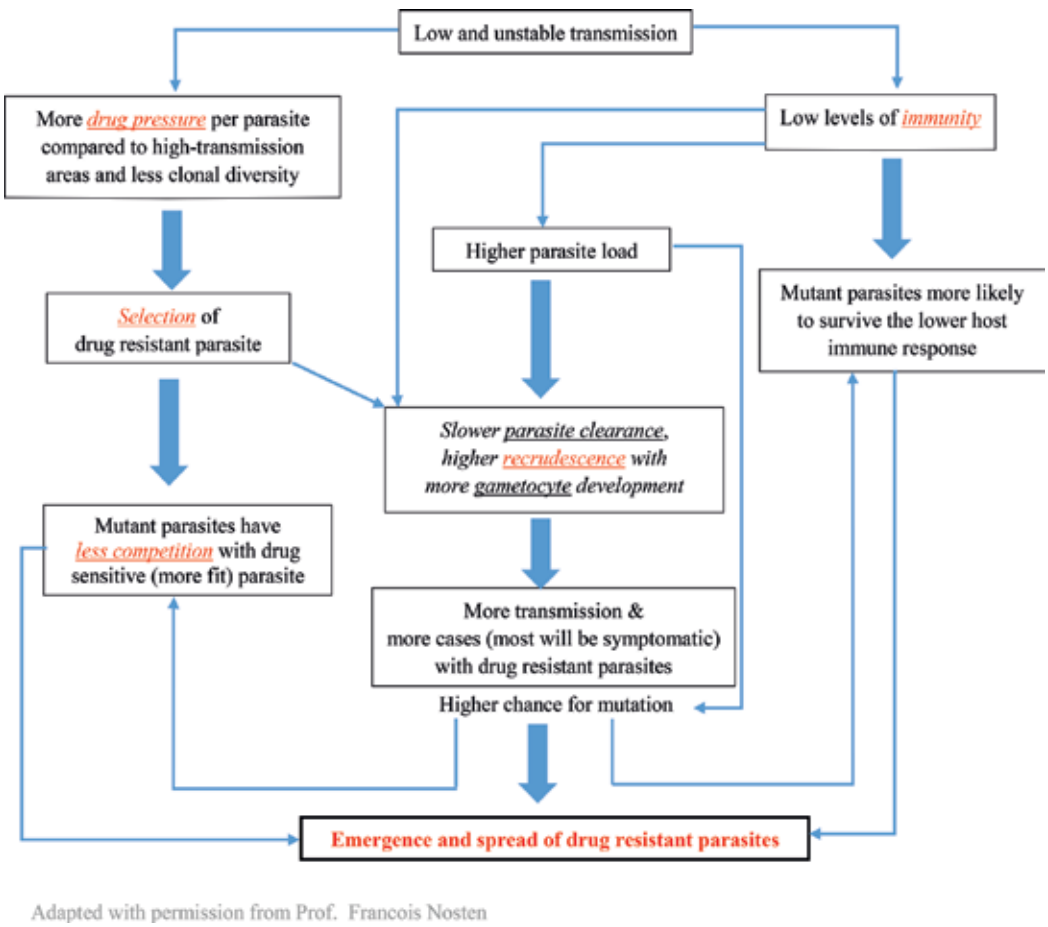


Figure 3. Postulated flow chart of emergence/spread of drug resistance (copyright permission from Prof Francois Nosten).

5. Prospects of elimination

With the declining transmission of malaria, the geographic clustering of both clinical and asymptomatic infections has become more apparent. Asymptomatic carriers represent a "reservoir" of parasites that are difficult to detect because the density of parasites is often below the sensitivity threshold of conventional diagnostic tools (Rapid Diagnostic Tests and microscopy). The size of these reservoirs of sub-microscopic infections (also called "hot-spots") can vary from a few households to large geographical areas. Clustering of these hotspots becomes more pronounced as transmission declines [111]. While considering malaria elimination, radical depletion of parasite reservoir (asymptomatic carriers with sub-microscopic parasitaemia) and gametocytes is a necessity. This can be achieved by two functional components: (1) early diagnosis with treatment (EDT) of the symptomatic patients (preferably within 48 hour of symptoms before the development of gametocytaemia) and (2) early detection and treatment targeting the reservoirs of sub-microscopic infections through Mass Drug Administration (MDA) [112, 113].

The intervention for the first element is to set up or reinforce and sustain malaria control program hence reducing the number of clinical episodes as much as possible through increased access to EDT where the use of efficacious antimalarial regimen is critical [114]. As the drug resistance worsen, the rising number of clinical cases due to increasing gametocyte carriage in the community will be inevitable. MDA or mass screening and treatment (MSAT) is only accelerating the malaria elimination alongside EDT, by eliminating the sub-microscopic reservoir [115]. The effectiveness of MDA or MSAT significantly relies on the therapeutic efficacy of the drug in use, the coverage and the total number of rounds of MDA. In turn, this means that a careful and well-conducted community engagement is primordial for enhanced coverage [115, 116].

6. Choice of drug for malaria elimination: is the pipeline empty?

The current malaria elimination program along the Thai-Myanmar border is using artemether-lumefantrine (AL) for treating the clinical cases at the village malaria posts or by malaria workers [114], whereas dihydroartemisinin-piperaquine (DP) is deployed in MDA activities [117]. In this area, the third ACT, mefloquine-artesunate combination, is already failing [25], and the prospect of elimination program is highly dependent on the therapeutic efficacy of AL and DP. Recent emergence of piperaquine resistance following the artemisinin resistance has depleted the available ACTs to be deployed in malaria elimination programs. High failure rates of AL in Laos PDR and DP in Vietnam and Cambodia have cast doubts on the optimism of malaria elimination [10, 26, 87, 118].

There are very few new compounds in the development pipeline. The front runners are OZ439, a synthetic endoperoxide, structurally related to artemisinin, and KAF156 belonging to a new class of antimalarial (imidazolopiperazines) and the spiroindolone cipargamin (formerly KAE609). However, these short-acting drugs will have to be deployed in combination therapies and their full development will take many years.

http://dx.doi.org/10.5772/intechopen.76519

As a stopgap measure, two triple ACTs (mefloquine plus DP and amodiaquine plus AL) are under multicentre trial, using the inverse correlation between susceptibility to amodiaquine and lumefantrine as well as between piperaquine and mefloquine. The trial has completed the patient recruitment and the results are promising with high cure rates. However, recent increasing prevalence of parasite isolates with potential resistance to both mefloquine and piperaquine has questioned the longevity of the triple ACT [20].

7. Drug resistance in *P. vivax*

For the *P. vivax*, chloroquine remains the first line of treatment in majority of the endemic countries. However, after the first report from Papua New Guinea in 1989, chloroquine resistance has reached northern Papua and Indonesia. Later on, data with recurrences (by day-28 of chloroquine treatment) greater than 10% have also been reported from Myanmar, Thailand, Cambodia, India, Vietnam, Turkey, South America, Ethiopia and Madagascar [119]. Resistance in *P. vivax* is more difficult to document than for *P. falciparum* because of the relapses from liver stages. The most robust proof of resistance is given when a circulating parasite is detected in the peripheral blood in the presence of therapeutic chloroquine concentrations (i.e. >100 ng/ml). The absence of long-term parasite culture for *P. vivax* further complicates the efficacy testing in the laboratory, but short-term assays have been developed in recent years.

8. Regional artemisinin resistance initiative (RAI)

The six countries of the Greater Mekong Subregion (GMS), Thailand, Myanmar, Cambodia, Laos, Vietnam and China (Yunnan Province), are part of a larger community, the Association of Southeast Asian Nations (ASEAN). Despite political pledges to fight artemisinin resistance and eliminate malaria, coordination remains hampered by deep political, economic and geographical gaps. The WHO strategic plans to counter artemisinin resistance failed to prevent its spread to the entire sub-region. In 2013, the Global Fund launched the Regional Artemisinin-resistance Initiative to provide financial support to the five countries affected by this new treat. This initiative came in addition to the contributions of the Global Fund to the Malaria National Program and contributed to the decrease in malaria-related mortality and morbidity in the region. However, these efforts have been compromised by the fragmentation in the public health policies, the disparities in the infrastructures and human resources as well as corruption. In terms of treatment policies, all GMS countries had already adopted ACTs long before the emergence of resistance, but poor monitoring in some countries meant that monotherapies and sub-standard or counterfeit drugs continued to circulate until recently. The relative absence of entomological data in some parts of SEA explains that there is no coherent strategy for containment of local disease vectors. Large budgets continue to be spent on long-lasting impregnated nets (LLINs) despite the absence of evidence of their effectiveness.

9. Conclusions

Artemisinin resistance in *P. falciparum* has emerged 10 years ago in SEA and spread in the entire GMS. Parasite populations resistant to all ACTs are now circulating in Cambodia, triggering a resurgence of the disease. Current gains in malaria control/elimination program are heavily relying upon the efficacy of ACTs. The emergence of artemisinin and partner drug resistance is a serious threat to the global prospect of malaria elimination. The recent decline in the number of clinical cases in the region is encouraging but by no means a victory. Current resurgence of malaria in Cambodia and the existence of large reservoirs of sub-microscopic infections must be seen as warnings that malaria could make a devastating comeback. Efforts must continue and accelerate to eliminate the parasite and this will only be possible with stronger political will and sustained financial support. The three main programmatic components are EDT, elimination of the reservoirs and adapted vector control measures. The few antimalarials in the development pipeline are promising, though these compounds will not be ready on time to replace the ACTs [120]. The spread of the ACT-resistant malaria has so far outpaced the malaria containment measures and time is running out. There are not many options but to accelerate the current malaria elimination efforts.

Acknowledgements

We acknowledge the insightful suggestions of two editors from InTech Open Access. The Shoklo Malaria Research Unit and Myanmar Oxford Clinical Research Unit are part of the Mahidol Oxford Tropical Medicine Research Unit-Tropical health network funded by the Wellcome Trust.

List of acronyms

ACT	artemisinin combination therapy
AL	artemether-lumefantrine
ASEAN	Association of Southeast Asian Nations
CNV	(gene) copy number variation
CHQ	chloroquine
DDT	dichlorodiphenyltrichloroethane
DP	dihydroartemisinin-piperaquine
EDT	early diagnosis and treatment
GMS	Greater Mekong Subregion
K-13	Kelch 13 gene of *P. falciparum*

LLINs	long-lasting impregnated nets
LMF	lumefantrine
MDA	mass drug administration
MFQ	mefloquine
MSAT	mass screening and treatment
Pfcrt	*P. falciparum* chloroquine resistance transporter
Pfmdr-1	*P. falciparum* multi-drug resistant gene-1
PfPI3K	*P. falciparum* phosphatidylinositol-3-kinase
Pgh1	P-glycoprotein homologue 1
RBC	red blood cell
SEA	Southeast Asia
SNP	single nucleotide polymorphism
SP	sulfadoxine-pyrimethamine
UPR	unfolded protein response pathway (UPR)
WWARN	Worldwide Antimalarial Resistance Network
WHO	World Health Organisation

Author details

Aung Pyae Phyo[1,2]* and François Nosten[1,3]

*Address all correspondence to: aungpyaephyo@shoklo-unit.com

1 Shoklo Malaria Research Unit, Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Mae Sot, Thailand

2 Myanmar Oxford Clinical Research Unit, Yangon, Myanmar

3 Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, UK

References

[1] Plowe CV, Wellems TE. Molecular approaches to the spreading problem of drug resistant malaria. In: Jungkind DL, Mortensen JE, Fraimow HS, Calandra GB, editors. Antimicrobial Resistance: A Crisis in Health Care. Boston, MA, USA: Springer; 1995. pp. 197-209

[2] Wellems TE, Plowe CV. Chloroquine-resistant malaria. The Journal of Infectious Diseases. 2001;**184**(6):770-776

[3] Najera JA, Gonzalez-Silva M, Alonso PL. Some lessons for the future from the global malaria eradication programme (1955-1969). PLoS Medicine. 2011;**8**(1):e1000412

[4] Wellems TE, Hayton K, Fairhurst RM. The impact of malaria parasitism: From corpuscles to communities. The Journal of Clinical Investigation. 2009;**119**(9):2496-2505

[5] Wongsrichanalai C, Pickard AL, Wernsdorfer WH, Meshnick SR. Epidemiology of drug-resistant malaria. The Lancet Infectious Diseases. 2002;**2**(4):209-218

[6] Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. The New England Journal of Medicine. 2009; **361**(5):455-467

[7] Nosten F, ter Kuile F, Chongsuphajaisiddhi T, Luxemburger C, Webster HK, Edstein M, et al. Mefloquine-resistant falciparum malaria on the Thai-Burmese border. Lancet. 1991;**337**(8750):1140-1143

[8] Boudreau EF, Webster HK, Pavanand K, Thosingha L. Type II mefloquine resistance in Thailand. Lancet. 1982;**2**(8311):1335

[9] Gates Foundation. Available from: https://www.gatesfoundation.org/What-We-Do/Global-Health/Malaria

[10] WHO. World malaria report 2017. Geneva: World Health Organization; 2017

[11] Culleton RL, Abkallo HM. Malaria parasite genetics: Doing something useful. Parasitology International. 2015;**64**(3):244-253

[12] White NJ. Antimalarial drug resistance. The Journal of Clinical Investigation. 2004; **113**(8):1084-1092

[13] Maïga O, Djimdé AA, Hubert V, Renard E, Aubouy A, Kironde F, et al. A shared Asian origin of the triple-mutant dhfr allele in *Plasmodium falciparum* from sites across Africa. Journal of Infectious Diseases. 2007;**196**(1):165-172

[14] Roper C, Pearce R, Nair S, Sharp B, Nosten F, Anderson T. Intercontinental spread of pyrimethamine-resistant malaria. Science. 2004;**305**

[15] Cerqueira GC, Cheeseman IH, Schaffner SF, Nair S, McDew-White M, Phyo AP, et al. Longitudinal genomic surveillance of *Plasmodium falciparum* malaria parasites reveals complex genomic architecture of emerging artemisinin resistance. Genome Biology. 2017;**18**(1):78

[16] Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, Khim N, et al. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. Nature. 2014;**505** (7481):50-55

[17] Miotto O, Amato R, Ashley EA, MacInnis B, Almagro-Garcia J, Amaratunga C, et al. Genetic architecture of artemisinin-resistant *Plasmodium falciparum*. Nature Genetics. 2015;**47**(3):226-234

[18] Thomsen TT, Madsen LB, Hansson HH, Tomás EV, Charlwood D, Bygbjerg IC, et al. Rapid selection of *Plasmodium falciparum* chloroquine resistance transporter gene and multidrug resistance gene-1 haplotypes associated with past chloroquine and present artemether-lumefantrine use in Inhambane District, southern Mozambique. The American Journal of Tropical Medicine and Hygiene. 2013;**88**(3):536-541

[19] Kublin JG, Cortese JF, Njunju EM, Mukadam G, RA WJJ, Kazembe PN, et al. Reemergence of chloroquine-sensitive *Plasmodium falciparum* malaria after cessation of chloroquine use in Malawi. The Journal of Infectious Diseases. 2003;**187**(12):1870-1875

[20] Rossi G, De Smet M, Khim N, Kindermans J-M, Menard D. Emergence of *Plasmodium falciparum* triple mutant in Cambodia. The Lancet Infectious Diseases. 2017;**17**(12):1233

[21] Payne D. Did medicated salt hasten the spread of chloroquine resistance in *Plasmodium falciparum*? Parasitology Today. 1988;**4**(4):112-115

[22] Verdrager J. Localized permanent epidemics: The genesis of chloroquine resistance in *Plasmodium falciparum*. The Southeast Asian Journal of Tropical Medicine and Public Health. 1995;**26**(1):23-28

[23] Verdrager J. Epidemiology of the emergence and spread of drug-resistant falciparum malaria in South-East Asia and Australasia. Journal of Tropical Medicine and Hygiene. 1986;**89**(6):277-289

[24] Ataíde R, Powell R, Moore K, McLean A, Phyo AP, Nair S, et al. Declining transmission and immunity to malaria and emerging artemisinin resistance in Thailand: A longitudinal study. The Journal of Infectious Diseases. Sep 15, 2017;**216**(6):723-731

[25] Phyo AP, Ashley EA, Anderson TJC, et al. Declining efficacy of artemisinin combination therapy against *P. falciparum* malaria on the Thai-Myanmar Border (2003-2013): The role of parasite genetic factors. Clinical Infectious Diseases. 2016;**63**:784-791

[26] Amato R, Lim P, Miotto O, et al. Genetic markers associated with dihydroartemisinin-piperaquine failure in *Plasmodium falciparum* malaria in Cambodia: A genotype–phenotype association study. The Lancet Infectious Diseases. 2017;**17**:164-173

[27] Simpson BB, Conner-Ogorzaly M. Economic Botany: Plants in our World. 3rd ed. Dubuque, Iowa: McGraw-Hill; 2000

[28] da Silva AF, Benchimol JL. Malaria and quinine resistance: A medical and scientific issue between Brazil and Germany (1907-1919). Medical History. 2014;**58**(1):1-26

[29] Werner H. Beobachtungen über relative Chininresistenz bei Malaria aus Brasilien. Deutsche Medizinische Wochenschrift. 1910;**36**(34):1557-1560

[30] Ménard D, Andriantsoanirina V, Khim N, Ratsimbasoa A, Witkowski B, Benedet C, et al. Global analysis of *Plasmodium falciparum* Na(+)/H(+) exchanger (pfnhe-1) allele polymorphism and its usefulness as a marker of *In vitro* resistance to quinine. International Journal for Parasitology, Drugs and Drug Resistance. 2013;**3**:8-19

[31] Menard D, Yapou F, Manirakiza A, Djalle D, Matsika-Claquin MD, Talarmin A. Polymorphisms in pfcrt, pfmdr1, dhfr genes and *In vitro* responses to antimalarials in *Plasmodium falciparum* isolates from Bangui, Central African Republic. The American Journal of Tropical Medicine and Hygiene. 2006;**75**(3):381-387

[32] Mayxay M, Barends M, Brockman A, Jaidee A, Nair S, Sudimack D, et al. *In vitro* antimalarial drug susceptibility and pfcrt mutation among fresh *Plasmodium falciparum* isolates from the Lao PDR (Laos). The American Journal of Tropical Medicine and Hygiene. 2007;**76**(2):245-250

[33] Legrand E, Volney B, Meynard J-B, Mercereau-Puijalon O, Esterre P. *In vitro* monitoring of *Plasmodium falciparum* drug resistance in French Guiana: A synopsis of continuous assessment from 1994 to 2005. Antimicrobial Agents and Chemotherapy. 2008;**52**(1):288-298

[34] Achan J, Talisuna AO, Erhart A, Yeka A, Tibenderana JK, Baliraine FN, et al. Quinine, an old anti-malarial drug in a modern world: Role in the treatment of malaria. Malaria Journal. 2011;**10**(1):144

[35] Myint HY, Tipmanee P, Nosten F, Day NP, Pukrittayakamee S, Looareesuwan S, et al. A systematic overview of published antimalarial drug trials. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2004;**98**(2):73-81

[36] Coatney GR. Pitfalls in a discovery: The chronicle of chloroquine. The American Journal of Tropical Medicine and Hygiene. 1963;**12**:121-128

[37] Ecker A, Lehane AM, Clain J, Fidock DA. PfCRT and its role in antimalarial drug resistance. Trends in Parasitology. 2012;**28**(11):504-514

[38] Fidock DA, Nomura T, Talley AK, Cooper RA, Dzekunov SM, Ferdig MT, et al. Mutations in the *P. falciparum* digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. Molecular Cell. 2000;**6**(4):861-871

[39] Dzekunov SM, Ursos LMB, Roepe PD. Digestive vacuolar pH of intact intraerythrocytic *P. falciparum* either sensitive or resistant to chloroquine. Molecular and Biochemical Parasitology. 2000;**110**(1):107-124

[40] Ariey F, Fandeur T, Durand R, Randrianarivelojosia M, Jambou R, Legrand E, et al. Invasion of Africa by a single pfcrt allele of south East Asian type. Malaria Journal. 2006;**5**(1):34

[41] Payne D. Spread of chloroquine resistance in *Plasmodium falciparum*. Parasitology Today. 1987;**3**(8):241-246

[42] Peters W. Resistance in human malaria IV: 4-aminoquinolines and multiple resistance. Chemotherapy and Drug Resistance in Malaria. 1987;**2**:659-786

[43] Wongsrichanalai C, Sirichaisinthop J, Karwacki JJ, Congpuong K, Miller RS, Pang L, et al. Drug resistant malaria on the Thai-Myanmar and Thai-Cambodian borders. The Southeast Asian Journal of Tropical Medicine and Public Health. 2001;**32**(1):41-49

[44] Pinichpongse S, Doberstyn EB, Cullen JR, Yisunsri L, Thongsombun Y, Thimasarn K. An evaluation of five regimens for the outpatient therapy of falciparum malaria in Thailand 1980-81. Bulletin of the World Health Organization. 1982;**60**(6):907-912

[45] Cowman AF, Morry MJ, Biggs BA, Cross GA, Foote SJ. Amino acid changes linked to pyrimethamine resistance in the dihydrofolate reductase-thymidylate synthase gene of *Plasmodium falciparum*. Proceedings of the National Academy of Sciences of the United States of America. 1988;**85**(23):9109-9113

[46] Peterson DS, Walliker D, Wellems TE. Evidence that a point mutation in dihydrofolate reductase-thymidylate synthase confers resistance to pyrimethamine in falciparum malaria. Proceedings of the National Academy of Sciences of the United States of America. 1988;**85**(23):9114-9118

[47] Sirawaraporn W, Sathitkul T, Sirawaraporn R, Yuthavong Y, Santi DV. Antifolate-resistant mutants of *Plasmodium falciparum* dihydrofolate reductase. Proceedings of the National Academy of Sciences of the United States of America. 1997;**94**(4):1124-1129

[48] Brooks DR, Wang P, Read M, Watkins WM, Sims PF, Hyde JE. Sequence variation of the hydroxymethyldihydropterin pyrophosphokinase: Dihydropteroate synthase gene in lines of the human malaria parasite, *Plasmodium falciparum*, with differing resistance to sulfadoxine. European Journal of Biochemistry/FEBS. 1994;**224**(2):397-405

[49] Triglia T, Cowman AF. Primary structure and expression of the dihydropteroate synthetase gene of *Plasmodium falciparum*. Proceedings of the National Academy of Sciences of the United States of America. 1994;**91**(15):7149-7153

[50] Nair S, Williams JT, Brockman A, Paiphun L, Mayxay M, Newton PN, et al. A selective sweep driven by pyrimethamine treatment in southeast asian malaria parasites. Molecular Biology and Evolution. 2003;**20**(9):1526-1536

[51] Roper C, Pearce R, Bredenkamp B, Gumede J, Drakeley C, Mosha F, et al. Antifolate antimalarial resistance in Southeast Africa: A population-based analysis. The Lancet. 2003;**361**(9364):1174-1181

[52] Venkatesan M, Gadalla NB, Stepniewska K, Dahal P, Nsanzabana C, Moriera C, et al. Polymorphisms in *Plasmodium falciparum* chloroquine resistance transporter and multidrug resistance 1 genes: Parasite risk factors that affect treatment outcomes for *P. falciparum* malaria after artemether-lumefantrine and artesunate-amodiaquine. The American Journal of Tropical Medicine and Hygiene. 2014;**91**(4):833-843

[53] Folarin OA, Bustamante C, Gbotosho GO, Sowunmi A, Zalis MG, Oduola AMJ, et al. *In vitro* Amodiaquine resistance and its association with mutations in pfcrt and pfmdr1 genes of *Plasmodium falciparum* isolates from Nigeria. Acta Tropica. 2011;**120**(3):224-230

[54] Happi C, Gbotosho G, Folarin O, Bolaji O, Sowunmi A, Kyle D, et al. Association between mutations in *Plasmodium falciparum* chloroquine resistance transporter and *P. falciparum* multidrug resistance 1 genes and *in vivo* amodiaquine resistance in *P. falciparum* malaria–infected children in Nigeria. The American Journal of Tropical Medicine and Hygiene. 2006;**75**(1):155-161

[55] Holmgren G, Gil JP, Ferreira PM, Veiga MI, Obonyo CO, Björkman A. Amodiaquine resistant *Plasmodium falciparum* malaria *in vivo* is associated with selection of pfcrt 76T and pfmdr1 86Y. Infection, Genetics and Evolution. 2006;**6**(4):309-314

[56] Young MD. Amodiaquine and hydroxychloroquine resistance in *Plasmodium falciparum*. The American Journal of Tropical Medicine and Hygiene. 1961;**10**:689-693

[57] Young MD. Failure of chloroquine and amodiaquine to suppress *Plasmodium falciparum*. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1962;**56**(3):252-256

[58] Gascón J, Soldevila M, Merlos A, Bada JL. Chloroquine and amodiaquine resistant falciparum malaria in Rwanda. The Lancet. 1985;**326**(8463):1072

[59] Doberstyn EB, Phintuyothin P, Noeypatimanondh S, Teerakiartkamjorn C. Single-dose therapy of falciparum malaria with mefloquine or pyrimethamine-sulfadoxine. Bulletin of the World Health Organization. 1979;**57**(2):275-279

[60] Pinichpongse S, Suebsaeng L, Malikul S, Doberstyn E, Rooney W. The operational introduction of mefloquine, a new anti-malarial drug by the malaria program of Thailand. The Journal of Communicable Diseases – Thai. 1987;**13**:411-424

[61] Wongsrichanalai C, Prajakwong S, Meshnick SR, Shanks GD, Thimasarn K. Mefloquine – Its 20 years in the Thai malaria control program. The Southeast Asian Journal of Tropical Medicine and Public Health. 2004;**35**(2):300-308

[62] Thimasarn K, Sirichaisinthop J, Vijaykadga S, Tansophalaks S, Yamokgul P, Laomiphol A, et al. *In vivo* study of the response of *Plasmodium falciparum* to standard mefloquine/sulfadoxine/pyrimethamine (MSP) treatment among gem miners returning from Cambodia. The Southeast Asian Journal of Tropical Medicine and Public Health. 1995;**26**(2):204-212

[63] Nosten F, Imvithaya S, Vincenti M, Delmas G, Lebihan G, Hausler B, et al. Malaria on the Thai-Burmese border: Treatment of 5192 patients with mefloquine-sulfadoxine-pyrimethamine. Bulletin of the World Health Organization. 1987;**65**(6):891-896

[64] Anderson TJ, Patel J, Ferdig MT. Gene copy number and malaria biology. Trends in Parasitology. 2009;**25**(7):336-343

[65] Alker AP, Lim P, Sem R, Shah NK, Yi P, Bouth DM, et al. Pfmdr1 and *in vivo* resistance to artesunate-mefloquine in falciparum malaria on the Cambodian-Thai border. The American Journal of Tropical Medicine and Hygiene. 2007;**76**(4):641-647

[66] Lim P, Alker AP, Khim N, Shah NK, Incardona S, Doung S, et al. Pfmdr1 copy number and arteminisin derivatives combination therapy failure in falciparum malaria in Cambodia. Malaria Journal. 2009;**8**:11

[67] Price RN, Uhlemann AC, Brockman A, McGready R, Ashley E, Phaipun L, et al. Mefloquine resistance in *Plasmodium falciparum* and increased pfmdr1 gene copy number. Lancet. 2004;**364**(9432):438-447

[68] Price RN, Cassar C, Brockman A, Duraisingh M, van Vugt M, White NJ, et al. The pfmdr1 gene is associated with a multidrug-resistant phenotype in *Plasmodium falciparum*

from the western border of Thailand. Antimicrobial Agents and Chemotherapy. 1999; **43**(12):2943-2949

[69] Preechapornkul P, Imwong M, Chotivanich K, Pongtavornpinyo W, Dondorp AM, Day NP, et al. *Plasmodium falciparum* pfmdr1 amplification, mefloquine resistance, and parasite fitness. Antimicrobial Agents and Chemotherapy. 2009;**53**(4):1509-1515

[70] Sidhu AB, Uhlemann AC, Valderramos SG, Valderramos JC, Krishna S, Fidock DA. Decreasing pfmdr1 copy number in *Plasmodium falciparum* malaria heightens susceptibility to mefloquine, lumefantrine, halofantrine, quinine, and artemisinin. The Journal of Infectious Diseases. 2006;**194**(4):528-535

[71] Lim P, Dek D, Try V, Sreng S, Suon S, Fairhurst RM. Decreasing pfmdr1 copy number suggests that *Plasmodium falciparum* in Western Cambodia is regaining *In vitro* susceptibility to mefloquine. Antimicrobial Agents and Chemotherapy. 2015;**59**(5):2934-2937

[72] Looareesuwan S, Chulay JD, Canfield CJ, Hutchinson DB. Malarone (atovaquone-proguanil hydrochloride): A review of its clinical development for treatment of malaria. Malarone Clinical Trials Study Group. The American Society of Tropical Medicine and Hygiene. 1999;**60**(4):533-541

[73] Looareesuwan S, Viravan C, Webster HK, Kyle DE, Hutchinson DB, Canfield CJ. Clinical studies of atovaquone, alone or in combination with other antimalarial drugs, for treatment of acute uncomplicated malaria in Thailand. The American Journal of Tropical Medicine and Hygiene. 1996;**54**(1):62-66

[74] Gil JP, Nogueira F, Stromberg-Norklit J, Lindberg J, Carrolo M, Casimiro C, et al. Detection of atovaquone and Malarone resistance conferring mutations in *Plasmodium falciparum* cytochrome b gene (cytb). Molecular and Cellular Probes. 2003;**17**(2-3):85-89

[75] Korsinczky M, Chen N, Kotecka B, Saul A, Rieckmann K, Cheng Q. Mutations in *Plasmodium falciparum* cytochrome b that are associated with atovaquone resistance are located at a putative drug-binding site. Antimicrobial Agents and Chemotherapy. 2000;**44**(8):2100-2108

[76] Gassis S, Rathod PK. Frequency of drug resistance in *Plasmodium falciparum*: A nonsynergistic combination of 5-fluoroorotate and atovaquone suppresses *In vitro* resistance. Antimicrobial Agents and Chemotherapy. 1996;**40**(4):914-919

[77] Rathod PK, McErlean T, Lee PC. Variations in frequencies of drug resistance in *Plasmodium falciparum*. Proceedings of the National Academy of Sciences of the United States of America. 1997;**94**(17):9389-9393

[78] Srivastava IK, Morrisey JM, Darrouzet E, Daldal F, Vaidya AB. Resistance mutations reveal the atovaquone-binding domain of cytochrome b in malaria parasites. Molecular Microbiology. 1999;**33**(4):704-711

[79] Pradines B, Mamfoumbi MM, Parzy D, Medang MO, Lebeau C, Mbina JM, et al. *In vitro* susceptibility of African isolates of *Plasmodium falciparum* from Gabon to pyronaridine. The American Journal of Tropical Medicine and Hygiene. 1999;**60**(1):105-108

[80] Wells TN, van Huijsduijnen RH, Van Voorhis WC. Malaria medicines: A glass half full? Nature Reviews Drug Discovery. 2015;**14**(6):424-442

[81] Leang R, Canavati SE, Khim N, Vestergaard LS, Fuhrer IB, Kim S, et al. Efficacy and safety of pyronaridine-artesunate for treatment of uncomplicated *Plasmodium falciparum* malaria in western Cambodia. Antimicrobial Agents and Chemotherapy. 2016;**60**(7):3884-3890

[82] Pascual A, Madamet M, Bertaux L, Amalvict R, Benoit N, Travers D, et al. *In vitro* piperaquine susceptibility is not associated with the *Plasmodium falciparum* chloroquine resistance transporter gene. Malaria Journal. 2013;**12**(1):1-6

[83] Briolant S, Henry M, Oeuvray C, Amalvict R, Baret E, Didillon E, et al. Absence of association between piperaquine *In vitro* responses and polymorphisms in the pfcrt, pfmdr1, pfmrp, and pfnhe genes in *Plasmodium falciparum*. Antimicrobial Agents and Chemotherapy. 2010;**54**

[84] Eastman RT, Dharia NV, Winzeler EA, Fidock DA. Piperaquine resistance is associated with a copy number variation on chromosome 5 in drug-pressured *Plasmodium falciparum* parasites. Antimicrobial Agents and Chemotherapy. 2011;**55**(8):3908-3916

[85] Duru V, Khim N, Leang R, Kim S, Domergue A, Kloeung N, et al. *Plasmodium falciparum* dihydroartemisinin-piperaquine failures in Cambodia are associated with mutant K13 parasites presenting high survival rates in novel piperaquine *In vitro* assays: Retrospective and prospective investigations. BMC Medicine. 2015;**13**(1):1

[86] Amaratunga C, Lim P, Suon S, Sreng S, Mao S, Sopha C, et al. Dihydroartemisinin–piperaquine resistance in *Plasmodium falciparum* malaria in Cambodia: A multisite prospective cohort study. The Lancet Infectious Diseases. 2016;**16**(3):357-365

[87] Witkowski B, Duru V, Khim N, Ross LS, Saintpierre B, Beghain J, et al. A surrogate marker of piperaquine-resistant *Plasmodium falciparum* malaria: A phenotype–genotype association study. The Lancet Infectious Diseases. 2017;**17**(2):174-183

[88] Haldar K, Bhattacharjee S, Safeukui I. Drug resistance in *Plasmodium*. Nature Reviews Microbiology. 2018;**16**(3):156-170

[89] Mohon AN, Alam MS, Bayih AG, Folefoc A, Shahinas D, Haque R, et al. Mutations in *Plasmodium falciparum* K13 propeller gene from Bangladesh (2009-2013). Malaria Journal. 2014;**13**:431

[90] Mita T, Tachibana S-I, Hashimoto M, Hirai M. *Plasmodium falciparum* kelch 13: A potential molecular marker for tackling artemisinin-resistant malaria parasites. Expert Review of Anti-infective Therapy. 2016;**14**(1):125-135

[91] Mbengue A, Bhattacharjee S, Pandharkar T, Liu H, Estiu G, Stahelin RV, et al. A molecular mechanism of artemisinin resistance in *Plasmodium falciparum* malaria. Nature. 2015;**520**(7549):683-687

[92] Bhattacharjee S, Stahelin RV, Speicher KD, Speicher DW, Haldar K. Endoplasmic reticulum PI(3)P lipid binding targets malaria proteins to the host cell. Cell. 2012;**148**(1-2):201-212

[93] Tawk L, Chicanne G, Dubremetz JF, Richard V, Payrastre B, Vial HJ, et al. Phosphatidylinositol 3-phosphate, an essential lipid in *Plasmodium*, localizes to the food vacuole membrane and the apicoplast. Eukaryotic Cell. 2010;**9**(10):1519-1530

[94] Vaid A, Ranjan R, Smythe WA, Hoppe HC, Sharma P. PfPI3K, a phosphatidylinositol-3 kinase from *Plasmodium falciparum*, is exported to the host erythrocyte and is involved in hemoglobin trafficking. Blood. 2010;**115**(12):2500-2507

[95] Cheeseman IH, Miller BA, Nair S, Nkhoma S, Tan A, Tan JC, et al. A major genome region underlying artemisinin resistance in malaria. Science. 2012;**336**(6077):79-82

[96] Miotto O, Almagro-Garcia J, Manske M, Macinnis B, Campino S, Rockett KA, et al. Multiple populations of artemisinin-resistant *Plasmodium falciparum* in Cambodia. Nature Genetics. 2013;**45**(6):648-655

[97] Takala-Harrison S, Clark TG, Jacob CG, Cummings MP, Miotto O, Dondorp AM, et al. Genetic loci associated with delayed clearance of *Plasmodium falciparum* following artemisinin treatment in Southeast Asia. Proceedings of the National Academy of Sciences of the United States of America. 2013;**110**(1):240-245

[98] Painter HJ, Campbell TL, Llinas M. The Apicomplexan AP2 family: Integral factors regulating *Plasmodium* development. Molecular and Biochemical Parasitology. 2011;**176**(1):1-7

[99] Mok S, Ashley EA, Ferreira PE, Zhu L, Lin Z, Yeo T, et al. Drug resistance. Population transcriptomics of human malaria parasites reveals the mechanism of artemisinin resistance. Science. 2015;**347**(6220):431-435

[100] Nosten F. Waking the sleeping beauty. Journal of Infectious Diseases. 2010;**202**(9): 1300-1301

[101] Anderson TJ, Nair S, McDew-White M, Cheeseman IH, Nkhoma S, Bilgic F, et al. Population parameters underlying an ongoing soft sweep in southeast Asian malaria parasites. Molecular Biology and Evolution. Jan 2017;**34**(1):131-144

[102] White N. Antimalarial drug resistance and combination chemotherapy. Philosophical Transactions of the Royal Society of London Series B, Biological Sciences. 1999;**354** (1384):739-749

[103] Jeffery GM, Eyles DE. Infectivity to mosquitoes of *Plasmodium falciparum* as related to gametocyte density and duration of infection. The American Journal of Tropical Medicine and Hygiene. 1955;**4**(5):781-789

[104] Bousema T, Drakeley C. Epidemiology and infectivity of *Plasmodium falciparum* and *Plasmodium vivax* gametocytes in relation to malaria control and elimination. Clinical Microbiology Reviews. 2011;**24**(2):377-410

[105] Barnes KI, White NJ. Population biology and antimalarial resistance: The transmission of antimalarial drug resistance in *Plasmodium falciparum*. Acta Tropica. 2005;**94**(3): 230-240

[106] Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, et al. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. The New England Journal of Medicine. 2014;**371**(5):411-423

[107] Barnes KI, Little F, Mabuza A, Mngomezulu N, Govere J, Durrheim D, et al. Increased gametocytemia after treatment: An early parasitological indicator of emerging sulfadoxine-pyrimethamine resistance in falciparum malaria. The Journal of Infectious Diseases. 2008;**197**(11):1605-1613

[108] Hallett RL, Dunyo S, Ord R, Jawara M, Pinder M, Randall A, et al. Chloroquine/sulphadoxine-pyrimethamine for gambian children with malaria: Transmission to mosquitoes of multidrug-resistant *Plasmodium falciparum*. PLoS Clinical Trials. 2006;**1**(3):e15

[109] Méndez F, Muñoz Á, Carrasquilla G, Jurado D, Arévalo-Herrera M, Cortese JF, et al. Determinants of treatment response to sulfadoxine-pyrimethamine and subsequent transmission potential in falciparum malaria. American Journal of Epidemiology. 2002;**156**(3):230-238

[110] Price R, Nosten F, Simpson JA, Luxemburger C, Phaipun L, Ter Kuile F, et al. Risk factors for gametocyte carriage in uncomplicated falciparum malaria. The American Journal of Tropical Medicine and Hygiene. 1999;**60**(6):1019-1023

[111] Bousema T, Griffin JT, Sauerwein RW, Smith DL, Churcher TS, Takken W, et al. Hitting hotspots: Spatial targeting of malaria for control and elimination. PLoS Medicine. 2012;**9**(1):e1001165

[112] Cotter C, Sturrock HJ, Hsiang MS, Liu J, Phillips AA, Hwang J, et al. The changing epidemiology of malaria elimination: New strategies for new challenges. Lancet. 2013;**382**(9895):900-911

[113] Sturrock HJ, Hsiang MS, Cohen JM, Smith DL, Greenhouse B, Bousema T. Targeting asymptomatic malaria infections: Active surveillance in control and elimination. PLoS Medicine. 2013;**10**(6):e1001467

[114] Landier J, Parker DM, Thu AM, Carrara VI, Lwin KM, Bonnington CA, et al. The role of early detection and treatment in malaria elimination. Malaria Journal. 2016;**15**(1):363

[115] Kajeechiwa L, Thwin MM, Nosten S, Tun SW, Parker D, von Seidlein L, et al. Community engagement for the rapid elimination of malaria: The case of Kayin State, Myanmar. Wellcome Open Research. Jul 28, 2017;**2**:59

[116] Landier J, Parker D, Thu AM, Lwin KM, Delmas G, Nosten F. A regional *P. falciparum* malaria elimination programme in Eastern Myanmar: Impact of generalized access to early diagnosis and treatment and targeted mass drug administration. The Lancet. 2018 (in press)

[117] Parker DM, Landier J, Thu AM, Lwin KM, Delmas G, Nosten FH. Scale up of a *Plasmodium falciparum* elimination program and surveillance system in Kayin State, Myanmar. Wellcome Open Research. 2017;**2**:98

[118] Thanh NV, Thuy-Nhien N, Tuyen NTK, Tong NT, Nha-Ca NT, Quang HH, et al. Rapid decline in the susceptibility of *Plasmodium falciparum* to dihydroartemisinin–piperaquine in the south of Vietnam. Malaria Journal. 2017;**16**(1):27

[119] Price RN, von Seidlein L, Valecha N, Nosten F, Baird JK, White NJ. Global extent of chloroquine-resistant *Plasmodium vivax*: A systematic review and meta-analysis. The Lancet Infectious Diseases. 2014;**14**(10):982-991

[120] Phyo AP, Seidlein L. Challenges to replace ACT as first-line drug. Malaria Journal. 2017;**16**(1):296

[121] WHO. Artemisinin and Artemisinin based Combination Therapy Resistance: World Health Organization. 2016

[122] Srimuang K, Miotto O, Lim P, Fairhurst RM, Kwiatkowski DP, Woodrow CJ, et al. Analysis of anti-malarial resistance markers in pfmdr1 and pfcrt across Southeast Asia in the tracking resistance to Artemisinin collaboration. Malaria Journal. 2016;**15**(1):541

[123] Mishra N, Bharti RS, Mallick P, Singh OP, Srivastava B, Rana R, et al. Emerging polymorphisms in falciparum Kelch 13 gene in Northeastern region of India. Malaria Journal. 2016;**15**(1):583

[124] Tun KM, Jeeyapant A, Imwong M, Thein M, Aung SS, Hlaing TM, et al. Parasite clearance rates in upper Myanmar indicate a distinctive artemisinin resistance phenotype: A therapeutic efficacy study. Malaria Journal. 2016;**15**(1):185

[125] Imwong M, Suwannasin K, Kunasol C, Sutawong K, Mayxay M, Rekol H, et al. The spread of artemisinin-resistant *Plasmodium falciparum* in the greater Mekong subregion: A molecular epidemiology observational study. The Lancet Infectious Diseases. May 2017;**17**(5):491-497

[126] Ye R, Hu D, Zhang Y, Huang Y, Sun X, Wang J, et al. Distinctive origin of artemisinin-resistant *Plasmodium falciparum* on the China-Myanmar border. Scientific Reports. 2016;**6**:20100

[127] Arrow KJ, Panosian C, Gelband H, editors. In: Saving Lives, Buying Time: Economics of Malaria Drugs in an Age of Resistance. Washington (DC): National Academies Press; 2004. ISBN: 0309092183

[128] Wernsdorfer WH, Payne D. The dynamics of drug resistance in *Plasmodium falciparum*. Pharmacology & Therapeutics. 1991;**50**(1):95-121

[129] Maberti S. Desarrollo de Resistencia a Ia pirimetamina. Presentación de 15 casos estudiados en Trujillo, Venezuela. Archivos Venezolanos de Medicina Tropical y Parasitología Médica. 1960;**3**:239-259

[130] Moore DV, Lanier JE. Observations on two *Plasmodium falciparum* infections with an abnormal response to chloroquine. The American Journal of Tropical Medicine and Hygiene. 1961;**10**:5-9

[131] Berliner RW. Studies on the chemotherapy of the human malarias; of the human malarias the physiological disposition, antimalarial activity, and toxicity of several derivatives of 4-aminoquinoline. Journal of Clinical Investigation. 1948;**27**(3 Pt1):98-107

[132] Hoekenga MT. Camoquin treatment of malaria: A preliminary report. The American Journal of Tropical Medicine and Hygiene. 1950;**s1–s30**(1):63-69

[133] Covell G. "Paludrine" (Proguanil) in prophylaxis and treatment of malaria. British Medical Journal. 1949;**1**(4593):88-91

[134] Kay HEM. Resistance to proguanil. The Lancet. 1949;**253**(6556):712

[135] Harinasuta T, Viravan C, Reid HA. Sulphormethoxine in chloroquine-resistant falciparum malaria in Thailand. Lancet. 1967;**1**(7500):1117-1119

[136] Canfield CJ, Hall AP, MacDonald BS, Neuman DA, Shaw JA. Treatment of falciparum malaria from Vietnam with a phenanthrene methanol (WR 33063) and a quinoline methanol (WR 30090). Antimicrobial Agents and Chemotherapy. 1973;**3**(2):224-227

[137] Davis TME, Hung TY, Sim IK, Karunajeewa HA, Ilett KF. Piperaquine: A resurgent antimalarial drug. Drugs. 2005;**65**(1):75-87

[138] Liu DQ. Surveillance of antimalarial drug resistance in China in the 1980s–1990s. Infectious Diseases of Poverty. 2014;**3**(1):8

[139] Tu Y. The discovery of artemisinin (qinghaosu) and gifts from Chinese medicine. Nature Medicine. 2011;**17**(10):1217-1220

[140] Nosten F, Luxemburger C, ter Kuile FO, Woodrow C, Eh JP, Chongsuphajaisiddhi T, et al. Treatment of multidrug-resistant *Plasmodium falciparum* malaria with 3-day artesunate-mefloquine combination. The Journal of Infectious Diseases. 1994;**170**(4):971-977

[141] Wongsrichanalai C, Meshnick SR. Declining artesunate-mefloquine efficacy against falciparum malaria on the Cambodia-Thailand border. Emerging Infectious Diseases. 2008;**14**(5):716-719

[142] Premji ZG. Coartem®: The Journey to the Clinic. Malaria Journal. 2009;**8**(1):S3

[143] Denis MB, Tsuyuoka R, Lim P, Lindegardh N, Yi P, Top SN, et al. Efficacy of artemether-lumefantrine for the treatment of uncomplicated falciparum malaria in Northwest Cambodia. Tropical Medicine & International Health. 2006;**11**(12):1800-1807

[144] Song J, Socheat D, Tan B, Seila S, Xu Y, Ou F, et al. Randomized trials of artemisinin-piperaquine, dihydroartemisinin-piperaquine phosphate and artemether-lumefantrine for the treatment of multi-drug resistant falciparum malaria in Cambodia-Thailand border area. Malaria Journal. 2011;**10**:231

[145] Denis MB, Davis TM, Hewitt S, Incardona S, Nimol K, Fandeur T, et al. Efficacy and safety of dihydroartemisinin-piperaquine (Artekin) in Cambodian children and adults with uncomplicated falciparum malaria. Clinical Infectious Diseases. 2002;**35**(12):1469-1476

[146] Leang R, Taylor WR, Bouth DM, Song L, Tarning J, Char MC, et al. Evidence of *Plasmodium falciparum* malaria multidrug resistance to Artemisinin and Piperaquine in western Cambodia: Dihydroartemisinin-piperaquine open-label multicenter clinical assessment. Antimicrobial Agents and Chemotherapy. 2015;**59**(8):4719-4726

[147] Spring MD, Lin JT, Manning JE, Vanachayangkul P, Somethy S, Bun R, et al. Dihydroartemisinin-piperaquine failure associated with a triple mutant including kelch13 C580Y in Cambodia: An observational cohort study. The Lancet Infectious Diseases. 2015;**15**(6):683-691

Chapter 3

Preparing for the Next Global Threat: A Call for Targeted, Immediate Decisive Action in Southeast Asia to Prevent the Next Pandemic in Africa

Colin Ohrt, Thang Duc Ngo and
Thieu Quang Nguyen

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.78261

Abstract

Global investments have had great impact on malaria—these are now at risk of being reversed. Cambodia is where drug resistance historically emerges and spreads globally to drive resulting pandemics—we are currently watching history repeat itself. Despite large investments and recent success in driving down overall rates of malaria, high levels of resistance to nearly all antimalarial drugs are now widespread in Cambodia. Malaria cases are again rising in both Cambodia and Vietnam. Nearly incurable malaria in this region is a real and present threat. Critical actions to prevent further spread of the emerging incurable parasites are: (1) Commitment and real sense of urgency through declaration of a "Public Health Emergency of International Concern" or a similar set of directives; (2) Establish leadership with sufficient authority, respect, expertise and operational funding; (3) Engage affected security forces to stop disease transmission and support elimination operations; (4) Utilize surveillance as a core intervention with result-based funding targeting malaria transmission foci with rapid and effective action. Immediate decisive action is needed in Southeast Asia to prevent the next malaria pandemic. This chapter highlights persistent gaps in the region with methods to address them. In 2015–2016, our collaboration with NIMPE pilot tested tools to intervene in actual forest transmission foci. Our study district saw a 96% decrease in malaria from 2014 to 2017, with the entire province seeing the largest decrease in Central Vietnam in this same timeframe. We describe methods to tackle transmission foci, with both an integrated prevention and treatment package. We call on all stakeholders to make changes to current investments to address this critical challenge.

Keywords: malaria elimination, multidrug-resistance, surveillance, information systems, public health emergency of international concern (PHEIC), Cambodia, Vietnam, Greater Mekong Subregion (GMS)

1. Introduction

Considerable global and domestic investments have averted millions of malaria-attributable deaths since 2000 [1, 2]. Nevertheless, one person (usually a child) still dies every 1.2 minutes from this disease, with no reduction in mortality between 2015 and 2016 [1]. Progress is further threatened by failure to contain artemisinin resistance, as confirmed by the Regional Artemisinin Initiative (RAI) mid-term review [3]. In Cambodia, between mid-2014 and mid-2015, *Plasmodium falciparum* (Pf) malaria increased by 65%, with further substantial rise in 2017, especially along the border with Vietnam [4]. High rates of clinical treatment failures to nearly all antimalarial drugs—including artemisinin derivatives—are now widespread in the country [3] and there is evidence of its spread into southern Vietnam (from western Cambodia) [5]. Multidrug-resistant malaria parasites could cause a global health catastrophe if they were to spread to other countries, particularly malaria-endemic nations in Africa [4, 6–9]. Spread may be facilitated by international peacekeeping missions and migration for work [10–13]. Given available evidence of this current threat, urgent and decisive action is essential.

We are currently watching history repeat itself. In the late 1950s, resistance to the antimalarial chloroquine emerged in Cambodia. It eventually spread to and throughout Africa during the 1980s [14, 15], resulting in a two- to six-fold increase in malaria-related mortality [14]. Subsequent waves of resistance to two other antimalarial drugs followed the same pattern of spread [6]. It was not until the early 2000s, when international bodies finally took affirmed action against malaria, that mortality rates began to decrease globally. But as the World Malaria Report 2017 states: "*although malaria case incidence has fallen globally since 2010, the rate of decline has stalled and even reversed in some regions since 2014; mortality rates have followed a similar pattern*" [1].

Today, a two-fold increase in mortality [14] would cause in the order of 7 million malaria deaths over a decade. The threat will also be magnified by widespread resistance to insecticides already reported in Africa [16] (again history is repeating itself, in the "malaria eradication" era of 1955–1972, it was DDT resistance [17]). The concomitant threat of insecticide resistance, particularly as it affects the efficacy of insecticide-treated bed nets (ITNs) and household spraying, is widely recognized.

Drug-resistant strains of malaria will likely spread to Africa much faster in the current era. Some security forces in Southeast Asia (SEA) are a reservoir of multidrug-resistant (MDR) malaria parasites due to their occupational risk. In 2010, 5% of Cambodian army personnel, screened for research purposes, tested positive for Pf [18] using DNA (PCR) screening. Additional testing revealed failure to the latest artemisinin-based combination therapy (ACT) used at that time [18]. Despite investment by the Bill and Melinda Gates (BMGF) to stop malaria transmission in these troops, the positive cases for Pf malaria had doubled to 10% by 2016 [19, 20]. During this same time frame, more than 3300 Cambodian soldiers were deployed on UN peacekeeping missions—without prior adequate (PCR) screening for malaria—to eight countries, including five in sub-Saharan Africa [10, 21]. Such missions could lead to

rapid spread of resistance across the endemic areas of the world. Despite being an obvious conduit for the spread of multidrug-resistant (MDR) malaria, the use of standard malaria prevention measures has not yet been put into practice by most militaries in SEA. This is due, in part, to the fact that they are a neglected population receiving little aid [22].

The spread of disease by peacekeepers is well documented. For example, following the 2010 earthquake in Haiti, United Nations troops from Nepal were the source of a cholera epidemic with over 730,000 cases and 8700 deaths, and an estimated economic cost of US$2.2 billion [10]. A single infected peacekeeper could potentially serve as the source for the next malaria pandemic [10].

A similar threat is posed by migration of workers from SEA to sub-Saharan Africa. For example, nearly 20,000 Vietnamese are legally working in Angola [23], and an unknown number are there illegally. These migrants are importing malaria from Angola into Vietnam [13]; the converse could also happen. Chinese workers are employed in mining and construction industries in the forests of Cambodia. They are hard to access, and malaria prevention and treatment practices are of uncertain quality (CO, personal communications). It is quite probable that some workers will move on to the African continent, where there is huge Chinese investment in these industries. Due to the high burden of malaria in Africa, it is likely that malaria strains originating from Asia would go undetected until the next pandemic is underway.

With immediate decisive action, we believe the transmission of the MDR strains can be interrupted approaching the 2020 target (see box, **Table 1**) [24]. The World Health Organization (WHO)'s declaration of a Public health emergency of international concern (PHEIC) led to impressive increases in resources for both Ebola and Zika epidemics, and may be essential to address the current MDR malaria threat. Together, Ebola and Zika claimed fewer than 12,000 lives [25]. Yet, with MDR malaria, millions of lives are at stake. In 2015, WHO published a document warning that MDR malaria "…*has reached alarming levels in several areas of the GMS"* and that malaria "…*could become untreatable with currently available drugs within a few years*" [24]. In 2014, Bill Gates commented: "*There's the potential for a real nightmare scenario here. If a strain of malaria that's resistant to artemisinin were to spread to Africa*"… "*it would be the worst ever disaster in malaria control*" [7]. Chris Plowe, former president of American Society of Tropical Medicine and Hygiene, argues that, "*The danger of untreatable malaria is real and*

Recommendations for rapid elimination of emerging incurable malaria

The world needs to focus efforts to eliminate incurable malaria strains by 2020. The critical remaining actions are:

1. Commitment and real sense of urgency through declaration of multidrug-resistant malaria as a "Public health emergency of international concern" or similar set of directives;

2. Establish response leadership with sufficient authority, respect, expertise and operational funding;

3. Engage affected security forces to stop disease transmission and support elimination operations;

4. Utilize surveillance as a core intervention with result-based funding to drive the targeting malaria transmission foci with rapid, localized and effective action.

Table 1. Critical actions to eliminate emerging incurable malaria strains.

present" [6]. If the WHO's call for "urgent action" is to be answered, a PHEIC or similar set of directives would be hugely important. The definition of PHEIC is "an extraordinary event which is determined to constitute a public health risk to other States through the international spread of disease and potentially require a coordinated international response" [26–28]. We argue that almost incurable malaria in Cambodia, and now Vietnam, with the threat to other countries and regions, undoubtedly fits this definition. WHO contends the regulations were designed to address acute public health conditions and the public health community has known of the emergence and spread of multidrug resistance for a number of years [29]. We claim that if a PHEIC had been declared in 2014, when WHO first learned of high ACT treatment failure rates crossing into Vietnam, malaria would be nearly eliminated now in these countries. The new "extraordinary event" is a marked increase in malaria in eight provinces of Cambodia in 2017, which is continuing in five provinces in early 2018 (CO, personal communication) despite substantial Global Fund resources in the region. Furthermore, in the province where parasites crossed into Vietnam, malaria was up 2.5 fold in 2017, with an extraordinary annual parasite index of about 55. It is not too late to call a PHEIC. If a PHEIC declaration is politically not achievable, a set of similar instructions could generate both commitment and urgency. One example would be a US Department of Defense (DoD) directive [30]—this should be achievable, as malaria is the number one infectious disease threat for US troops [31] and the US DoD continues to invest large sums of money in new antimalarial drug and vaccine development. We are currently at risk of losing all drugs for both malaria prophylaxis and treatment, which should prompt a major investment in direct support of elimination of these strains. The current Defense Malaria Elimination Program [32] was intended by the lead author to be modeled after the DoD Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome Prevention Program (DHAPP) to Support Foreign Militaries directive [30]; instead it turned out to be just more research funding (CO, unpublished observations).

In the SEA context, the artemisinin-based drugs are only one of several classes of previously effective drugs. Over time, malaria parasites in Cambodia have accumulated multiple resistance mechanisms against all of these drug classes. Global health stakeholders and donors must learn from past experience, and urgently recognize the continually evolving malaria parasites in Cambodia as an emergency that must be halted.

A short-term solution to the challenge is the return of malaria sensitivity to the prior artemisinin combination treatment (ACT) partner drug mefloquine (Lariam®) following its reintroduction in Cambodia. One expert predicts that continued mefloquine efficacy will be short-lived [33]. When used as a monotherapy, mefloquine remained efficacious for <5 years in the Thailand–Cambodia border areas [34]; now, two years after its reintroduction in Cambodia as part of drug combinations, it fortunately appears to remain efficacious (CO, unpublished observations). It is currently being paired with artesunate, to which resistance has emerged, essentially leaving mefloquine again as monotherapy. Moreover, mefloquine is a drug greatly challenged because of both gastrointestinal and neuropsychiatric side-effects, which will undoubtedly affect compliance (adherence, or people completing the recommended three-day regimen), as effective monitoring of adherence is lacking (CO, unpublished observations in northern and eastern Cambodia). Additional solutions are currently undergoing research, including one with a combination of three drugs that are or were failing in other combinations [35]. In addition, molecular marker evidence reveals that the theory of reciprocal cross-resistance is probably not a factor [36]. Another proposed solution is to return to the use of drug combinations that previously

proved inefficacious in initial clinical trials in Cambodia [37]. However, these are unlikely to be more than very temporarily solutions. When international leaders and policy-makers, ministries of health, donors and the philanthropic and scientific communities understand the gravity of this situation, only then can there be a concerted, effective push to finally eliminate malaria in SEA.

Under the late Alan Magill's previous leadership, the BMGF made great strides in its ambitious goal to eradicate malaria; tragically, Dr. Magill passed away before his plan was fully implemented [38, 39]. Two of his key concepts are presented in **Figures 1** and **2**.

In 2014, Bill & Melinda Gates, Alan Magill and a team visited the epicenter of MDR malaria in western Cambodia. In Gates' notes from that trip, they reported the threat as outlined above [7].

Recently, Mr. Gates has proposed a global strategy to prepare for "the next epidemic" [41]. We developed "Recommendations for rapid elimination of emerging incurable malaria" (**Table 1**) based on his article. A key recommendation of Mr. Gates is that it should be "*coordinated by a global institution given enough authority and funding to be effective…*". He also noted that cooperation among various nations' militaries should be a priority. In 2014, SEA military

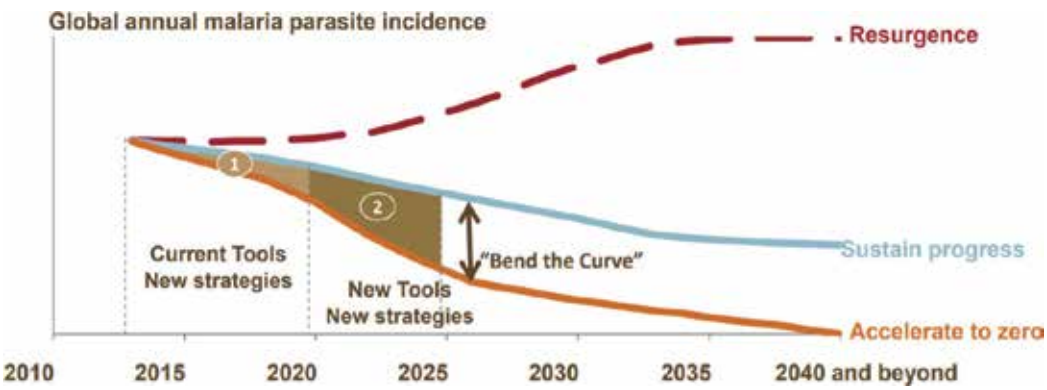


Figure 1. Three potential future trajectories for malaria as per the late Alan Magill from his "Accelerate to Zero" presentation [40]. He recommended to "bend the curve." One of his specific goals was Pf elimination "East of Bangkok" by 2020. 1 and 2 in circles represent additional reduction of malaria cases in the timeframe represented (reproduced with permission from the Bill & Melinda Gates Foundation). We are currently on the resurgence curve in Cambodia and may soon be worldwide if effective action is not taken.

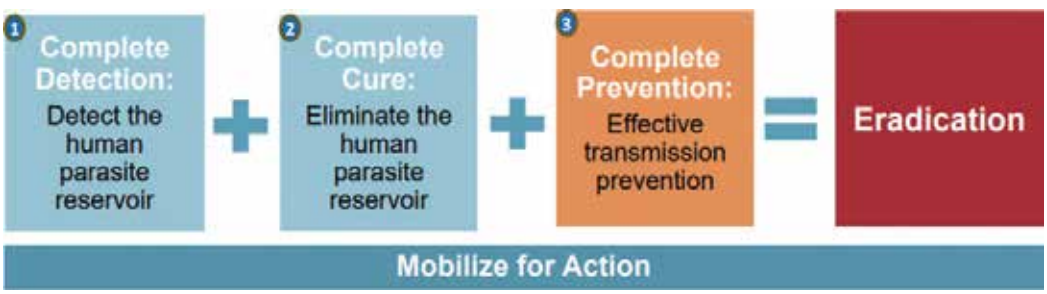


Figure 2. Mobilize for action. How to accelerate the trajectory to malaria eradication by concurrently achieving three goals: (1) identifying the human reservoir of infection in asymptomatic persons + (2) eliminating the human parasite reservoir + (3) combined with geographically and temporally targeted transmission prevention and strengthened surveillance and response [40] (reproduced with permission from the Bill & Melinda Gates Foundation).

leaders met twice and indicated their willingness to support malaria elimination efforts [42], but there has not been effective funding forthcoming to protect troops from malaria nor for them to assist with the much needed effective response. Another meeting was held in 2016, but the only available funding was for research (P. Smith, personal communication), not for an effective treatment and prevention package at scale, nor the needed direct support for malaria elimination operations that militaries can provide. A well-led military response to the MDR malaria crisis would be a large part of the solution to achieve WHO goals.

Gates also called for a warning and response system to enable fast decision-making. A surveillance/information system of the sort envisioned in a recent background paper [43] should be rapidly implemented across the SEA region. This is not an untested assertion, as a simple smart phone-based system has already been successfully pilot tested in central Vietnam [44]. A key 2016 finding was that each household had received an average of 4.3 treated bed nets; despite very high coverage at the household level (where there is little or no transmission), only 16% reported using a treated net when traveling to areas of elevated risk [9]. Similarly, low rates of treated net usage have been anecdotally observed by authors in four provinces in Cambodia recently and in other SEA countries [45, 46]. A smart phone-based system can provide quality monitoring including picture and video evidence to help leadership ensure that those in need of treated bed nets are actually using them and that treatments are being completed [44].

The mechanisms underlying emergence of drug resistance are not fully understood and are believed to be multifactorial [47]. A key factor leading to resistance may be incomplete treatment—effective adherence monitoring is still not in place and must be a priority. Until recently, Vietnam made standby ACT-treatment available to forest-goers, during which partial treatment may frequently occur. The same problem arises when people with symptoms of fever are provided antimalarial drugs by individuals who do not stress the need for complete treatment (e.g., private drug sellers and others). As artemisinin-derivative components have decreased efficacy in the GMS, a greater selection pressure is placed on the partner drugs. Whenever possible, all combination therapies should include a fully curative dose of each component, which, for short half-life drugs, requires seven days of therapy. Seven days of monitored treatment is feasible in the region if patients are renumerated for lost work time.

The role of mosquito vectors in the spread of drug-resistant malaria is also not fully understood. In the GMS, it is clear that malaria transmission is closely associated with the forest and forest-fringe vectors, i.e., *An. dirus* s.l. and *An. minimus* s.l. Fortunately, extensive insecticide resistance has not yet emerged to these vectors. Humans are likely the main transmission reservoir as they can infect mosquitoes for months if not cured of their malaria infection, while mosquitoes are relatively short-lived (lifespan of *Anopheles* female: three to four weeks) [48].

Lastly, no child should currently die from malaria in Africa, as all strains there are fully curable with ACT treatments. The high death rates are due to ineffective prevention and/or delayed/inappropriate treatment as a result of weak health infrastructure. Targeted and decisive action should be taken in Africa to reduce the overall public health impact of malaria while the most commonly used antimalarial drugs remain effective. This can be done using modifications of the same approaches outlined in this chapter. Because of the threat to Africa, where the

combination of both ineffective drugs and weak health infrastructure will lead to another public health emergency, decisive actions must be taken immediately in Asia.

2. Achieve both local and international commitment and a real sense of urgency—a "Public Health Emergency of International Concern (PHEIC) or similar set of directives"

In the 1980s, nearly incurable malaria parasites emerged on the Thailand-Cambodia border. Mefloquine failed four years after introduction in 1985 [34]. The only treatment option at the time was quinine-tetracycline [49]. Quinine is a very poorly tolerated drug requiring three daily doses for seven days—meaning almost no one completes it (e.g., poor adherence or compliance) unless every dose is monitored. Fortunately, artemisinin derivatives and other drugs became available—all of which either did not work at the time of their introduction (e.g., lumefantrine, pyronaridine [50–52]) or have lost therapeutic efficacy (e.g., piperaquine [53]). Presently, the pipeline of new antimalarial drugs is not keeping pace with the emergence of drug resistant strains [37]. Now in Cambodia, mefloquine sensitivity has returned, but is likely to be short-lived (e.g., two more years or less), by which time we may be back to quinine-doxycycline (a tetracycline derivative). We have recently learned that the Vietnam Border Guard Forces have provided doxycycline prophylaxis to more than ½ million people over the last decade (CO, personal communication)—which means this drug might as well be rendered ineffective by now. Identification of the combination partner drugs or the new seven-day regimens must be a priority. Ineffective or incomplete treatment will result in people carrying malaria parasites, an increased transmission reservoir, cases and deaths. The US Center for Disease Control and Prevention (CDC) reported the direct costs for malaria (e.g., illness, treatment, premature death) to be at least $12 billion per year. The cost in lost economic growth is many times more than that [54]. If key leadership does not act rapidly and effectively now, these costs will be much higher. If a PHEIC was declared, these emerging incurable parasites can be rapidly eliminated using the approaches presented here. If not, we will likely have a slight upgrade of "business as usual" with this critical window of opportunity lost.

As advocated early in this chapter and by others [4, 55] (Rear Admiral C. Chinn, personal communication), we believe that the best way to handle this threat is to declare a PHEIC or similar directives. The failure of nearly all drugs in Cambodia, the crossing of these parasites into Vietnam and current increasing Pf malaria in Cambodia arguably constitute such an "extraordinary event" based on the three prongs identified by the International Health Regulations (IHRs). First, the failure of standard treatment options for deadly communicable disease constitutes a public health threat. While all forms of malaria are still currently treatable, the imminent failure of last ACT poses a public health risk, especially for sub-Saharan Africa. This situation would make the deadliest form of malaria untreatable, which would at least be comparable to the "events" that have triggered previous PHEIC declarations [56, 57]. Second, drug-resistant malaria is at risk of spreading internationally as a result of the substantial presence of security personnel from the GMS in sub-Saharan Africa such as Cambodian and military personnel and workers traveling to Africa. There is greater risk that these challenging new parasites will reach both India and Bangladesh soon. These countries have substantial

numbers of their troops in Northeast India and Eastern Bangladesh, where these parasites will first arrive, and are currently the 2nd and 3rd largest contributors to international peacekeeping missions [58]. Third, a coordinated international response is absolutely required to manage this risk. In SEA, there are now fortunately few deaths from malaria. Leadership here has other pressing health issues to address, with malaria now becoming a low priority. Both international leadership and assistance for the countries involved, especially with security forces, is needed to ensure proper response to this peril most directly threatening Africa.

3. Establish mission leadership with enough authority, respect, expertise and functional funding

We urge Bill & Melinda Gates Foundation, the US government and the Asian Development Bank to fill the leadership vaccuum at the operational level, as the newly resistant parasites, if they reach Africa, will make the goal of malaria eradication very much more challenging. We believe these are the only organizations with the authority, resources, and respect to make this happen. Local governmental personnel with the responsibility for the mission must be empowered. Future, on-the-ground non-govermental leadership must be carefully selected to ensure they have the needed commitment, authority, respect, and expertise to be effective [41]. The WHO Emergency Response to Artemisinin Resistance (ERAR) in the GMS hub was established in 2013 [59] to strengthen the response to artemisinin resistant malaria by coordinating action, technical leadership and catalyzing resource mobilization. The ERAR hub was "transitioned" to the Mekong Malaria Elimination program in 2016 [60]. Why containment failed must be objectively evaluated to insure that the new leadership is effective. Public Health Emergency Operations Centers could be a good solution [61].

The elimination of malaria is not as difficult as it appears on the surface. It boils down to prevention and effective treatment of malaria patients in or traveling from the actual transmission foci. GMS original forest areas are now shrinking, which is making the mission easier. Bill Gates himself and others have effectively outlined the actions needed [41, 55]. Cambodia is the epicenter of emerging incurable malaria; the needed policies and guidelines are now in place in this country [24, 62]. What is needed now is quality implementation of relatively straightforward interventions in the field. In 2013, the RAI, a three-year $100 million grant, was launched by the Global Fund (GF) to contain artemisinin-resistant malaria (http://www.raifund.org/). Many of the key impediments were clearly outlined in the 2015 RAI mid-term report [3]. Unfortunately, we were denied permission by Global Fund leadership to publish the conclusions and recommendations from their report in this chapter (A Joubert, personal communication). We were allowed to publish only the map, which revealed Pf was going up at that time (**Figure 3**).

With a new leadership team, given a passion for the mission, along with authority, expertise and sufficient/effective funding, we strongly believe that Pf elimination goals can be achieved near to the WHO targets in the GMS [24]. Determining if the RAI mid-term review results have been effectively addressed should be top priority [3]. The results of routine, truly independent quality monitoring from the field must be a key component. Quality monitoring must focus on what is most important (e.g., effective prevention and treatment in actual transmission foci). Targeted supportive supervision for partners experiencing implementation challenges is also

http://dx.doi.org/10.5772/intechopen.78261

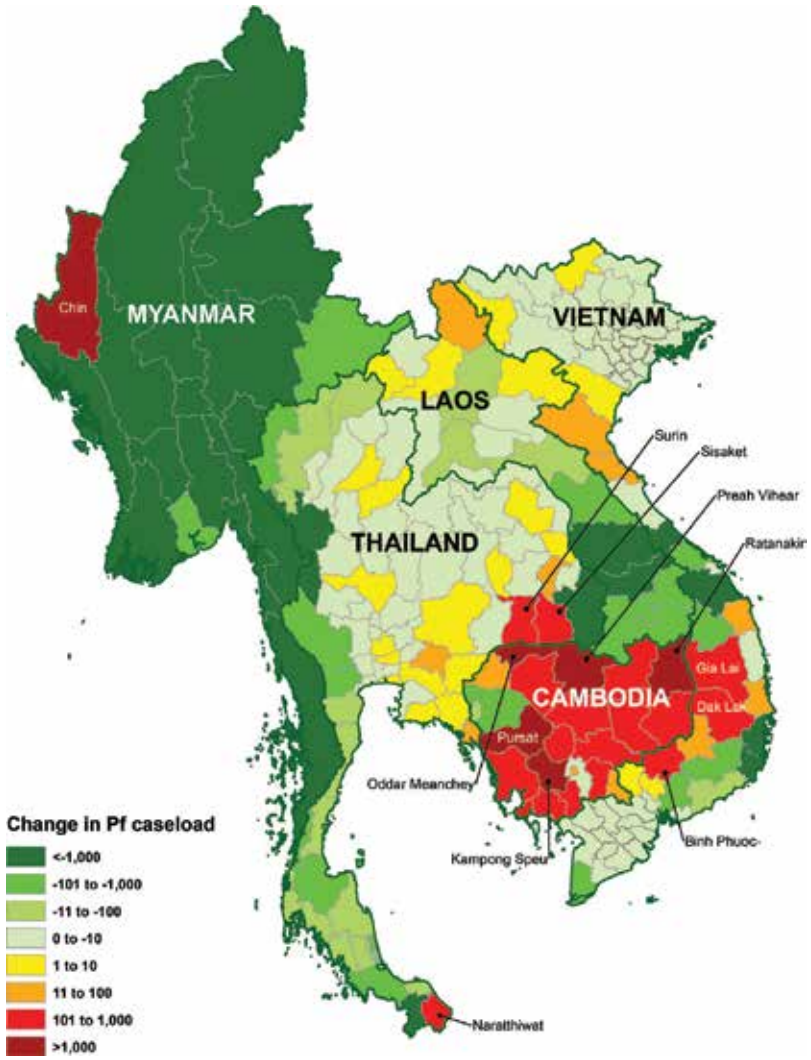


Figure 3. Pf caseload increase between July 2014–June 2015 [3]. Malaria was again rising in 2017–2018 in several provinces of Cambodia.

essential to achieve elimination targets. Our new "Red-to-Green, Keep-It-Green" Information System is an example of the ability to provide near real-time feedback to leadership with image and video documentation of what is really happening in the field (see Section 5.1.1) for both independent quality monitoring and supportive supervision.

GF is by far the largest investor in malaria in the region; their funding, however, is not nearly as effective as it could be for malaria in the GMS, especially in Cambodia. GF has evolved to be a mega-donor. As with all large organizations, this brings bureaucracy; in addition, it has the added challenge of accountabilty for very large sums of money. Malaria in the GMS represents only a small part of the overall portofolio, but has the same rules that apply to all funding, impacting timely and effective intervention implementation. For example, a sub-contractor receiving Presidential Malaria Initiative (PMI) funding reports PMI funds are hard to use, but

GF funds are 10 times worse (CO, person communication). GF funding is focused on process and financial accountability, not on timely, effective and quality implementation of interventions in transmission foci despite WHO's call for "urgent action" [24]. For example, in a province in Eastern Cambodia where malaria increased 2.5 fold in 2017, nets were first delivered to the lower risk villagers in early 2018 and the high risk mobile and migrant populations will not receive nets until at least mid-2018 following the set process. Furthermore, in the villages, a fixed number of treated nets are being provided, resulting in households with more than one forest-goer often not having enough nets. Response to new cases is in the village, which does not make sense with transmission being in the forest. Malaria in the region is an occupational disease for those working in the forest, while most programs have been designed around presumed household exposure. Lastly, Cambodia returned more GF funds unused than any other country in the region in the cycle that ended in December, 2017 (CO, unpublished observation).

Available financial resources must be used much more effectively. Despite large amounts of funding in the region, basic intervention coverage in forest transmission foci is poor. There is markedly disproportionate financial support provided for partner organizations in the region. Each international support staff often costs hundreds of thousands of dollars per person per year, including overhead and allowances. Yet, incentives for good performance are not allowed for malaria-endemic country government staff who have salaries that are not enough for subsistence. For example, district-level health staff typically make $200–$300/month, while salaries for NMCEP staff at the national level are in the range of $300–$1200/month. While GF guidelines do allow for incentives to government employees [63], we have been informed that this policy does not apply in the GMS (CO, personal communications). Undoubtedly, this leads to resentment by those expected to execute malaria elimination operations, which are often beyond their normal duties and may put their own job security at risk. A main argument against incentives is "sustainability in the context of decreasing external financing for malaria" (The RAI-Regional Steering Committee, personal communication), despite 7+ million lives being at risk. Malaria "East of Bangkok" can be rapidly eliminated, making long-term sustainability a non-issue if the recommendations in this chapter are followed.

Leadership must also address many conflicts of interest, which are often subtle in this setting, especially with research. As per the former Pacific Command Surgeon, "[elimination of malaria] is an action problem, not a research problem" (Rear Admiral C. Chinn, personal communication), yet the US Army continues to do only research with substantial increases in funding for malaria. Researchers, to be successful, must enhance their own *curriculum vitae* and malaria research usually requires substantial disease transmission, resulting in disincentives to facilitate elimination. The lead author is most familiar with the malaria research being conducted by the US military, which is expensive, frequently wasteful, often duplicative, many times not impactful and sometimes actually counterproductive (CO, unpublished observations). A mechanism must be in place for research prioritization, independent review by experts who understand the needs and challenges, and for timely action based on important findings [20].

The lead author of this chapter helped to identify funding to protect security forces in the GMS from malaria. His intent was for this to be modeled after the DoD directive to prevent and treat HIV-AIDS [30]. Following the realization that new funding was more for research, the following feedback was received … "I am aware that you're in disagreement… want to see us more

aggressively target malaria elimination. As we've discussed, we see this ultimately as a host country responsibility…" (M. Fukuda, personal communication). Many in the region are being misled that researchers are actually helping to protect host country militaries from malaria, when in practice, only small research studies are being conducted to fund research staff and to generate publications, with no action being taken based on the results. Leadership must take corrective action as militaries are both a key malaria transmission reservoir and can direclty support elimination operations (see Section 4). Malaria is the largest infectious disease threat for the US DoD and action can be taken as exemplified by an HIV prevention program to support foreign militaries and a DoD directive [30]. The US Army should engage with an institution that is not research-focused to rapidly help eliminate the emerging incurable parasites and ensure than any malaria research funding in the region is focused on rapidly stopping falciparum malaria transmission.

Vietnam can serve as an example for effective leadership and health system strengthening leading to rapid reduction and preparation for elimination of malaria. With intensive implementation of malaria control measures over the past decades, the burden of malaria is decreasing rapidly, and the disease is becoming increasingly focal. Between 2000 and 2016, the number of malaria cases was reduced by 94.4% (74,316 down to 4161) and number of deaths reduced by 97.9% (142 down to 3) [64] (**Figure 4**).

Key factors leading to the success of the program are as follows: (1) strong commitment and substantial investments by the Government of Vietnam and its international development partners, (2) a strong and comprehensive health network from central to community levels, (3) a vertical, well organized and functional program (e.g., health staff specialized in malaria control activities are working effectively at all levels down to village), (4) extensive vector control measures with high coverage of ITNs and indoor residual spraying (IRS), (5) availability of highly effective medicines for malaria treatment at all levels, (6) engagement of multisectoral partners (see Section 4.2).

Although great success towards malaria elimination has been made, Vietnam is now faced with a critical window of opportunity to achieve the elimination of malaria as mandated in

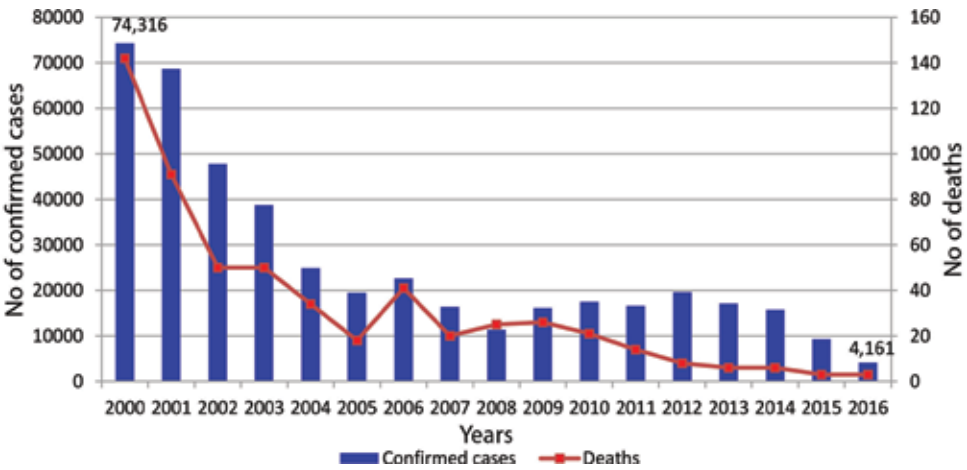


Figure 4. Decreasing malaria transmission trends in Vietnam between 2000 and 2016.

the "National Strategy for Malaria Control and Elimination 2011-2020 and Vision for 2030" [65]. This critical window includes the following factors: (1) efficacious antimalarial combinations still exist but are failing fast; (2) potent tools for vector control are available but could be undermined quickly by the development of insecticide resistance; and (3) financial support from external funding partners continues to flow but is likely time bound. The Government of Vietnam will also need to take bold steps and intensify national malaria elimination efforts to ensure that malaria is eliminated from Vietnam for good before this window of opportunity closes.

In summary, with a new leadership team, given a passion for the mission, along with authority, expertise and sufficient/effective funding, we believe WHO's Pf elimination goals can be achieved near the set target date of 2020. Vietnam is an example of a success story, which can serve as a model for other country programs. However, Vietnam cannot eliminate malaria until neighboring countries also do so. We urge BMGF, Asian Development Bank (ADB), PMI, the US military and other philanthropists to take action to address the challenges presented here in order to drive malaria elimination in the region.

4. Engage security forces to prevent disease spread and support elimination operations

4.1. Security forces as a neglected population contributing to the malaria transmission reservoir and spread of drug-resistant parasites

The GMS security forces are a neglected population group that are at greater risk of contracting malaria, [22, 63] and serving as a transmission reservoir [20, 66]. They are certainly spreading the disease in the region and are the most obvious direct conduit of the current parasites to Africa [10]. In Africa, peacekeeping forces, deployed from many malaria endemic countries, work together, which could cause rapid spread of the new drug-resistant parasites. PCR-based screening of UN peacekeeping troops from Cambodia was initiated in 2015 (PCR is the only method sensitive enough to detect asymptomatic parasite carriers). In 2017, it was learnt that this practice had been discontinued at some point, but with inputs from key leaders to WHO, this was fortunately re-initiated. This process must be monitored so that lapses in screening do not recur. It must also be extended to include other militaries in the region that soon will also be at risk of spreading these parasites.

We have evidence that the Cambodian army has high malaria infection rates, and not been receiving optimal malaria prevention or treatment, and are serving as the primary malaria transmission reservoir in an area of Northwestern Cambodia [67, 68]. In 2010, 5% of troops were reported positive for Pf by PCR during a malaria screening [68]. The study for which the screening was done provided direct evidence, although with small numbers, early warning that dihydroartemisinin-piperaquine was failing as treatment. The lead author urged the US Army to act to stop transmission in these troops, but to no avail. He then contacted BMGF in 2012 to provide funding to stop transmission in these troops; funding was awarded, but not used until 2016, by which time the number positive for Pf had doubled to 10% [20]. It is unclear why the US Army does not act much more quickly given the threat to US troops.

In the 2016 intervention study, Pf transmission was stopped, providing direct evidence that the Cambodian army was the primary Pf transmission reservoir in the area of the study [20]. We believe there is sufficient evidence to scale the interventions that were proven to be effective (e.g., permethrin-treated uniforms, see **Table 2**). The impressive results from the study, however, have unfortunately not yet been acted upon.

Additional evidence of security forces being a transmission reservoir comes from the area where the most resistant parasites cross-border into Vietnam. Forest rangers in the Bu Gia Map National Park screened positive for Pf malaria at about ~11% for Pf (very similar to the Cambodian army in Northwestern Cambodia, see above) [66]. This population is probably a significant malaria transmission reservoir in this area. From the publication, the rangers appear neither to be receiving effective prevention measures nor routine screening and treatment. No funding in the GF 2018–2020 budget was allocated for such activities in this population. The only way it will be possible is to request unused GF year-end funding be reallocated, which is not an easy process (CO, personal communication). Furthermore, since permethrin-treated uniforms are not yet WHO-prequalified (despite standard of care in Western militaries and recent impressive evidence in the Cambodian army), GF funding may not be able to be used for this intervention (CO, personal communications).

Permethrin is very inexpensive, well-tolerated, and widely used for uniform treatment by Western militaries for malaria prevention [69]. The evidence for efficacy of treated uniforms/clothing is summarized in **Table 2**. Based on available evidence, and in light of emerging incurable parasites and pyrethroid sensitivity of the main transmitting mosquitoes (M. Macdonald, personal communication), we believe treated uniforms should be rapidly scaled up for all security forces in the GMS as one component of an integrated vector control package.

In Cambodia, there are three types of government security personnel working in the forest—army, forest rangers, and border police; each falling under different ministries. We are aware of a pilot project in Northwestern Cambodia where an ADB-funded project is working with all of these groups for malaria prevention and treatment (CO, personal communications). We believe this initiative should be taken to scale as quickly as possible in the region.

4.2. GMS security forces can provide direct support if given a mission and properly resourced

The Vietnam People's Army, including the Vietnam Border Defense Force, provides an excellent model for security personnel supporting health interventions. From 2005 to 2015, the Combined Military Medical Program contributed to improve health, hunger elimination and poverty reduction [75]. Example accomplishments for health include: (1) vaccination of 5.1 million children with Ministry of Health-recommended vaccines, (2) family planning for more than 3.7 million people, (3) malaria prevention education for more than 1.8 million people, and (4) IRS of nearly 26 million square meters of housing with National Malaria Control Program with provided pyrethroids.

More than 1300 health stations were strengthened with military staffing, including 1044 health stations in remote and isolated areas (>10% of all nationwide; in locations where it is hard to recruit civilian staff). The system includes 152 border clinics, which also serves as a border surveillance system for early detection of epidemics.

First author and study location	Level of evidence[a]	Study population	Study Design[b]	Intervention groups (n)	Control group (n)	% Failure intervention[c]	% Failure control	Protective efficacy (95% CI)[d]	Reference/ notes[e]
Moore (2019), Tanzania	1	Public Service Corps	CR	Permethrin-treated uniforms (n~500), DEET (N~250)	Untreated uniforms (n~500), DEET placebo (N~250)	NYA	NYA	NYA	1
Wojnarski (2016), Cambodia	2	Military	CR	Permethrin-treated uniforms (n = 125)	Untreated uniforms (n = 143)	10%	24%	56% (21–76%)	[20] 2
Additional arm in Wojnarski (2016)	‘“	““	““	Permethrin-treated uniforms + partially effective prophylaxis (n = 130)	Untreated uniforms (n = 143)	15%	24%	35% (−7–61%)	[20] 2
Soto (1995), Columbia	3	Military	RDBCT	Permethrin-treated uniforms, socks and hat (n = 86)	Water-treatment of the same (n = 86)	3%	14%	75% (15–93%)	[70] 3
Rowland (1999), Pakistan	4	Afghan refugees	RCT (to household)	Permethrin-impregnated headscarves/top sheets (n = 438, 51 families)	Placebo EC formulation (n = 387, 51 families)	25%	38%	36% (21–48%)	[71] 4
Kimani (2006), Kenya	5	Somali refugees	CR	Permethrin-impregnated clothing & sheets (n = 90)	Plain water-impregnated clothing & sheets (n = 91)	38%	66%	43% (22–58%)	[72]

First author and study location	Level of evidence[a]	Study population	Study Design[b]	Intervention groups (n)	Control group (n)	% Failure intervention[c]	% Failure control	Protective efficacy (95% CI)[d]	Reference/ notes[e]
Eamsila (1994), Thailand	6	Military	Unclear, probably CR	Permethrin-treated uniforms (n = 249)	Placebo-treated uniforms (n = 414)	27%	29%	4% (−23–26%)	[73] 5
Most (2016), French Guiana	7	Military	Unclear "conditions identical"	Long-lasting polymer-coated permethrin-impregnated uniforms (n = 25)	Untreated uniforms (n = 125)	0%	9%	100% (NA)	[74]

[a]1 is strongest, 7 least strong.

[b]CR: Cluster randomized trial, RDBCT: randomized, double blind clinical trial.

[c]NYA: Not yet available.

[d]NA: Not available.

[e]Reference in [], notes by number: (1) Trial planned to execute in 2018, (2) True efficacy higher as estimate is confounded with *Plasmodium vivax* (Pv) relapses; efficacy only for Pv as Pf transmission was interrupted by interventions; final clinical study report pending. (3) Instructed to wear their uniforms day and night; under garments also treated, two adverse events in the permethrin arm requiring topical treatment but able to continue in study, no treatment needed in placebo arm; only trial with adverse events reported, (4) < 20 years had ~64% Pf protective efficacy, pooled efficacy underestimated because of Pv relapses, headscarf's were worn by women outside during waking hours and used by the family as bed sheets at night, noted efficacy almost as good a treated bed nets in a prior trial in the same population. (5) Pv cases despite CQ prophylaxis indicated non-adherence; noted incomplete ability to monitoring uniform use, possible randomization leading to unequal exposure (e.g. permethrin-treated uniforms had more malaria than placebo in one area).

Table 2. Review of protective efficacy of permethrin treated clothing for malaria prevention.

In conclusion, we urge donors and NMCEPs to help supporting security forces take action. This should include first to make sure security forces are using appropriate prevention and treatment packages. GMS security forces absolutely can directly support elimination operations. They best understand the mobile and migrant populations (MMPs) and terrain of the remaining forest in the region, understand military planning and have a structured workforce in many of the challenging areas. We call on donors to provide the leadership and funding by making sure that security forces are not a transmission reservoir and are engaged in the fight against malaria.

5. Utilize surveillance as a core intervention linked to results-based funding (SCI-RBF) to achieve rapid and effective action in actual transmission foci

5.1. Development of an innovative information (surveillance) system to eliminate emerging incurable malaria

In 2015–2016, our research team worked in two provinces in Vietnam (**Figure 5**) to field test a surveillance/information system envisioned in a background paper written for the BMGF [43]. We partnered with National Institute of Malariology, Parasitology and Entomology (NIMPE) and the Phu Yen Provincial Health Department (PHD) to develop and pilot test the information system linked to a pay-for-performance system. We also developed concepts to target forest malaria transmission foci and/or people traveling to these locations with effective integrated prevention and treatment.

5.1.1. 2015 results

We defined transmission locations, intervention usage, risk groups/factors and desired interventions. This was first done with a household survey of the identifiable malaria patients from the most recent 100 cases and a sample of nearby houses (**Table 3**). This table illustrates total cases and risk by work-type and that the reported use of program-provided long lasting insecticidal nets (LLINs) in actual transmission areas was very low [9]. For example, most malaria cases (49%) were coming from paper plantations, but farmers and charcoal producers were at higher risk (75 and 80%, respectively). LLIN and overall treated nets in use were only 6 and 19%, respectively. Households with malaria were ~three-fold less likely to use treated nets, Odds Ratio 3.2; $p < 0.01$) [76]. The actual transmission locations were determined by where people reported sleeping one to two weeks before they developed fever (malaria incubation period). The lack of transmission at the village level was confirmed by the lack of malaria patients who remained in the village during the incubation time for malaria.

These results were next confirmed and extended through geographic reconnaissance of sleeping sites of malaria patients (**Figure 6**) [8, 9, 44]. At each sleeping site, GPS coordinates, with images the sleeping structure and the nets being used (if any), were captured (**Figure 6**). The pictures were used to validate self-reported data (e.g., **Figures 7** and **8**). Interviews revealed forest rangers reported taking partial malaria treatment when ill, malaria infection was common and lack of prevention measures for work in the forest at night. Based on this information, we suspected this population was the primary transmission reservoir in this area, similar to that recently reported from forest rangers in a nearby province [66].

http://dx.doi.org/10.5772/intechopen.78261

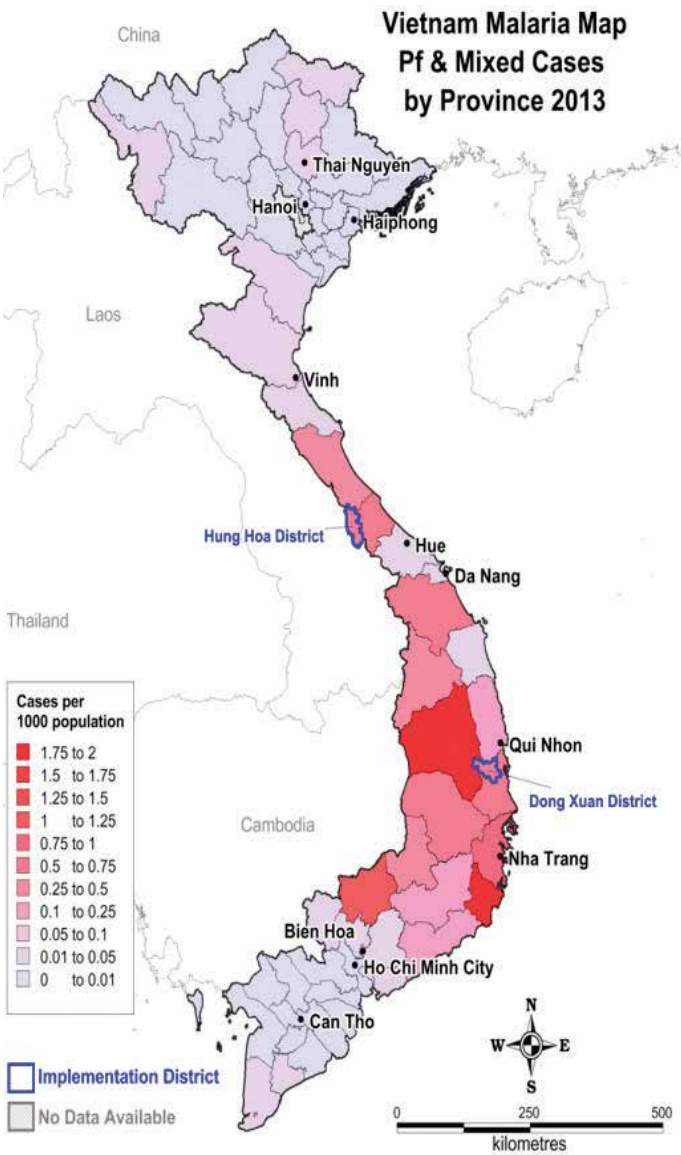


Figure 5. Vietnam study districts in Quang Tri (Hung Hoa District) and Phu Yen Provinces (Dong Xuan District) (blue outlined areas).

Figure 6 illustrates that actual forest transmission locations were readily identifiable; 80% were accessible within one hour by motorcycle. For the remaining 20%, the optimal placement of malaria posts to access those at risk was determined. The primary factors for low treated net use were the fact that 92% desired a hammock net (with few provided) and 83% desired a zip-type hammock net [76]. The hard-type LLINs that were provided were a type that was strongly disliked by 85% of those surveyed. Of the forest-goers surveyed, 89% reported they would be willing to use mosquito repellent and 91% malaria prophylaxis [76]. Based on these results, we believe the provision of nets of a type that people want to use, net retreatment

Main work type	n	Percent interviewed	Number of malaria cases	Percent total malaria (n = 93)	Percent risk malaria[a]	Nets currently used (n = 186)		Net types currently used (n = 189)	
						Any net[b]	At least a treated net type[d]	At least a zip hammock type	At least a LLIN type
Paper plantation	94	49%	44	47%	47%	65%	11%	41%	2%
Agarwood harvesting	39	21%	14	15%	36%	95%	15%	36%	8%
Farmer	16	8%	12	13%	75%	80%	80%	0%	38%
Trapper	16	8%	6	6%	38%	44%	25%	13%	6%
Charcoal production	10	5%	8	9%	80%	70%	0%	50%	0%
Timber harvesting	9	5%	4	4%	44%	33%	11%	11%	0%
Hunter	3	2%	3	3%	100%	0%	0%	0%	0%
Other	3	2%	2	2%	67%	67%	67%	0%	0%
Total or mean	**190**	**100%**	**93**	**100%**	**49%**	**68%**[c]	**19%**	**32%**	**6%**

[a]Cases of malaria/people interviewed.

[b]Number reporting any net reported being used at forest sleeping site/number interviewed in work type.

[c]Number reporting any net reported being used at forest sleeping site/total interviewed.

[d]Program was providing annual net retreatment accounting for the difference between 6% for LLIN.

Table 3. Malaria-risk populations and bed net use in Phu Yen Province, Vietnam in 2015.

for self-purchased products, repellent availability, along with on-going education and use monitoring will enable high usage of an integrated prevention package.

Figure 6 also illustrates the smart-phone based information system, which greatly improved our ability to conduct near-real time data capture and quality control from Hanoi, while the team was working in the field. Information was captured using a smartphone app with the data fed into an on-line server when Internet access was available to the field staff. Ona.io server, KoboToolbox (http://www.kobotoolbox.org/) and other systems are inexpensive, easy-to-program/use, powerful new tools for the fight against malaria.

In **Table 4**, we outline the interventions we believe are needed for rapid malaria elimination. We illustrate linking of successful execution of the interventions to incentive pay, which was successfully pilot tested. Data captured from each intervention can constitute "surveillance as core intervention (SCI)." Incentive payments can be linked to the smartphone-captured data following quality checks. We believe surveillance as an intervention with results-based funding (SCI-RBF) will motivate staff to make sure patients complete antimalarial treatment and effectively intervene in transmission foci (both of which are still largely lacking).

http://dx.doi.org/10.5772/intechopen.78261

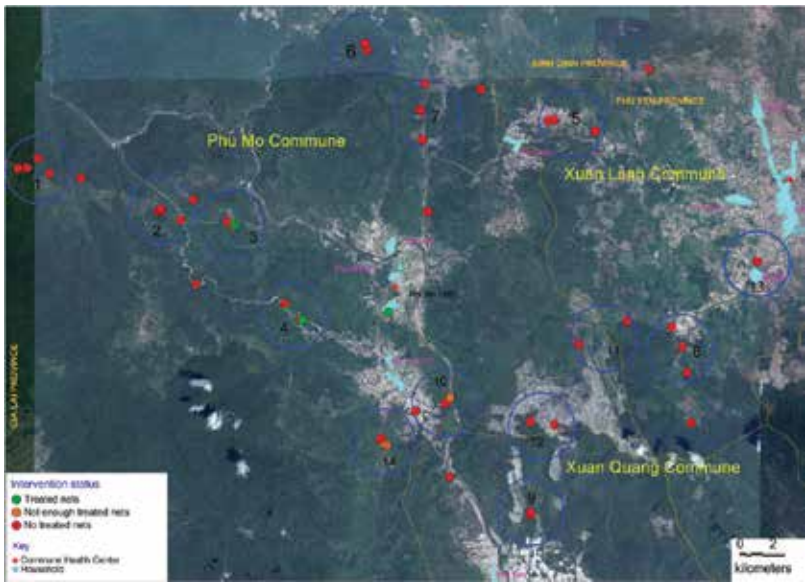


Figure 6. Baseline malaria transmission location map with insecticide treated net (ITN) usage in Phu Yen Province, 2015. The turquoise color represents 4700 households. In 2016, provincial health records showed 4.3 treated nets per household. Dots are the forest sleeping locations 1–2 weeks earlier, including 95% of 2015 cases. The color of the dots reveals very low use of nets in actual transmission areas, despite very high coverage in the village (where there was no transmission). The light purple circles are transmission foci, defined here as two cases within a 1 km radius, which captured 80% of all cases.

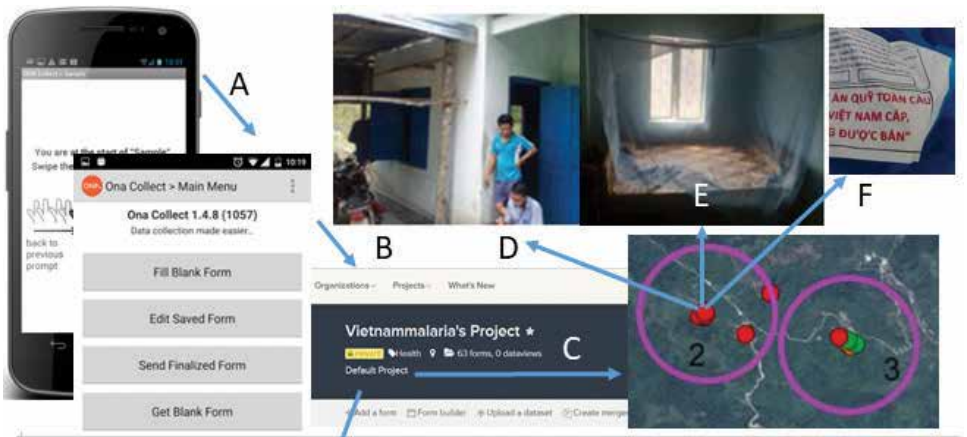
Lastly, a concept was developed for a malaria elimination task force (METF) led and implemented by PHD staff, implemented by mobile malaria workers (MMWs). The METF should routinely have challenge-solving workshops with NMCEP staff to improve the quality of data and responses.

5.1.2. Conclusions

Despite very high household insecticide-treated net coverage, their use in risk areas is very low. Forest transmission sites are identifiable and targetable directly and/or at forest pathway points. The described transformative smart-phone based information technology will facilitate rapid malaria elimination allowing near real-time monitoring to improve the quality and targeting of interventions. Urgent action must be taken to improve the selection of interventions of products benefitting people at risk and for those working in actual transmission areas.

5.1.3. Broader impact

Based in part on our work in 2015–2017, Phu Yen Province saw the largest drop (89%) in malaria of any province in the region of the south-central coast and central highlands in this time frame. In 2016, as cases decreased, the cases spatially clustered into two areas to prioritize (pink dots in **Figure 9**). In our study district, malaria reduction is striking. In 2017, only 13 cases were reported, compared to 52 (**Figure 9**), 133, 292, and 291 in the years 2016–2013, respectively, a



G

	n	Work type (%)	Net Type (%)		
			No net	Any net	Any treated net
Transmission site survey (119 malaria case)					
Paper Planation Workers	28	24%	79%	29%	14%
Agarwood harvesters	26	22%	77%	8%	4%
Trappers	12	10%	67%	33%	17%
Timber harvesters	12	10%	67%	33%	17%
Charcoal	2	3%	67%	0%	0%
Farmers	28	24%	29%	57%	29%
Other	10	8%	40%	40%	20%
Total or Mean	**119**	**100%**	**61%**	**32%**	**16%**

Figure 7. Example use of smart phone technology to capture information, images and video linked to GPS coordinates. A. Smart phones with good data coverage are becoming ubiquitous, Ona.oi smartphone data capture; B. Ona.io internet interface; C. Transmission focus map with global positioning system (GPS) coordinates of sleeping locations of malaria patients; D-F. Pictures linked to GPS coordinates for quality control, D. Forest ranger station, E. How the net should look not how did it look, F. Tag on the net to confirm if it is an LLIN or not; G. Example of key data. All will allow for near-real time monitoring of intervention quality with regularly updated maps to allow malaria elimination staff, donors, and key leaders to understand what is happening where and when, from anywhere with internet access for the first time—this technology "changes the game".

96% reduction from baseline. The malaria lead for Phu Yen Province reported our contribution, noting the health staff "operated more effectively" during and following our project.

5.2. New approaches to "leap forward" to achieve more rapid malaria elimination

5.2.1. Development of a "Red-to-Green, Keep-it-Green" information system to achieve high adherence with both integrated vector control and treatment interventions

Based on what we learned in 2015, an information system using the "Red light-Green light" approach, as envisioned by Alan Magill, was developed. Based on 2015 case mapping (**Figure 6**), a simple system was developed to prioritize actual transmission areas for targeting interventions (**Figure 10**). The dot in each circle represents prevention status and the triangle treatment status in each focus. Those presented are treated net usage (dots) and time to ACT treatment (triangles), but must be enhanced to include both an integrated prevention and treatment package (**Table 5**).

Figure 8. Image evidence of LLIN usage. A–C represent probable use and D-F, non-use. Note in E, chickens inside.

One can visualize the low usage of treated bed nets (red dots). In addition, all but two sites are within one hour by motorcycle of a health center for ACT treatment (green triangles). The green triangles also reflect that most of the forest transmission sites are directly accessible for both responses to new cases and for on-going monitoring. The sites with an orange and a red triangle need proper placement of malaria posts or mobile malaria workers to capture people going deeper into the forest.

The map on the bottom of **Figure 10** illustrates perfect, 100% "Green" status. We believe 60–70% "Green" targets will be sufficient to rapidly eliminate malaria. These maps can be

No.	Intervention/item	Description[a]	Cost/form[b]	Cost/intervention/ case[b]	Results-based funding (cost/year)[b]
1	Rapid case report, initial investigations and initial response	Full interview and interventions at initial patient encounter	$ 4.15	$ 4.15	$ 4149
2	Treatment plan and follow-up	Document adherence and late treatment success	$ 20.47	$ 51	$ 51,182
3.1	Foci response (Village)	Screen & treat, treat nets, new nets, BCC	$ 3.75	$ 113	$ 67,500
3.2	Foci response (Forest)	Screen & treat, treat nets, new nets, BCC, IT-ASSBA	$ 15.26	$ 305	$ 122,054
3.3	Foci response (Cross-border)	Screen & treat, treat nets, new nets, BCC, IT-ASSBA	$ 30.51	$ 610	$ 61,027
4	Foci monitoring	Screen & treat, treat nets, new nets, BCC, IT-ASSBA	$ 7.63	$ 76	$ 76,284
5	Forest entry point/ work place monitoring	Screen & treat, treat nets, new nets, BCC, IT-ASSBA	$ 3.75	$ 38	$ 37,500
6	On-going provider quality monitoring	Routine visits to document diagnosis, treatment, prevention and reporting	$ 16.00	$ 2500	$ 30,000
	Total				$ 449,696

[a]Screen: screening with new highly sensitive rapid diagnostic tests (hsRDTs); BCC: behavior change communication; IT-ASSBA: insecticide treatment around sleeping, sitting, sleeping and bathing areas (most sleeping structures are huts without walls).

[b]These are example costs; true costs will be estimated in the field in Cambodia from May–August 2018. Some of these costs are currently provided as travel incentives and monthly stipends for field staff which are not focused on results; the estimates do not include commodities.

Table 4. Example of surveillance as an intervention with results-based funding (SCI-RBF) for the first year of implementation.

regularly updated in an on-line information system for donors, national programs and implementers to monitor progress near real-time.

5.2.2. Mitigation of forest malaria transmission with more effective and complete prevention/ treatment packages

We believe a more comprehensive prevention and treatment package targeting malaria transmission foci, and the people working or traveling there, will have rapid impact, especially when those constituting the primary transmission reservoir are targeted. Pf must be the priority as this species is causing the public health emergency. Both Pf and Pv are transmitted by the same vector species. Pv remains a challenge for cure because of the dormant liver stage (hypnozoites). All of the prevention interventions will also be efficacious for control of Pv, and will drive down transmission in parallel. Pv residual transmission will often remain when Pf has been eliminated. The same resources can be used to mop up residual Pv transmission, which will also ensure that Pf has been truly eliminated.

ITNs are the cornerstone for malaria prevention worldwide, but have inadequate efficacy (e.g., in areas of unstable malaria transmission, 62 and 43% efficacy is reported with no or untreated nets for Pf prevention, respectively [77]). ITNs lack effect when not in use as illustrated by

http://dx.doi.org/10.5772/intechopen.78261

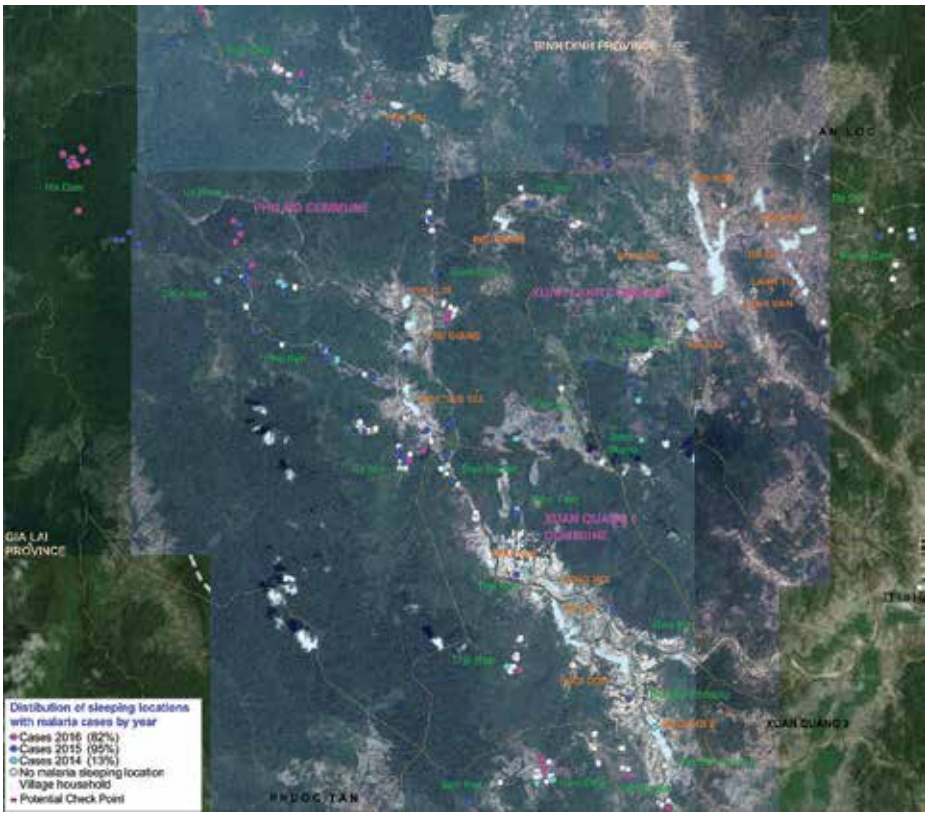


Figure 9. Actual malaria transmission locations by year in Dong Xuan District, Phu Yen Province, Vietnam. Please note the marked clustering of the pink dots as malaria transmission decreased in 2016 to 52 cases from 133 in 2015. Based in part on our effort to demonstrate actual transmission locations and lack of treated nets at these sites, malaria cases continued to decrease to 13 cases in 2017 (a 96% reduction from baseline in 2013-2014).

Prevention	Treatment
>90% using a treated net	Complete treatment and follow-up
Treated clothing	Targeted malaria posts to forest entry points
Mosquito repellent	Mobile malaria workers to access hot-spots
Safer sleeping, sitting and bathing areas	Screening with highly sensitive RDTs

Each of these measures has partial efficacy—they must be used in combination with adherence monitoring to achieve high effectiveness. With all drugs soon to be lost, a focus must be placed on use of an integrated vector control package. New vector control products are also in the pipeline, which should be added when effectiveness is demonstrated.

Table 5. Immediately available interventions for integrated prevention and treatment in transmission foci.

Durnez and Coosemans [78] (**Figure 11**). Additional tools are available—achieving adherence with all prevention tools is the critical challenge, which we believe can be addressed with SCI-RBF (see Section 5.1.1).

In **Table 5**, immediately available prevention and treatment measures are outlined. We believe the use of treated nets can be greatly increased in actual transmission areas with smart phone

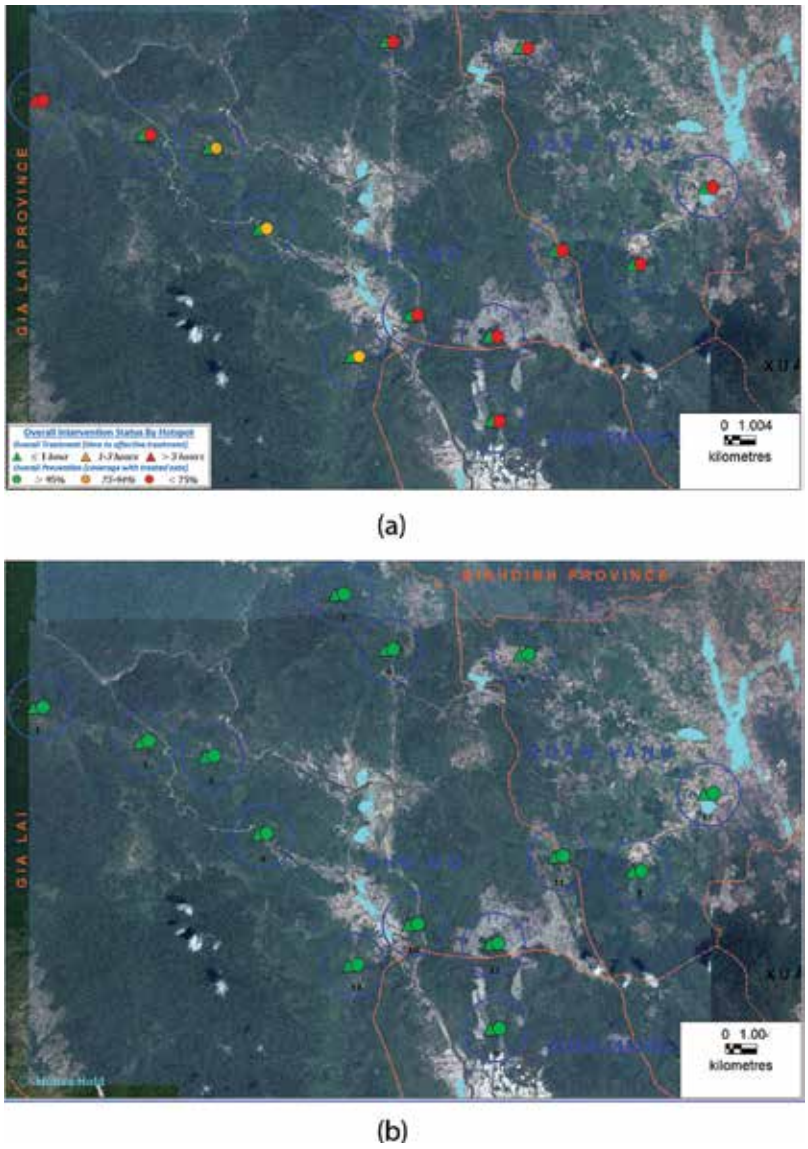


Figure 10. "Red-to-Green, Keep-it-Green" information system (see https://ConsortiumHA.org); (a) the blue circles are transmission areas to prioritize; here classified as 2 cases within a 1 km radius, which captured 80% of cases. Within the blue circles, a small circle represents the prevention package and a triangle represents the treatment package. The top of this figure represents the actual status of Dong Xuan District, Phu Yen Province, Vietnam in 2015. The prevention package was only the use of a treated net or not, and the treatment package was only time to access effective malaria treatment. As you can see, treated net use is poor, but all but two triangles are green, illustrating that all but two transmission foci are within one hour of a health center by motorcycle. This also means they are directly accessible for interventions, both when a new case occurs and for on-going monitoring of use of malaria elimination tools. (b) the theoretical desired 100% green status. 100% will never by achieved – we believe perhaps 60-70% usage of an integrated prevention and treatment package will be enough to rapidly reduce transmission.

monitoring. The SCI-RBF will also allow iterative testing and improvement of methods until high usage is achieved, both with ITNs and an integrated vector control package. There is now direct evidence of substantial efficacy of permethrin-treated uniforms in the Cambodian

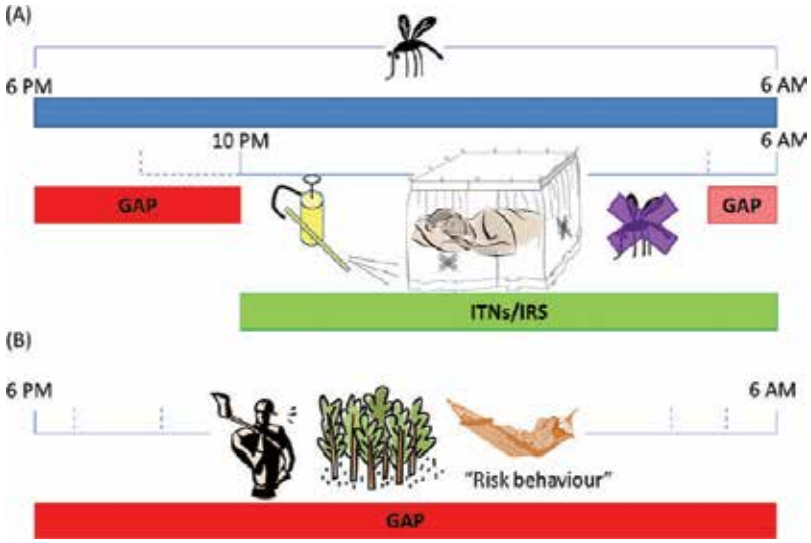


Figure 11. Protection "gap" when only indoor insecticide-based vector control measures are applied. *Anopheles* mosquitoes bite between 6 PM and 6 AM. A "gap" exists while people are not sleeping (A) and for people conducting night-time outdoor activities (B) (courtesy of Durnez and Coosemans [78]). Additional note: even the green part of the figure is also often red in the GMS because of lack of use of ITNs in risk areas (see **Figures 6** and **10**).

military, including contributing to the interruption of Pf transmission [20]. The literature-based evidence of efficacy of permethrin-treated clothing is presented in **Table 2**. Based on the available data and the growing body of evidence that those working in the forest at night in the GMS are a significant transmission reservoir, we believe treated uniforms/clothing should be scaled up as rapidly as possible. We believe retreatment of clothing, self-purchased nets and insecticide treatment around sleeping, sitting and bathing areas (IT-ASSBA) should be routine in hot-spots. Re-treatment will also allow for on-going education and monitoring. Treated netting when used with partial coverage in sleeping areas has been shown to reduce *An. dirus* (the main forest vector in the GMS) bites by 50% in Eastern Vietnam (Marchand R, unpublished data). Some efficacy has also been seen for other insecticide-treated products [79, 80].

Topical insect repellent clearly prevents mosquito bites when used correctly, especially for outdoor biting mosquitoes such as *An. dirus* that are otherwise hard to control with traditional indoor methods (ITN, IRS); however, getting people to regularly use the product in risk areas (be adherent or compliant) is the challenge, probably being the primary factor leading to a lack of efficacy in recent trials in the GMS [81]. Locally available DEET-containing repellent was well accepted and prevented *An. dirus* bites in forest transmission areas all night in a single application in Eastern Vietnam (Marchand R, unpublished data).

Prompt, complete and correct treatment is the cornerstone for malaria therapy. The effective ACT regimen should be used for both Pf and Pv blood stages. Primaquine in standard dosages should be used for Pf gametocytes and Pv hypnozoites in countries where these doses are already being used as standard of care (e.g., Vietnam) or low-dose [82] for Pf gametocytes in areas where there are safety concerns. We believe that all patients in the GMS should have visits on days 28 and 42 to detect late-treatment failure, as well as to have malaria patients and their work groups be transmission-stopping ambassadors. In addition, improved tools are just becoming available to

identify asymptomatic malaria carriers through active case detection (ACD). The BMGF funded the development of a highly sensitive rapid diagnostics test (hsRDT), which is specific for Pf [83]. It is much more sensitive than standard rapid diagnostic tests (RDT), but not as sensitive as PCR (which is probably not necessary) [84, 85]. Alere (www.alere.com, now Abbott) has made these hsRDTs commercially available for $0.95/each; approvals in GMS are in process. The same company also markets a malaria antibody-based RDT for both Pf and Pv [86]. The usefulness of these new tools can be rapidly demonstrated during scale-up; the hsRDT will hopefully obviate the need for mass drug administration [87]. Both of these new RDTs should facilitate defining risk populations.

If the package of available tools does not rapidly stop malaria transmission, other more aggressive tools can be added as they become available [85]. Currently available drugs are problematic for prophylaxis—primaquine and doxycycline require daily dosing; doxycycline has already been widely used along the Vietnam border—its current efficacy is unknown. Mefloquine is poorly tolerated and its use for prophylaxis may accelerate its demise. Tafenoquine [88, 89] and RTS,S malaria vaccine [90] should be accelerated to play a role for prevention in the region.

The last remaining parasites will be the most drug-resistant—alternative regimens are urgently needed. Tafenoquine [91] (unpublished observations for Pf), azithromycin and methylene blue [92–95] are currently under recognized, but could also play a role in combination treatment when no alternatives remain, which may be very soon. New regimens should be urgently evaluated; all should be seven days, as some or all of the drugs will have short half-lives. With seven-day regimens, adherence will be very challenging. Hospitalization with appropriate incentives should become the norm to achieve very high treatment adherence in the near future, including the current three-day regimens in areas where outpatient treatment monitoring is not successful.

6. Conclusion/call to action

Emerging incurable malaria in the GMS is a grave public health threat. We call for targeted, immediate decisive action by international and host country governments to establish mission leadership, enough authority, respect, and expertise at each operational level. Security forces must be engaged. Commitment and a real sense of urgency will be most effectively achieved with a PHEIC. We call on the major donors (BMGF, United States Agency for International Development, ADB, the US military) and other philanthropists/donors to fulfill the need for efficient funding. In this chapter, we have proposed the methodology to achieve elimination of the nearly incurable malaria parasites "East of Bangkok" near the WHO target of 2020 [24]. With the currently available tools, each partially effective, but when used together with smart phone-based quality monitoring of appropriate use in the actual transmission areas—we believe the mission can be accomplished near the target date. We call for the critical actions, focusing resources to where they will have most impact to help prevent the next pandemic.

Acknowledgements

We wish to thank NIMPE and Phu Yen Provincial Health leadership and staff for planning executing the work described in Vietnam (especially Professor Duong Thanh Tran, Dung

Viet Dang and Thinh Duc Ngo). Drs. Nam Dinh Nguyen, Tuong Trinh Dinh, Global Fund, Hanoi, Vietnam provided technical support, data analysis and illustration preparation. Dr. Hung Pham, Consortium for Health Action, Savage, MN, USA and Hanoi, Vietnam provided project leadership and coordination. Dr. Gerard Kelly, Research School of Population Health, Canberra, Australia for geographic information system (GIS) planning and support. We also would like to thank Michael MacDonald for critical review of this chapter. We thank Ms. Tuyen Pham for providing illustration editing for this chapter. This work was supported in part by funding from the US Department of Defense. The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the US Department of Defense, nor the US or Vietnamese Government. Much of this work was inspired by the late Alan Magill (former Director of Malaria, Bill & Melinda Gates Foundation).

Acronyms

ACD	active case detection
ACT	artemisinin-based combination therapy
ADB	Asian Development Bank
BCC	behavior change communication
BMGF	Bill & Melinda Gates Foundation
CDC	US Center for Disease Control and Prevention
ConsortiumHA	Consortium for Health Action
CR	cluster randomized trial
DHAPP	Defense HIV/AIDS Prevention Program
DoD	Department of Defense
ERAR	emergency response to artemisinin resistance
GF	Global Fund
GIS	Geographic Information System
GMS	Greater Mekong Subregion
GPS	global positioning system
hsRDT	highly sensitive rapid diagnostic test
IHRs	International Health Regulations
IRS	indoor residual spraying
IT-ASSBA	insecticide treatment around sleeping, sitting and bathing areas
ITN	insecticide treated nets
LLIN	long lasting insecticidal nets
MDR	multidrug-resistant

METF	malaria elimination task force
MMPs	mobile and migrant populations
MMW	mobile malaria worker
NIMPE	National Institute of Malariology, Parasitology and Entomology
NMC(E)P	National Malaria Control (and Elimination) Program
PCR	polymerase chain reaction
PHEIC	Public Health Emergency of International Concern
Pf	*Plasmodium falciparum*
PHD	Provincial Health Department
PMI	presidential malaria initiative
PPS	pay-for-performance system
Pv	*Plasmodium vivax*
RAI	Regional Artemisinin Initiative
RBF	results-based funding
RDT	rapid diagnostic test
SCI	surveillance as a core intervention
SCI-RBF	surveillance as a core intervention linked to results-based funding
SEA	Southeast Asia
WHO	World Health Organization

Author details

Colin Ohrt[1]*, Thang Duc Ngo[2] and Thieu Quang Nguyen[3]

*Address all correspondence to: colin@consortiumha.org

1 Consortium for Health Action, Savage, MN, USA

2 Department of Epidemiology, National Institute of Malariology, Parasitology and Entomology (NIMPE), Hanoi, Vietnam

3 National Institute of Malariology, Parasitology and Entomology (NIMPE), Hanoi, Vietnam

References

[1] WHO. World Health Organization World Malaria Report 2017. Geneva: World Health Organization; 2017

[2] Alonso P, Noor AM. The global fight against malaria is at crossroads. The Lancet. 2017;**390**:2532-2534. DOI: 10.1016/S0140-6736(17)33080-5

[3] Garcia R, Hewitt S, Tulloch J. Report of the Mid-Term Review of the Global Fund Regional Artemisinin Initiative (RAI). Geneva: Global Fund; 2015

[4] Ives M, McNeil D. As malaria resists treatment, experts warn of global crisis. In: The New York Times. 2017

[5] Imwong M, Hien TT, Thuy-Nhien NT, Dondorp AM, White NJ. Spread of a single multidrug resistant malaria parasite lineage (PfPailin) to Vietnam. The Lancet Infectious Diseases. 2017;**17**:1022-1023. DOI: 10.1016/S1473-3099(17)30524-8

[6] Plowe CV. The Danger of Untreatable Malaria Is Real and Present. In: CfSaI S, editor. Washington, DC: Center for Strategic and International Studies; 2014

[7] Gates B. What it takes to wipe out malaria. In: Gatesnotes. 2014

[8] Ohrt C, Ngo TD, Bui PQ, Bertrand W, Pham HQ, Nguyen ND, et al. Emerging incurable malaria in Southeast Asia–A call for targeted, decisive action in the region. American Society of Tropical Medicine and Hygiene Annual Meeting, Abstract 1561. 2016

[9] Ohrt C. Preparing for the next global threat–A call for targeted, decisive action now in Southeast Asia to prevent the next pandemic in Africa. Joint International Tropical Medicine Meeting, Bangkok, Thailand; 2016

[10] Houston S, Houston A. Screening and treating UN peacekeepers to prevent the introduction of artemisinin-resistant malaria into Africa. PLoS Medicine. 2015;**12**:e1001822. DOI: 10.1371/journal.pmed.1001822

[11] Cambodia marks 10th anniversary of peacekeeper deployment Royal Embassy of Cambodia to UK, Denmark, Finland, Ireland, Norway, Sweden, Ethiopia and African Union; 2016

[12] Sovuthy K. Peacekeeper dies from malaria in Mali. In: Cambodia Daily. 2015

[13] Van Hong N, Amambua-Ngwa A, Tuan NQ, Cuong Do D, Giang NT, Van Dung N, et al. Severe malaria not responsive to artemisinin derivatives in man returning from Angola to Vietnam. Emerging Infectious Diseases. 2014;**20**:1199-1202. DOI: 10.3201/eid2007.140155

[14] Trape JF. The public health impact of chloroquine resistance in Africa. The American Journal of Tropical Medicine and Hygiene. 2001;**64**:12-17

[15] Verdrager J. Localized permanent epidemics: The genesis of chloroquine resistance in *Plasmodium falciparum*. The Southeast Asian Journal of Tropical Medicine and Public Health. 1995;**26**:23-28

[16] malERA refresh consultative panel on insecticide and drug resistance. malERA: An updated research agenda for insecticide and drug resistance in malaria elimination and eradication. PLoS Medicine. 2017;**14**:e1002450. DOI: 10.1371/journal.pmed.1002450

[17] Greenwood BM, Fidock DA, Kyle DE, Kappe SH, Alonso PL, Collins FH, et al. Malaria: Progress, perils, and prospects for eradication. The Journal of Clinical Investigation. 2008;**118**:1266-1276. DOI: 10.1172/JCI33996

[18] Lon C, Manning JE, Vanachayangkul P, So M, Sea D, Se Y, et al. Efficacy of two versus three-day regimens of dihydroartemisinin-piperaquine for uncomplicated malaria in military personnel in northern Cambodia: An open-label randomized trial. PLoS One. 2014;**9**:e93138. DOI: 10.1371/journal.pone.0093138

[19] Sok S, Wojnarski M, Prom S, Chann S, Spring M, Gosi P, et al. Comparison of mass drug administration vs. mass screening and treatment high-risk, military mobile populations to support malaria elimination in Cambodia. American Society of Tropical Medicine and Hygiene, Annual Meeting, Abstract 1216. 2016

[20] Wojnarski M, Sok S, Prom S, et al: Review of comparative efficacy and acceptance of interventions of monthly prophylaxis vs. screening and treatment in high-risk, military mobile populations to support malaria elimination in Cambodia. Joint International Tropical Medicine Meeting 2016. Bangkok, Thailand, Poster 93. 2016

[21] Prashanth Parameswaran TD: Cambodia Marks Milestone in UN Peacekeeping Contributions. 2017

[22] Manning JE, Satharath P, Gaywee J, Lopez MN, Lon C, Saunders DL. Fighting the good fight: The role of militaries in malaria elimination in Southeast Asia. Trends in Parasitology. 2014;**30**:571-581. DOI: 10.1016/j.pt.2014.10.002

[23] Angola and Vietnam to Jointly Combat Illegal Immigration. 2016-08-30 Ed. Luanda, Angola: Angola Press Agency; 2016

[24] WHO: Strategy for Malaria Elimination in the Greater Mekong Subregion (2015-2030). 2015

[25] WHO Ebola Response Team. After Ebola in West Africa–Unpredictable risks, preventable epidemics. The New England Journal of Medicine. 2016;**375**:587-596. DOI: 10.1056/NEJMsr1513109

[26] WHO: International Health Regulations and Emergency Committees. 2016

[27] WHO. International Health Regulations (2005). 3rd ed. Geneva; 2016

[28] WHO. Implementation of the International Health Regulations. 2005 Report of the Review Committee on Second Extensions for Establishing National Public Health Capacities and on IHR Implementation. 2015

[29] WHO. Antimalarial Drug Resistance in the Greater Mekong Subregion: How Concerned should we Be? Q&A with Dr Pedro Alonso, Director of the Global Malaria Programme. 2017

[30] Department of Defense Directive 6485. 02E: DoD Human Immunodeficiency Virus (HIV)/Acquired Immune Deficiency Syndrome (AIDS) Prevention Program (DHAPP) to Support Foreign Militaries. US Department of Defense; 2013

[31] Department of Defense Names WRAIR Scientist "Lab Scientist of the Quarter". 2018

[32] President's Malaria Initiative, Thailand, Lao PDR and Regional Malaria Operational Plan FY2018. 2017

[33] Roberts L. Drug resistance triggers war to wipe out malaria in the Mekong region. Science American Association for the Advancement of Science; 2016

[34] Boudreau EF, Fleckenstein L, Pang LW, Childs GE, Schroeder AC, Ratnaratorn B, et al. Mefloquine kinetics in cured and recrudescent patients with acute falciparum malaria and in healthy volunteers. Clinical Pharmacology and Therapeutics. 1990;**48**:399-409

[35] Maxmen A. Back on TRAC: New trial launched in bid to outpace multidrug-resistant malaria. Nature Medicine. 2016;**22**:220-221. DOI: 10.1038/nm0316-220

[36] Rossi G, De Smet M, Khim N, Kindermans JM, Menard D. Emergence of *Plasmodium falciparum* triple mutant in Cambodia. The Lancet Infectious Diseases. 2017;**17**:1233. DOI: 10.1016/S1473-3099(17)30635-7

[37] Phyo AP, von Seidlein L. Challenges to replace ACT as first-line drug. Malaria Journal. 2017;**16**:296. DOI: 10.1186/s12936-017-1942-5

[38] Remembering Alan Magill. The American Journal of Tropical Medicine and Hygiene. 2015;**93**:893. DOI: 10.4269/ajtmh.935mem

[39] Kirby T. Obituary—Alan Magill. The Lancet Infectious Diseases. 2015;**15**:1266. DOI: 10.1016/S1473-3099(15)00365-5

[40] Magill A. Accelerate to zero: Strategies to eliminate malaria. In: MMV Stakeholders Meeting, Medicines for Malaria Venture; 2015

[41] Gates B. The next epidemic–Lessons from Ebola. The New England Journal of Medicine. 2015;**372**:1381-1384. DOI: 10.1056/NEJMp1502918

[42] US PACOM Regional Forum on Drug Resistant Malaria among Military Populations. PACOM Regional Military Meeting Forum. 2014

[43] Ohrt C, Roberts KW, Sturrock HJ, Wegbreit J, Lee BY, Gosling RD. Information systems to support surveillance for malaria elimination. The American Journal of Tropical Medicine and Hygiene. 2015;**93**:145-152. DOI: 10.4269/ajtmh.14-0257

[44] Ngo T, Tran D, Martin N, Nguyen P, Pham H, Dang D, et al. An innovative information system to eliminate emerging incurable malaria. American Society of Tropical Medicine and Hygiene, Annual Meeting, Abstract 1565; 2016

[45] Grietens KP, Xuan XN, Ribera J, Duc TN, Bortel W, Ba NT, et al. Social determinants of long lasting insecticidal hammock use among the Ra-glai ethnic minority in Vietnam: Implications for forest malaria control. PLoS One. 2012;**7**:e29991. DOI: 10.1371/journal.pone.0029991

[46] Aung T, Wei C, McFarland W, Aung YK, Khin HS. Ownership and use of insecticide-treated nets among people living in malaria endemic areas of eastern Myanmar. PLoS One. 2016;**11**:e0162292. DOI: 10.1371/journal.pone.0162292

[47] Menard D, Dondorp A. Antimalarial drug resistance: A threat to malaria elimination. Cold Spring Harbor Perspectives in Medicine. 2017;**7**:1-24. DOI: 10.1101/cshperspect.a025619.

http://publicationslist.org/data/didier.menard/ref-216/Menard%20and%20Dondorp,%20Cold%20Spring%20Harb%20Perspect%20Med_2017.pdf

[48] Manguin S, Carnevale P, Mouchet J, Coosemans M, Julvez J, Richard-Lenoble D, et al. Biodiversity of Malaria in the World. Montrouge, France: John Libbey Eurotext; 2008

[49] Achan J, Talisuna AO, Erhart A, Yeka A, Tibenderana JK, Baliraine FN, et al. Quinine, an old anti-malarial drug in a modern world: Role in the treatment of malaria. Malaria Journal. 2011;**10**:144. DOI: 10.1186/1475-2875-10-144

[50] Leang R, Canavati SE, Khim N, Vestergaard LS, Borghini Fuhrer I, Kim S, et al. Efficacy and safety of pyronaridine-artesunate for the treatment of uncomplicated *Plasmodium falciparum* malaria in western Cambodia. Antimicrobial Agents and Chemotherapy. 2016; **60**:3884-3890. DOI: 10.1128/AAC.00039-16. http://aac.asm.org/content/60/7/3884 . short

[51] Denis MB, Tsuyuoka R, Lim P, Lindegardh N, Yi P, Top SN, et al. Efficacy of artemether-lumefantrine for the treatment of uncomplicated falciparum malaria in Northwest Cambodia. Tropical Medicine & International Health. 2006;**11**:1800-1807. DOI: 10.1111/j.1365-3156.2006.01739.x

[52] Song J, Socheat D, Tan B, Seila S, Xu Y, Ou F, et al. Randomized trials of artemisinin-piperaquine, dihydroartemisinin-piperaquine phosphate and artemether-lumefantrine for the treatment of multi-drug resistant falciparum malaria in Cambodia-Thailand border area. Malaria Journal. 2011;**10**:231. DOI: 10.1186/1475-2875-10-231

[53] Thanh NV, Thuy-Nhien N, Tuyen NT, Tong NT, Nha-Ca NT, Dong LT, et al. Rapid decline in the susceptibility of *Plasmodium falciparum* to dihydroartemisinin-piperaquine in the south of Vietnam. Malaria Journal. 2017;**16**:27. DOI: 10.1186/s12936-017-1680-8

[54] US CDC. Impact of malaria. In: Malaria GHDoPDa, editor. US Centers for Disease Control; 2017

[55] Lover AA, Gosling R, Feachem R, Tulloch J. Eliminate now: Seven critical actions required to accelerate elimination of *Plasmodium falciparum* malaria in the greater Mekong subregion. Malaria Journal. 2016;**15**:518. DOI: 10.1186/s12936-016-1564-3

[56] Heymann DL, Hodgson A, Sall AA, Freedman DO, Staples JE, Althabe F, et al. Zika virus and microcephaly: Why is this situation a PHEIC? Lancet. 2016;**387**:719-721. DOI: 10.1016/S0140-6736(16)00320-2

[57] Siedner MJ, Gostin LO, Cranmer HH, Kraemer JD. Strengthening the detection of and early response to public health emergencies: Lessons from the west African Ebola epidemic. PLoS Medicine. 2015;**12**:e1001804. DOI: 10.1371/journal.pmed.1001804

[58] Troop and police contributors. United Nations Peacekeeping. 2017

[59] WHO. All systems "go" for ERAR hub in Phnom Penh, Cambodia. World Health Organization (WHO); 2014

[60] WHO. Countries of the Greater Mekong Making Inroads in the Race Towards Elimination. World Health Organization (WHO); 2017

[61] Brencic DJ, Pinto M, Gill A, Kinzer MH, Hernandez L, Pasi OG. CDC support for global public health emergency management. Emerging Infectious Diseases. 2017;**23**. DOI: 10.3201/eid2313.170542

[62] Cambodia Malaria Elimination Action Framework (2016-2020). MESA; 2016

[63] The Global Fund. Guidelines for Grant Budgeting; 2017

[64] Vietnam National Malaria Control Program Annual Reports. Hanoi, Vietnam: National Institute of Malariology, Parasitology and Entomology; 2000-2016

[65] National Strategy for Malaria Control and Elimination in the Period 2011-2020 and Orientation to 2030. Issued with Decision No. 1920/QĐ-TTg. Government Socialist Republic of Vietnam; 2011

[66] Son DH, Thuy-Nhien N, von Seidlein L, Le Phuc-Nhi L, Phu NT, NTK T, et al. The prevalence, incidence and prevention of *Plasmodium falciparum* infections in forest rangers in Bu Gia map National Park, Binh Phuoc province, Vietnam: A pilot study. Malaria Journal. 2017;**16**:444. DOI: 10.1186/s12936-017-2091-6

[67] Edwards HM, Canavati SE, Rang C, Ly P, Sovannaroth S, Canier L, et al. Novel cross-border approaches to optimise identification of asymptomatic and artemisinin-resistant *Plasmodium* infection in mobile populations crossing Cambodian borders. PLoS One. 2015;**10**:e0124300. DOI: 10.1371/journal.pone.0124300

[68] Manning J, Vanachayangkul P, Lon C, Spring M, So M, Sea D, et al. Randomized, double-blind, placebo-controlled clinical trial of a two-day regimen of dihydroartemisinin-piperaquine for malaria prevention halted for concern over prolonged corrected QT interval. Antimicrobial Agents and Chemotherapy. 2014;**58**:6056-6067. DOI: 10.1128/AAC.02667-14

[69] Banks SD, Murray N, Wilder-Smith A, Logan JG. Insecticide-treated clothes for the control of vector-borne diseases: A review on effectiveness and safety. Medical and Veterinary Entomology. 2014;**28**(Suppl 1):14-25. DOI: 10.1111/mve.12068

[70] Soto J, Medina F, Dember N, Berman J. Efficacy of permethrin-impregnated uniforms in the prevention of malaria and leishmaniasis in Colombian soldiers. Clinical Infectious Diseases. 1995;**21**:599-602

[71] Rowland M, Durrani N, Hewitt S, Mohammed N, Bouma M, Carneiro I, et al. Permethrin-treated chaddars and top-sheets: Appropriate technology for protection against malaria in Afghanistan and other complex emergencies. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1999;**93**:465-472

[72] Kimani EW, Vulule JM, Kuria IW, Mugisha F. Use of insecticide-treated clothes for personal protection against malaria: A community trial. Malaria Journal. 2006;**5**:63. DOI: 10.1186/1475-2875-5-63

[73] Eamsila C, Frances SP, Strickman D. Evaluation of permethrin-treated military uniforms for personal protection against malaria in northeastern Thailand. Journal of the American Mosquito Control Association. 1994;**10**:515-521

[74] Most B, Pommier de Santi V, Pages F, Mura M, Uedelhoven WM, Faulde MK. Long-lasting permethrin-impregnated clothing: Protective efficacy against malaria in hyperendemic foci, and laundering, wearing, and weathering effects on residual bioactivity after worst-case use in the rain forests of French Guiana. Parasitology Research. 2017;**116**:677-684. DOI: 10.1007/s00436-016-5333-6

[75] Huỳnh QH: The Military Combined Medical Contributed Much to the Success of the Health Sector. 2016

[76] Martin N, Ngo T, Tran D, Dang D, Pham H, Bui T, et al.: U.S navy-NIMPE collaborations and operations research support of the malaria elimination program in Vietnam. American Society of Tropical Medicine and Hygiene Annual Meeting, Abstract 1564 [Internet]; 2016. Available from: https://consortiumhaorg/wp-content/uploads/2016/12/2016_10_31_NMAr_V03_ASTMH-Posterpdf [Accessed: 2017-12-28]

[77] Lengeler C. Insecticide-treated bednets and curtains for preventing malaria. Cochrane Database of Systematic Reviews. 2000:Cd000363. DOI: 10.1002/14651858.cd000363

[78] Durnez L, Coosemans M. Residual transmission of malaria: An old issue for new approaches. In: Manguin S, editor. *Anopheles* Mosquitoes–New Insights into Malaria Vectors. Rijeka, Croatia: InTech; 2013. pp. 672-704

[79] Mittal PK, Sreehari U, Razdan RK, Dash AP. Evaluation of the impact of ZeroFly(R), an insecticide incorporated plastic sheeting on malaria incidence in two temporary labour shelters in India. Journal of Vector Borne Diseases. 2011;**48**:138-143

[80] Burns M, Rowland M, N'Guessan R, Carneiro I, Beeche A, Ruiz SS, et al. Insecticide-treated plastic sheeting for emergency malaria prevention and shelter among displaced populations: An observational cohort study in a refugee setting in Sierra Leone. The American Journal of Tropical Medicine and Hygiene. 2012;**87**:242-250. DOI: 10.4269/ajtmh.2012.11-0744

[81] Sluydts V, Durnez L, Heng S, Gryseels C, Canier L, Kim S, et al. Efficacy of topical mosquito repellent (picaridin) plus long-lasting insecticidal nets versus long-lasting insecticidal nets alone for control of malaria: A cluster randomised controlled trial. The Lancet Infectious Diseases. 2016;**16**:1169-1177. DOI: 10.1016/S1473-3099(16)30148-7

[82] Leang R, Khu NH, Mukaka M, Debackere M, Tripura R, Kheang ST, et al. An optimised age-based dosing regimen for single low-dose primaquine for blocking malaria transmission in Cambodia. BMC Medicine. 2016;**14**:171. DOI: 10.1186/s12916-016-0701-8

[83] Das S, Jang IK, Barney B, Peck R, Rek JC, Arinaitwe E, et al. Performance of a high-sensitivity rapid diagnostic test for *Plasmodium falciparum* malaria in asymptomatic individuals from Uganda and Myanmar and naive human challenge infections. The American Journal of Tropical Medicine and Hygiene. 2017;**97**:1540-1550. DOI: 10.4269/ajtmh.17-0245

[84] Tripura R, Peto TJ, Chalk J, Lee SJ, Sirithiranont P, Nguon C, et al. Persistent *Plasmodium falciparum* and *Plasmodium vivax* infections in a western Cambodian population: Implications for prevention, treatment and elimination strategies. Malaria Journal. 2016;**15**:181. DOI: 10.1186/s12936-016-1224-7

[85] Lin JT, Ubalee R, Lon C, Balasubramanian S, Kuntawunginn W, Rahman R, et al. Microscopic *Plasmodium falciparum* Gametocytemia and infectivity to mosquitoes in Cambodia. The Journal of Infectious Diseases. 2016;**213**:1491-1494. DOI: 10.1093/infdis/jiv599

[86] Cho SJ, Lee J, Lee HJ, Jo HY, Sinniah M, Kim HY, et al. A novel malaria Pf/Pv Ab rapid diagnostic test using a differential diagnostic marker identified by network biology. International Journal of Biological Sciences. 2016;**12**:824-835. DOI: 10.7150/ijbs.14408

[87] Dondorp AM, Smithuis FM, Woodrow C, Seidlein LV. How to contain artemisinin- and multidrug-resistant falciparum malaria. Trends in Parasitology. 2017;**33**:353-363. DOI: 10.1016/j.pt.2017.01.004

[88] Walsh DS, Eamsila C, Sasiprapha T, Sangkharomya S, Khaewsathien P, Supakalin P, et al. Efficacy of monthly tafenoquine for prophylaxis of *Plasmodium vivax* and multidrug-resistant *P. falciparum* malaria. The Journal of Infectious Diseases. 2004;**190**:1456-1463. DOI: 10.1086/424468

[89] Edstein MD, Walsh DS, Eamsila C, Sasiprapha T, Nasveld PE, Kitchener S, et al. Malaria prophylaxis/radical cure: Recent experiences of the Australian Defence force. Médecine Tropicale (Mars). 2001;**61**:56-58

[90] Gosling R, von Seidlein L. The future of the RTS,S/AS01 malaria vaccine: An alternative development plan. PLoS Medicine. 2016;**13**:e1001994. DOI: 10.1371/journal.pmed.1001994

[91] Fukuda MM, Krudsood S, Mohamed K, Green JA, Warrasak S, Noedl H, et al. A randomized, double-blind, active-control trial to evaluate the efficacy and safety of a three day course of tafenoquine monotherapy for the treatment of *Plasmodium vivax* malaria. PLoS One. 2017;**12**:e0187376. DOI: 10.1371/journal.pone.0187376

[92] Noedl H, Krudsood S, Chalermratana K, Silachamroon U, Leowattana W, Tangpukdee N, et al. Azithromycin combination therapy with artesunate or quinine for the treatment of uncomplicated *Plasmodium falciparum* malaria in adults: A randomized, phase 2 clinical trial in Thailand. Clinical Infectious Diseases. 2006;**43**:1264-1271. DOI: 10.1086/508175

[93] Miller RS, Wongsrichanalai C, Buathong N, McDaniel P, Walsh DS, Knirsch C, et al. Effective treatment of uncomplicated *Plasmodium falciparum* malaria with azithromycin-quinine combinations: A randomized, dose-ranging study. The American Journal of Tropical Medicine and Hygiene. 2006;**74**:401-406

[94] Bhagavathula AS, Elnour AA, Shehab A. Alternatives to currently used antimalarial drugs: In search of a magic bullet. Infectious Diseases of Poverty. 2016;**5**:103. DOI: 10.1186/s40249-016-0196-8

[95] Ohrt C, Li Q, Obaldia N, Im-Erbsin R, Xie L, Berman J. Efficacy of intravenous methylene blue, intravenous artesunate, and their combination in preclinical models of malaria. Malaria Journal. 2014;**13**:415. DOI: 10.1186/1475-2875-13-415

Chapter 4

Challenges in the Control and Elimination of *Plasmodium vivax* Malaria

Puji BS Asih, Din Syafruddin and John Kevin Baird

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.77082

Abstract

The human malaria parasite *Plasmodium vivax* imposes unique challenges to its control and elimination. Primary among those is the hypnozoite reservoir of infection in endemic communities. It is the dominant source of incident malaria and exceedingly difficult to attack due to both inability to diagnose latent carriers and the potentially life-threatening toxicity of primaquine in patients with an inborn deficiency of G6PD, the only therapeutic option against hypnozoites. Large segments of endemic populations are not eligible for primaquine, and alternative strategies for managing the threat of relapse in any group have not been optimized or validated. Association of risk of primaquine failure against latent *P. vivax* with impaired alleles of P450 2D6 exacerbates the substantial pool of primaquine ineligibles. Resistance to chloroquine against acute *P. vivax* malaria commonly occurs; alternative therapies like ACTs are effective but seldom evaluated as a partner drug to primaquine in the essential radical cure. Many of the *Anopheles* mosquito vector of *P. vivax* in South and Southeast Asia, where >90% of infections occur, thrive in a diversity of habitats and exhibit wide ranges of feeding and breeding behavior. This chapter explores many of these challenges and possible approaches in controlling and eliminating endemic vivax malaria.

Keywords: *Plasmodium vivax*, malaria, latent malaria, hypnozoites, glucose-6-phosphtate dehydrogenase (G6PD), *Anopheles* mosquitoes, species-sanitation, control

1. Introduction

Human malaria caused by *Plasmodium vivax* currently has the widest geographical distribution among all malaria parasites with about 35% of the world population living at risk of this physically debilitating and sometime lethal infection [1–3]. **Figure 1** illustrates this

global distribution most heavily weighing upon South and Southeast Asia (SEA) [1]. In most endemic countries, chloroquine (CQ) remains the first-line therapy for acute vivax malaria after more than 70 years of continuous use. CQ-resistant *P. vivax*, documented nearly 30 years ago, now commonly occurs across much of SEA [4, 5]. Unlike the other dominant species causing human malaria, *P. falciparum*, some sporozoites (called bradysporozoites) of *P. vivax* develop into dormant forms in the liver called hypnozoites. This single feature—latency—defines and distinguishes the prevention, treatment, and control of vivax malaria. Other sporozoites (called tachysporozoites) immediately develop into actively dividing hepatic schizonts over the 7-day to 18-day incubation period and cause the primary parasitemia and acute attack of patent vivax malaria. Hypnozoites activate weeks, months, or even years later, causing a renewed clinical attack called relapses [6].

In natural endemic settings, it may not be known if any given patient presenting with patent acute vivax malaria is experiencing a tachysporozoite-borne primary attack or a bradysporozoite-borne relapse. This uncertainty poses a fundamental problem of interpretation of parasitemia that may follow therapy [5–8]. The origin of the parasitemia may be a consequence of new primary attack (reinfection), therapeutic failure against blood stages (recrudescence), or renewed latent malaria (relapse). These ambiguities may not be addressed by molecular genotyping techniques because relapses may be either homologous or heterologous to the primary infection event [9, 10]. Estimating the efficacy of blood schizonticidal therapy may thus be complex and difficult [11].

Another drug, a hypnozoitocide, is necessary to treat latent vivax malaria and prevent future attacks. Primaquine (PQ) has been the only available therapeutic option to kill hypnozoites since 1952. A single dose of 30 mg PQ within 48 hours of infection appears sufficient to kill stages of *P. vivax* or *P. falciparum* attempting to develop into hepatic schizonts or hypnozoites [12]. Beyond that period, presumably after formation of dormant hypnozoites, relatively large doses totaling 210 to 420 mg of PQ (delivered over 7 to 56 days) are required to prevent

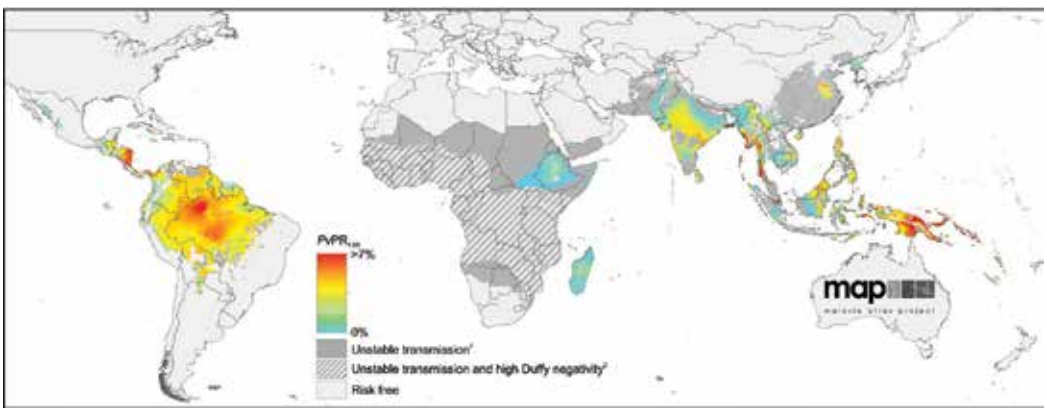


Figure 1. Distribution of *Plasmodium vivax* malaria in the world.

relapse [13]. Pharmacokinetic or pharmacodynamic interaction of blood schizonticidal and hypnozoitocidal therapies combined for the radical cure of vivax malaria has been observed and requires consideration in assessing the safety and efficacy of either or both in clinical use [14].

This chapter reviews the challenges vivax malaria poses in efforts to control and eliminate malaria in accordance with the Global Technical Strategy of the WHO [15] as they occur in many parts of the world. Experts advising the WHO formulated "*Plasmodium vivax* Control and Elimination: A Technical Brief" [16] highlighted the distinct character of this species in the context of control and elimination strategy. Conventional control aimed at diagnosing and treating of the acute attack and minimizing exposure to biting *Anopheles* mosquitoes will not suffice, largely due to the scale and importance of the latent hypnozoite reservoir in endemic communities. Decades of scientific, clinical, and public health neglect of this specific feature of vivax malaria leaves us poorly equipped to attack it safely and effectively. The biological basis of this problem is detailed with the aim of guiding discovery and development of sustainable solutions.

2. Biology of *Plasmodium vivax*

The broad and prolonged neglect of research on *P. vivax* has been highlighted by many researchers [17–20]. Although this lack of research certainly derives from complex and multiple factors, the misperception of this species as intrinsically benign perhaps dominates among them [21]. Today, we accept that a diagnosis of vivax malaria is sometimes associated with severe disease syndromes associated with fatal outcomes [16, 22]. The manner in which *P. vivax* threatens life with such typically low-grade parasitemias (usually tenfold lower than *P. falciparum*) is an important and relatively new question. Nonetheless, some researchers suggest that vivax malaria may be primarily an infection of hematopoietic tissues rather than of the vascular sinuses *per se* [21, 23, 24]. If most *P. vivax* biomass in the human host resides within tissues of the bone marrow and spleen, it would have far-reaching scientific, clinical, and public health implications with respect to measuring and combatting the threats imposed.

We already know the likely importance of the latent hypnozoite reservoir and sub-patent/asymptomatic parasitemias [25–27]. Adding an as-yet unacknowledged sequestered trophozoite reservoir—very few or no asexual parasites in vascular sinuses but many in the extravascular spaces of erythropoietic tissues—would greatly amplify concerns regarding the effectiveness of diagnosis and treatment in control and elimination. It is possible that most *P. vivax* parasites—certainly hypnozoites but perhaps also trophozoites—occur beyond the vascular sinuses in both asymptomatic and acutely ill patients and, therefore, also beyond the reach of standard diagnostics.

Although the Duffy antigen on the surface of the red blood cell has long been considered essential to *P. vivax* invasion—and its absence in many African populations thought to explain

the relative rarity of *P. vivax* on that continent—recent evidence from a variety of African locales has shown patent *P. vivax* parasitemia in patients who are negative for that molecule [28]. Moreover, *P. vivax* has been shown to be present in parts of Africa where it is not prevalent [29] and is indeed prevalent in other areas of that continent like Madagascar, the Horn, and across the northern Sahel [30].

3. Chloroquine-resistant acute *P. vivax*

Resistance to CQ by the asexual stages of *P. vivax* has been documented in most endemic regions [4, 5]. Resistant strains dominate the malarious Western Pacific and Indonesian archipelago and nations there have adopted highly efficacious ACTs [11] as first-line therapy. With the possible exception of artesunate combined with sulfadoxine-pyrimethamine, all ACTs have shown superb efficacy in killing asexual blood stages of *P. vivax* [31]. The safety and efficacy of PQ against relapse when combined with partner blood schizonticides other than CQ, quinine, or dihydroartemisinin-piperaquine [32, 33] require validation in clinical trials [14]. Elsewhere, for now, resistance appears sporadically and at relatively low frequencies. Despite substantial efforts to identify molecular markers of *P. vivax* resistance to antimalarial drugs, none have yet been validated. *In vivo* testing in patients or relatively difficult *ex vivo* drug testing procedures remain necessary [34]. The monitoring of antimalarial efficacy offers possible relief from risk of failure due to parasite resistance to specific therapies, but this is carried out relatively infrequently.

4. Latent and sub-patent *P. vivax*

The latent and sub-patent parasitemia caused by *P. vivax* is difficult or impractical to detect using available technologies. These unnoticed or invisible infections probably represent a dominant majority in most endemic settings. Thus, the primary blow to therapeutic effectiveness (the proportion of patients needing a particular therapy and receiving high-quality drug in a full and adequately absorbed dose) is simply the inability to identify those in need of therapy.

The human host also imposes important barriers to the effectiveness of antimalarial therapies in the real world. Clinical contraindications, patient adherence, provider prescribing practices, provider and patient access to the drug, and its quality and availability; all further chip away the realizable effectiveness of any given antimalarial agent. The contraindications are particularly important in the case of *P. vivax* and the crucial therapy against relapses with PQ, the only current therapeutic option for that clinical indication. Primaquine (and all other 8-aminoquinoline compounds evaluated) invariably provokes an acute hemolytic anemia in patients receiving therapeutic doses against relapse and having an inherited X chromosome-linked deficiency in glucose-6-phosphate dehydrogenase (G6PD) enzymatic activity [35]. This abnormality affects approximately 400 million people or 8% of people residing in malaria endemic countries [36].

Safe access to PQ for radical cure of vivax malaria may require access to point-of-care diagnostics for G6PD deficiency [37]. Even with such testing, however, there remains the problem of treating those diagnosed as G6PD-deficient, pregnant or lactating women, and infants below the age of 6 months [38]. There are no optimized or validated means of preventing relapse without 8-aminoquinoline drugs, e.g., by chemopreventive or presumptive periodic preventive therapeutic strategies [39, 40]. The 8-aminoquinoline drug, called tafenoquine, is in late clinical development and will likely soon offer a single-dose option to PQ, virtually eliminating the important adherence problem with that therapy [41, 42].

Another potential problem in the human host may be the inability to metabolize PQ to its active hypnozoite-killing metabolite by cytochrome P450 2D6 (CYP2D6) [43]. Natural polymorphism in the gene expressing CYP2D6 leads to a range of metabolic activities ranging anywhere between far above normal and null. Patients in need of PQ anti-relapse therapy and having significantly impaired or null CYP2D6 activity may relapse even with full compliance to good quality drug. We do not yet know the extent of this problem with regard to the frequencies of CYP2D6 alleles associated with PQ therapeutic failure, but the significantly impaired CYP2D6 *10 allele (a particular genetic variant of CYP2D6 gene) is relatively common among Southeast Asians, at about 35% frequency [44]. It may be that many Asians will be unable to adequately metabolize PQ and achieve successful radical cure [45].

The ambiguity of geographically variable frequency and timing of relapse—along with reinfection and recrudescence in recurrent *P. vivax* malaria after PQ therapy—makes estimating PQ efficacy in endemic settings very difficult. This is true even with directly observed therapy using high-quality drug. After decades of recommending a 5-day regimen of PQ against relapse, on the basis of observed low rates of relapse following therapy, investigators in India ultimately included a relapse control group (placebo) and discovered that efficacy to be nil [46]—the low rate of relapse was naturally occurring. John et al. [47] systematically reviewed recurrence rates after standard 0.25 mg/kg daily for 14-day regimen with rates of recurrence averaging about 8% at 1 month, 10% at 2–3 months, 14% at 4–6 months, and 20% at 7–12 months. In two randomized controlled trials of PQ given at high dose (0.5 mg/kg) to 257 Indonesian soldiers infected by *P. vivax* in eastern Indonesia and followed for a year where reinfection was not possible, 35 (14%) experienced at least one relapse [32, 33]. Among the 21 subjects whose CYP2D6 genotype and phenotype were examined, 20 showed evidence of significant functional impairment of CYP2D6 [48].

Evidence supports the notion of providing presumptive anti-relapse therapy to all patients diagnosed with any species of malaria agents, especially *P. falciparum*. In a retrospective analysis of over 10,000 research subjects naturally infected by *P. falciparum* in Thailand or Myanmar, 912 were treated with rapidly excreted blood schizonticides, and within 2 months, just over 50% experienced a *P. vivax* attack [49]. The people infected by one species in any given community must be considered at high risk of harboring latent and perhaps sub-patent infections of the other co-endemic species. Species-specific therapies, especially in an age of dominant CQ resistance among the plasmodia, may not be sensible in an elimination context.

Effective diagnosis and treatment represents the cornerstone of current control and elimination strategies, and the obstacles described here require consideration in realizing gains against this tenacious endemic problem. Indeed, such gains have been achieved both historically and recently. At the turn of the twentieth century, endemic vivax malaria occurred across much of southeastern North America, northern and southern Europe, the Middle East, and northern Australia—areas where it no longer appears. Much of this success was achieved applying environmental modifications against local *Anopheles* vectors, but more recent elimination successes using principally diagnosis and treatment strategy have occurred in nations like Turkey, Azerbaijan, and Sri Lanka, as examples [50]. The same had been achieved on the Korean Peninsula during the 1970s, but endemic vivax malaria transmission reappeared during the 1990s and persists today [51]. Post-elimination vigilance that includes not only diagnosis and treatment services but also vector control may be essential to protecting and sustaining the elimination of endemic vivax malaria [50].

5. Vector control in vivax malaria

Vector control of endemic vivax malaria may not have immediate impacts due to the hypnozoite reservoir contributing >80% of acute attacks of vivax malaria in low or high endemic settings [52, 53]. Success in reducing malaria incidence and local transmission to zero in a malaria endemic area, particularly where sympatric *P. falciparum* and *P. vivax* occur, may require greater sustainability of vector control measures. Vivax malaria transmission will outlast falciparum malaria, and reestablishment of local transmission may occur without imported cases, i.e., by local hypnozoites. Prevention of the seeding of new hypnozoites in liver cells by biting *Anopheles* mosquitoes obviously may contribute positively to the control and elimination of vivax malaria in the long term, but no randomized controlled trials yet affirm this. In one large cluster-randomized trial in Myanmar, insecticide-treated bed netting (ITN) had no impact whatsoever on the risk of malaria [54], an outcome attributed to the dominant *Anopheles* vector, *A. dirus* s.s., feeding predominantly outdoors and early in evening or morning [55]. Relatively modest effects were reported from a similarly cluster-randomized trial in Vietnam, again attributed to mosquito behaviors unfavorable to control by this means [56]. The main Asian vector species tend to feed early in the evening and outdoors where they also rest [57], minimizing their exposure to household insecticides. In other studies of strategies for minimizing exposure to *Anopheles,* much greater impacts against falciparum malaria were demonstrated relative to those against vivax malaria [58–60].

Over the last decade, attempts of using spatial repellents (SRs) to minimize exposure to biting insects have shown some success in diverse settings [61]. Repellency is distinct from the killing action of insecticides in more than one way, i.e., no direct contact is required, and lacking lethality does not select for resistance. SRs are effective irrespective of indoor or late-night feeding and resting behavior like conventional netting or indoor spraying. SRs should be evaluated for added benefit in areas where traditional long-lasting insecticidal net (LLIN) or

indoor residual spraying (IRS) of insecticides interventions may not offer full protection or have reached their efficacy limits—especially in areas with residual transmission or in areas where elimination may be considered feasible. Control of disease in these areas will require new approaches, and possibly spatial repellency would be practical and effective [62, 63]. SRs may be useful as stand-alone tools of personal or household protection where other interventions may not reach. Also, they may be combined with conventional interventions to augment their impacts.

Another vector control strategy for eliminating *P. vivax* in the Asian-Pacific region may be the method of environmental modification called "species sanitation." This approach offers prevention independently of the myriad problems and challenges of diagnosis and treatment or the limitations of insecticidal strategies. Species sanitation is simply sanitizing the environment against specific incriminated vector species by exploiting detailed knowledge of their bionomics (behavior and ecology) [64]. Malcolm Watson in British Malaya, along with Nicholas Swellengrebel and Raden Soesilo in the East Indies, invented, optimized, and validated species sanitation in malaria control [65]. A systematic analysis of 16 such interventions (most conducted before 1945) showed an average 88% reduction of malaria burden [66]. As new cases occur by relapse, reinfection, or importation, making the subsequent infection of mosquitoes improbable (by simply reducing their numbers) eventually suffocates transmission.

Although the implementation of LLIN, IRS, and species sanitation in different environmental settings rendered significant success rates [67], it is evident that the key determining factors for the success of any vector intervention selected is a thorough knowledge of the vector bionomics, local malaria transmission dynamics, and residual efficacy of choice insecticide. Knowledge of vector bionomics includes ascertaining breeding and resting preferences and feeding behavior of incriminated vector species. Transmission dynamics include information related to entomological inoculation rates, sibling species composition of vectors (based on reliable PCR identification assays), seasonality of malaria prevalence, and risk factors that may support the human-mosquito contact, while suitable insecticide means any available insecticide that renders knockdown effect and/or mortality to the incriminated vector population.

Another important issue to be considered is the ability of the *Anopheles* mosquitoes to adapt to the ongoing vector interventions by changing host-seeking behavior, such as from indoor to outdoor or *vice versa*, and selection of insecticide-resistant strain [68]. With current trends in globalization and population migration, deforestation, and resettlement of populations, reintroduction of malaria into areas that have been declared free from transmission is a clear and present risk. Therefore, no single intervention method may guarantee long-term efficacy; thus, regular monitoring of vector density and behavior should be a routine operation wherever this risk occurs. Most malaria control programs no longer have the entomological expertise needed to carry out these important tasks—addressing this problem may be the greatest and most important challenge within the context of a malaria elimination agenda.

6. Vaccination

A vaccine that prevents the seeding of human livers by both active schizonts and dormant hypnozoites of *P. vivax* would provide a conspicuously useful tool in eliminating this species. Mass or routine vaccination now seems impractical with non-sterilizing vaccines of short-lived immunity needing 3 or 4 doses. These may improve in the future, but even now a malaria vaccine could be applied in geographically or demographically narrowed settings to potentially great impacts. For example, high-risk and hard-to-reach populations like migrant workers or soldiers having sterile immunity to malaria (even if for just a season or two) may not only protect those people from harm but also greatly slow importation of malaria into receptive areas where transmission has been interrupted. Likewise, people living in areas prone to reintroduction of endemic malaria by high volumes of immigration from high-risk areas may be immunized and protected against very dangerous outbreaks and epidemics [69].

Today, there is no vaccine available that can prevent infection by *P. vivax* with high levels of sterilizing immune protection. That is also true for all other plasmodial species. The half-century-long efforts to develop a vaccine against *P. falciparum*—greatly aided by the ability to cultivate this species in continuous laboratory cultures since the late 1970s—culminated in the molecular subunit vaccine called RTS,S ASO1 (mimicking a protein-coating infectious sporozoites) [70] with the registered trademark name Mosquirix™ (GlaxoSmithKline). The vaccine did not prevent infection in the African infants and young children vaccinated but had the modest effects against higher parasitemias and signs of illness [67]. The modest efficacy combined with worrying and puzzling signals like increased risk of pneumococcal meningitis and significantly higher all-cause mortality among vaccinated females apparently explains the WHO position to withhold a favorable opinion on the vaccine until further studies involving targeted and limited rollout in several African nations are completed [71]. Molecular subunit vaccines targeting *P. vivax* molecules have not progressed beyond Phase 2a and show similar inability to achieve high levels of sterilizing protection [72].

Over the past decade, investigators applying live-attenuated sporozoites of *P. falciparum* have shown high levels of durable (~12 months) sterilizing protection in malaria-naïve adult volunteers in controlled human malaria infection (CHMI) experiments using a challenge strain homologous to the vaccine strain [73]. This approach relies on laboratory harvest of infectious sporozoites from laboratory-reared aseptic anopheline mosquitoes infected by *P. falciparum* maintained in the laboratory. Deriving live-attenuated sporozoites of *P. vivax* is possible [74] but exceedingly difficult, not strain-specific, and not sustainable as a source of vaccine. Nonetheless, immunization by irradiated sporozoites of the murine species *Plasmodium berghei* cross-protected against the murine species *Plasmodium yoelii* and *vice versa* in murine challenge models [75]. The possibility of sporozoites of *P. falciparum* cross-protecting against *P. vivax* challenge has not been examined directly, but proteomic analyses showed that these two human plasmodia species shared substantially more common probable T-cell epitopes than that between *P. berghei* and *P. yoelii*. A vaccine derived from laboratory-kept *P. falciparum*

systems offering protection against *P. vivax* would represent a quantum leap forward for vaccination against this species by effectively sidestepping the requirement for continuous laboratory cultivation for a live vaccine.

7. Challenges and recommendations

The greatest challenge in eliminating vivax malaria—the hypnozoite reservoir—may also be the greatest opportunity to accomplish the task. If >80% of incident malaria cases indeed derive from hypnozoites, then surely attacking and shrinking that reservoir would deliver substantial reductions in the burden of morbidity and mortality. Despite the availability of PQ for over 65 years, sustained and systematic assault on that reservoir has not been accomplished in the endemic tropics—largely due to the unsolved clinical problem of its hemolytic toxicity in G6PD-deficient patients.

Eliminating *P. vivax* malaria will require accepting the inadequacy of conventional falciparum malaria-focused control strategy, tactics, and tools and committing to the optimizing and validating of interventions suited to this stubborn parasite. This effectively means striving to solve the wrenchingly difficult problem of the hemolytic toxicity of PQ in G6PD-deficient patients by almost any means. The obstacles presented in managing populations and individual patients carrying this infection emphasize the great advantage of preventing it in the first place with an effective vector control strategy. In this context, species sanitation has proven highly effective against endemic Asian malarias a century ago [54] and would probably do so again.

Taking all these factors into considerations, we recommend the following measures for eliminating endemic vivax malaria:

1. Active case detection and early treatment are essential steps, fundamental to eliminating any endemic malaria; however, this measure alone will not lead to elimination—too many infections are latent, sub-patent, sequestered, and asymptomatic.
2. Adoption of safe and universal access to radical cure for cases of vivax malaria along with universal access to alternative means of relapse prevention for people ineligible for therapy with 8-aminoquinolines would accelerate progress to elimination. Achieving that will likely also require better diagnostics for both the parasite and G6PD deficiency than are currently available.
3. Adoption of radical cure with an 8-aminoquinoline and ACT with diagnosis of any species of malaria where *P. vivax* also occurs as a means of targeting likely carriers of hypnozoites.
4. Reduce new vivax infections/seeding of the liver with hypnozoites by substantially reducing human contact with malaria vectors, effectively stranding extant parasites in all stages of human infection—latent, sub-patent, patent, and eventually vanishing without *Anopheles* contact and onward transmission. Interrupting transmission by species sanitation measures may be the most durable and effective means of achieving this goal.

5. Examine the possibility of sterilizing immune protection against *P. vivax* provided by attenuated *P. falciparum* sporozoite vaccines providing an immediately highly relevant tool for eliminating endemic *P. vivax*.

8. Conclusion

Plasmodium vivax passes substantial challenges that may hinder achievement of global malaria elimination by 2030. The most challenging evidence is the lack of technology to detect the latent infection caused by hypnozoite. Therefore, the only tool to prevent *P. vivax* transmission originated from reactivation of hypnozoites is by vector control.

Acknowledgements

We gratefully acknowledge Prof. Amin Soebandrio MD, PhD, Clin. Microbiol, Director of the Eijkman Institute for Molecular Biology for his encouragement and advice, and Prof. A. Asadul I. Tahir, MD, PhD, FICS, Dean of the Faculty of Medicine, Hasanuddin University for the support to DS. JKB is supported by the Vietnam Major Overseas Programme grant from the Wellcome Trust.

List of acronyms

ACT	artemisinin-based combination therapy
CHMI	controlled human malaria infection
CQ	chloroquine
CYP2D6	cytochrome P450 2D6
G6PD	glucose-6-phosphate dehydrogenase
IRS	indoor residual spraying
ITN	insecticide-treated bed nets
LLIN	long-lasting insecticide treated nets
PQ	primaquine
SEA	Southeast Asia
SR	spatial repellent
WHO	World Health Organization

Author details

Puji BS Asih[1]*, Din Syafruddin[1,2] and John Kevin Baird[3,4]

*Address all correspondence to: puji@eijkman.go.id

1 Eijkman Institute of Molecular Biology, Jakarta, Indonesia

2 Hasanuddin University Medical Research Centre, Makassar, Indonesia

3 Eijkman Oxford Clinical Research Unit, Jakarta, Indonesia

4 Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom

References

[1] Gething PW, Elyazar IR, Moyes CL, Smith DL, Battle KE, Guerra CA, Patil AP, Tatem AJ, Howes RE, Myers MF, George DB, Horby P, Wertheim HF, Price RN, Müeller I, Baird JK, Hay SI. A long-neglected world malaria map: *Plasmodium vivax* endemicity in 2010. PLoS Neglected Tropical Diseases. 2012;**6**(9):1814. DOI: 10.1371/journal.pntd.0001814

[2] Battle KE, Gething PW, Elyazar IR, Moyes CL, Sinka ME, Howes RE, Guerra CA, Price RN, Baird KJ, Hay SI. The global public health significance of *Plasmodium vivax*. Advances in Parasitology. 2012;**80**:1-111. DOI: 10.1016/B978-0-12-397900-1.00001-3

[3] Rosalind EH, Katherine EB, Kamini NM, David LS, Richard EC, Kevin Baird J, Hay SI. Global epidemiology of *Plasmodium vivax*. The American Journal of Tropical Medicine and Hygiene. 2016;**95**(6):15-34. DOI: 10.4269/ajtmh.16-0141

[4] Price RN, von Seidlein L, Valecha N, Nosten F, Baird JK, White NJ. Global extent of chloroquine-resistant *Plasmodium vivax*: A systematic review and meta-analysis. The Lancet Infectious Diseases. 2014;**14**(10):982-991. DOI: 10.1016/S1473-3099(14)70855-2

[5] Baird JK. Resistance to therapies to infection by *Plasmodium vivax*. Clinical Microbiology Reviews. 2009;**22**:508-534. DOI: 10.1128/CMR.00008-09

[6] White NJ, Imwong M. Relapse. Advances in Parasitology. 2012;**80**:113-150. DOI: 10.1016/B978-0-12-397900-1.00002-5

[7] Baird JK, Leksana B, Masbar S, Fryauff DJ, Sutanihardja MA, Suradi, Wignall FS, Hoffman SL. Diagnosis of resistance to chloroquine by *Plasmodium vivax*: Timing of recurrence and whole blood chloroquine levels. The American Journal of Tropical Medicine and Hygiene. 1997;**56**:618-620. PMID: 9230792

[8] Asih PB, Syafruddin D, Leake J, Sorontou Y, Sadikin M, Sauerwein RW, Vinetz J, Baird JK. Phenotyping clinical resistance to chloroquine in *Plasmodium vivax* in northeastern

Papua, Indonesia. International Journal for Parasitology: Drugs and Drug Resistance. 2011;**1**(1):28-32. DOI: 10.1016/j.ijpddr.2011.08.001

[9] Chen N, Auliff A, Rieckmann K, Gatton M, Cheng Q. Relapses of *Plasmodium vivax* result from clonal hypnozoites activated at predetermined intervals. The Journal of Infectious Diseases. 2007;**195**:934-941. PMID: 17330782

[10] Imwong M, Snounou G, Pukrittayakamee S, Tanomsing N, Kim JR, Nandy A, Guthmann JP, Nosten F, Carlton J, Looareesuwan S, Nair S, Sudimack D, Day NP, Anderson TJ, White NJ. Relapses of *Plasmodium vivax* infection usually result from activation of heterologous hypnozoites. The Journal of Infectious Diseases. 2007;**195**:927-933. PMID: 17330781

[11] Baird JK, Valecha N, Duparc S, White NJ, Price RN. Diagnosis and treatment of *Plasmodium vivax* malaria. The American Journal of Tropical Medicine and Hygiene. 2016; **95**(6):35-51. DOI: 10.4269/ajtmh.16-0171

[12] Baird JK, Fryauff DJ, Hoffman SL. Primaquine for prevention of malaria in travellers. Clinical Infectious Diseases. 2003;**37**(12):1659-1667. DOI: https://doi.org/10.1086/379714

[13] Baird JK, Hoffman SL. Primaquine therapy for malaria. Clinical Infectious Diseases. 2004;**39**(9):1336-1345. DOI: https://doi.org/10.1086/424663

[14] Baird JK. Resistance to chloroquine unhinges vivax malaria therapeutics. Antimicrobial Agents and Chemotherapy. 2011;**55**(5):1827-1830. DOI: 10.1128/AAC.01296-10

[15] WHO. Global Technical Strategy for Malaria 2016-2030. Geneva, World Health Organization (WHO); 2015. http://apps.who.int/iris/bitstream/10665/176712/1/9789241564991_eng.pdf?ua=1&ua=1 [Accessed: 31 January 2018]

[16] WHO. Control and Elimination of *Plasmodium vivax*: A Technical Brief. Geneva, Switzerland: World Health Organization (WHO); 2015. http://www.searo.who.int/entity/malaria/control-and-elimination-plasmodium.pdf?ua=1 [Accessed: 31 January 2018]

[17] Mendis K, Sina BJ, Marchesini P, Carter R. The neglected burden of *Plasmodium vivax* malaria. The American Journal of Tropical Medicine and Hygiene 2001;**64**(1):97-106. PMID: 11425182

[18] Baird JK. Neglect of *Plasmodium vivax*. Trends in Parasitology 2007;**23**:533-539. PMID: 17933585

[19] Price RN, Tjitra E, Guerra CA, Yeung S, White NJ, Anstey NM. Vivax malaria: Neglected and not benign. The American Journal of Tropical Medicine and Hygiene 2007;**77**(6):79-87. PMID:18165478

[20] Mueller I, Galinski MR, Baird JK, Carlton JM, Kochar DK, Alonso PL, del Portillo HA. Key gaps in the knowledge of *Plasmodium vivax*, a neglected human malaria parasite. The Lancet Infectious Diseases. 2009;**9**(9):555-566. DOI: 10.1016/S1473-3099(09)70177-X

[21] Baird JK. Evidence and implications of mortality associated with acute *Plasmodium vivax* malaria. Clinical Microbiology Reviews. 2013;**26**(1):36-57. DOI: 10.1128/CMR.00074-12

[22] Anstey NM, Douglas NM, Poespoprodjo JR, Price RN. *Plasmodium vivax* clinical spectrum, risk factors and pathogenesis. Advances in Parasitology. 2012;**80**:151-201. DOI: 10.1016/B978-0-12-397900-1.00003-7

[23] Barber BE, William T, Grigg MJ, Parameswaran U, Piera KA, Price RN, Yeo TW, Anstey NM. Parasite biomass-related inflammation, endothelial activation, microvascular dysfunction and disease severity in vivax malaria. PLoS Pathogens. 2015;**11**. DOI: https://doi.org/10.1371/journal.ppat.1004558

[24] Malleret B, Li A, Zhang R, Tan KS, Suwanarusk R, Claser C, Cho JS, Koh EG, Chu CS, Pukrittayakamee S, Ng ML, Ginhoux F, Ng LG, Lim CT, Nosten F, Snounou G, Rénia L, Russell B. *Plasmodium vivax*: Restricted tropism and rapid remodeling of CD71-positive reticulocytes. Blood. 2015;**125**(8):1314-1324. DOI: 10.1182/blood-2014-08-596015

[25] Cheng Q, Cunningham J, Gatton ML. Systematic review of sub-microscopic *P. vivax* infections: Prevalence and determining factors. PLoS Neglected Tropical Diseases. 2015; **9**(1):3413. DOI: 10.1371/journal.pntd.0003413

[26] Moreira CM, Abo-Shehada M, Price RN, Drakeley CJ. A systematic review of submicroscopic *Plasmodium vivax* infection. Malaria Journal. 2015;**14**:360. DOI: 10.1186/s12936-015-0884-z

[27] Okell LC, Bousema T, Griffin JT, Ouedraogo AL, Ghani AC, Drakeley CJ. Factors determining the occurrence of submicroscopic malaria infections and their relevance for control. Nature Communications. 2012;**3**:1237. DOI: 10.1038/ncomms2241

[28] Menard D, Barnadas C, Bouchier C, Henry-Halldin C, Gray LR, Ratsimoasoa A, Thonier V, Carod JF, Domarle O, Colin Y, Bertrand O, Picot J, King CL, Grimberg BT, Mercereau-Puijalon O, Zimmerman PA. *Plasmodium vivax* clinical malaria is commonly observed in Duffy-negative Malagasy people. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**:5967-5971. DOI: 10.1073/pnas.0912496107

[29] Culleton R, Ndounga M, Zeyrek FY, Coban C, Casimiro PN, Takeo S, Tsuboi T, Yadava A, Carter R, Tanabe K. Evidence for the transmission of *Plasmodium vivax* in the Republic of the Congo, West Central Africa. The Journal of Infectious Diseases. 2009;**200**:1465-1469. DOI: 10.1086/644510

[30] Howes RE, Reiner RC Jr, Battle KE, Longbottom J, Mappin B, Ordanovich D, Tatem AJ, Drakeley C, Gething PW, Zimmerman PA, Smith DL, Hay SI. *Plasmodium vivax* transmission in Africa. PLoS Neglected Tropical Diseases. 2015;**9**:0004222. DOI: 10.1186/s12936-1118-8

[31] Gogtay N, Kannan S, Thatte UM, Olliaro PL, Sinclair D. Artemisinin-based combination therapy for treating uncomplicated *Plasmodium vivax* malaria. Cochrane Database of Systematic Reviews. 2013;**10**:CD008492. DOI: 10.1002/14651858.XCD008492.pub3

[32] Sutanto I, Tjahjono B, Basri H, Taylor WR, Putri FA, Meilia RA, Setiabudy R, Nurleila S, Ekawati LL, Elyazar I, Farrar J, Sudoyo H, Baird JK. Randomized, open-label trial of primaquine against vivax relapse in Indonesia. Antimicrob Agents Chemother. 2013;**57**:1128-1135. DOI: 1128/AAX.01879-12

[33] Nelwan EJ, Ekawati LL, Tjahjono B, Setiabudy R, Sutanto I, Chand K, Ekasari T, Djoko D, Basri H, Taylor WR, Duparc S, Subekti D, Elyazar I, Noviyanti R, Sudoyo H, Baird JK. Randomized trial of primaquine hypnozoitocidal efficacy when administered with artemisinin-combined blood schizontocides for radical cure of *Plasmodium vivax* in Indonesia. BMC Medicine. 2015;**13**:294. DOI: 10.1186/s12916-015-0535-9

[34] Commons RJ, Thriemer K, Humphreys S, Suay I, Sibley CH, Guerin PJ, Price RN. The vivax surveyor: Online mapping database for *Plasmodium vivax* clinical trials. International Journal for Parasitology: Drugs and Drug Resistance. 2017;**7**:181-190. DOI: 10.1016/ijpddr.2017.03.003

[35] Luzzatto L, Nannelli C, Notaro R. Glucose-6-phosphate dehydrogenase deficiency. Hematology/Oncology Clinics of North America. 2016;**30**(2):373-393. DOI: 10.1016/j.hoc.2015.11.006

[36] Howes RE, Piel FB, Patil AP, Nyangiri OA, Gething PW, Dewi M, Hogg MM, Battle KE, Padilla CD, Baird JK, Hay SI. G6PD deficiency prevalence and estimates of affected populations in malaria endemic countries: A geostatistical model-based map. PLoS Medicine. 2012;**9**:1001339. DOI: https://doi.org/10.1371/journal.pmed.1001339

[37] Baird JK. Point-of-care G6PD diagnostics for *Plasmodium vivax* malaria is a clinical and public health urgency. BMC Medicine. 2015;**13**:296. DOI: 10.1186/s12916-015-0531-0

[38] WHO. Guidelines for the treatment of malaria. 3rd ed. Geneva, World Health Organization (WHO); 2015. http://apps.who.int/iris/bitstream/10665/162441/1/9789241549127_eng.pdf [Accessed: 31 January 2018]

[39] Peters AL, Van Noorden CJ. Glucose-6-phosphate dehydrogenase deficiency and malaria: Cytochemical detection of heterozygous G6PD deficiency in women. The Journal of Histochemistry and Cytochemistry. 2009;**57**(11):1003-1011. DOI: 10.1369/jhc.2009.953828

[40] Padilla CD, Therrell BL. Working group of the Asia Pacific Society for human genetics on consolidating newborn screening efforts in the Asia Pacific R. Consolidating newborn screening efforts in the Asia Pacific region: networking and shared education. Journal of Community Genetics. 2012;**3**:35-45. DOI: 10.1007/s12687-011-0076-7

[41] Douglas NM, Poespoprodjo JR, Patriani D, Malloy MJ, Kenangalem E, Sugiarto P, Simpson JA, Soenarto Y, Anstey NM, Price RN. Unsupervised primaquine for the treatment of *Plasmodium vivax* malaria relapses in Southern Papua: A hospital-based cohort study. PLoS Medicine. 2017;**14**:1002379. DOI: 10.1371/journal.pmed.1002379

[42] Rjapaske S, Rodrigo C, Fernando SD. Tafenoquine for preventing relapse in people with *Plasmodium vivax* malaria. Cochrane Database of Systematic Reviews. 2015;**29**. DOI: CD010458, 10.1002/14651858.CD010458.pub2

[43] Marcsisin SR, Reichard G, Pybus BS. Primaquine pharmacology in the context of CYP 2D6 pharmacogenomics: Current state of the art. Pharmacology & Therapeutics. 2016;**161**:1-10. DOI: 10.1016/j.pharmthera.2016.03.011

[44] Sistonen J, Fuselli S, Palo JU, Chauhan N, Padh H, Sajantila A. Pharmacogenetic variation at CYP2C9, CYP2C19, and CYP2D6 at global and microgeographic scales. Pharmacogenetics and Genomics. 2009;**19**(2):170-179. DOI: 10.1097/FPC.0b013e32831ebb30

[45] Baird JK, Battle KE, Howes RE. Primaquine ineligibility in anti-relapse therapy of *Plasmodium vivax* malaria: The problem of G6PD deficiency and cytochrome P-450 2D6 polymorphisms. Malaria Journal. 2018;**17**:42. DOI: 10.1186/s12936-018-2190-z

[46] Gogtay NJ, Desai S, Kamtekar KD, Kadam VS, Dalvi SS, Kshirsagar NA. Efficacies of 5- and 14-day primaquine regimens in the prevention of relapses in *Plasmodium vivax* infections. Annals of Tropical Medicine and Parasitology. 1999;**93**:809-812. PMID: 9861412

[47] John GK, Douglas NM, von Seidlein L, Nosten F, Baird JK, White NJ, Price RN. Primaquine radical cure of *Plasmodium vivax*: A critical review of the literature. Malaria Journal. 2012;**11**:280. DOI: 10.1186/1475-2875-11-1280

[48] Baird JK, Louisa M, Noviyanti R, Ekawati L, Elyazar I, Subekti D, Chand K, Gayatri A, Instiaty, Soebianto S, Crenna-Darusallam C, Joki D, Silistyanto BP, Meriyenes D, Wesche D, Nelwan EJ, Sutanto I, Sudoyo H, Setiabudy R. Impaired cytochrom P-450 2D6 activity genotype and phenotype associated with primaquine treatment failure against latent *Plasmodium vivax* malaria: A nested case control study. JAMA Open Network, in press

[49] Douglas NM, Nosten F, Ashley EA, Phaiphun L, van Vugt M, Singhasivanon P, White NJ, Price RN. *Plasmodium vivax* recurrence following falciparum and mixed species malaria: Risk factors and effect of elimination kinetics. Clinical Infectious Diseases. 2011;**52**:612-620. DOI: 10.1093/cid/ciq249

[50] WHO. Eliminating Malaria. Global Malaria Program, World Health Organization, Geneva; 2016. WHO/HTM/GMP/2016.3

[51] Park JW, Jun G, Yeom JS. *Plasmodium vivax* malaria: Status in the Republic of Korea following re-emergence. The Korean Journal of Parasitology. 2009;**47**:S39-S50. DOI: 10.3347/kjp.2009.47.S.S39

[52] Adekunle AI, Pinkevych M, McGready R, Luxemburger C, White LJ, Nosten F, Cromer D, Davenport MP. Modeling the dynamics of *Plasmodium vivax* infection and hypnozoite reactivation *in vivo*. PLOS Neglected Tropical Diseases. 2015;**9**:0003595. DOI: https://doi.org/10.1371/journal.pntd.0003595

[53] Robinson LJ, Wampfler R, Betuela I, Karl S, White MT, CSN LWS, Hofmann NE, Kinboro B, Waltmann A, Brewster J, Lorry L, Tarongka N, Samol L, Mueller I. Strategies for understanding and reducing the *Plasmodium vivax* and *Plasmodium ovale* hypnozoite reservoir in Papua new guinean children: A randomised placebo-controlled trial and mathematical model. PLoS Medicine. 2015:1001891. DOI: https://doi.org/10.1371/journal.pmed.1001891

[54] Smithuis FM, Kyaw MK, Phe UO, van der Broek I, Katterman N, Rogers C, Almeida P, Kager PA, Stepniewska K, Lubell Y, Simpson JA, White NJ. The effect of insecticide-treated bed nets on the incidence and prevalence of malaria in children in an area of unstable seasonal transmission in Western Myanmar. Malaria Journal. 2013;**12**:363. DOI: https://doi.org/10.1186/1475-2875-12-363

[55] Smithuis FM, Kyaw MK, Phe UO, van der Broek I, Katterman N, Rogers C, Almeida P, Kager PA, Stepniewska K, Lubell Y, Simpson JA, White NJ. Entomological determinants of insecticide-treated bed net effectiveness in Western Myanmar . Malaria Journal. 2013;**12**:364 DOI: https://doi.org/10.1186/1475-2875-12-364

[56] Thang ND, Erhart A, Speybroeck N, Xa NX, Thanh NN, Ky PV, Hung LH, Thuan LK, Coosemans M, D'Alessandro U. Long-lasting insecticidal hammocks for controlling forest malaria: A community-based trial in a rural area of Central Vietnam. PLoS One. 2009;**4**(10):7369. DOI: http://doi.org/10.1371/journal.pone.0007369

[57] Sinka ME, Bangs MJ, Manguin S, Chareonviriyaphap T, Patil AP, Temperley WH, Gething PW, Elyazar IR, Kabaria CW, Harbach RE, Hay SI. The dominant vectors of human malaria in the Asia-Pacific region: Occurrence data, distribution maps and bionomics precis. Parasites & Vectors. 2011;**4**:89. DOI: 10.1186/1756-3305-4-89

[58] Ferreira MU, Castro MC. Challenges for malaria elimination in Brazil. Malaria Journal. 2016;**15**:284. DOI: 10.1186/s12936-016-1335-1

[59] Avikar AR, Shah N, Dhariwal AC, Sonal GS, Prdhan MM, Ghosh SK, Valecha N. Epidemiology of *Plasmodium vivax* in India. The American Journal of Tropical Medicine and Hygiene. 2016;**95**(6Suppl):108-120. DOI: 10.4269/ajtmh.16—163

[60] Sharma VP, Dev V, Phookan S. Neglected *Plasmodium vivax* malaria in northeastern states of India. Indian Journal of Medical Research. 2015;**141**:546-555. DOI: 10.4103/0971-5916.159511

[61] Syafruddin D, Bangs MJ, Sidik D, Elyazar I, Asih PB, Chan K, Nurleila S, Nixon C, Hendarto J, Wahid I, Ishak H, Bøgh C, Grieco JP, Achee NL, Baird JK. Impact of a spatial repellent on malaria incidence in two villages in Sumba, Indonesia. The American Journal of Tropical Medicine and Hygiene. 2014;**91**(6):1079-1087. DOI: 10.4269/ajtmh.13-0735

[62] Ogoma SB, Lorenz LM, Ngonyani H, Sangusangu R, Kitumbukile M, Kilalangongono M, Simfukwe ET, Mbeyela E, Roman D, Moore J, Kreppel K, Maia MF, Moore SJ. An experimental hut study to quantify the effect of DDT and airborne pyrethroids on entomological parameters of malaria transmission. Malaria Journal. 2014;**13**:131. DOI: https://doi.org/10.1186/1475-2875-13-131

[63] Achee NL, Bangs MJ, Farlow R, Killeen GF, Lindsay S, Logan JG, Moore SJ, Rowland M, Sweeney K, Torr SJ, Zwiebel LJ, Grieco JP. Spatial repellents: From discovery and development to evidence-based validation. Malaria Journal. 2012;**11**:164. DOI: http://doi.org/10.1186/1475-2875-11-164

[64] Baird JK. Malaria control by commodities without practical malariology. BMC Public Health. 2017;**17**:590. DOI: https://doi.org/10.1186/s12889-017-4454-x

[65] Keiser J, Singer BH, Utzinger J. Reducing the burden of malaria in different eco-epidemiological settings with environmental management: A systematic review. The Lancet Infectious Diseases. 2005;**5**(11):695-708. DOI: 10.1016/S1473-3099(05)70268-1

[66] Takken W, Snellen WB, Verhave JP, Knols BGJ, Atmosoedjono S. Environmental measures for malaria control in Indonesia -an historical review on species sanitation. Wageningen Agricultural University Papers. 1990;**90-7**:1-16. http://edepot.wur.nl/282643

[67] Lengeler C. Insecticide-treated bed nets and curtains for preventing malaria. Cochrane Database of Systematic Reviews. 2004;**2**:CD000363. DOI: 10.1002/14651858.CD000363.pub2

[68] Sokhna C, Ndiath MO, Rogier C. The changes in mosquito vector behaviour and the emerging resistance to insecticides will challenge the decline of malaria. Clinical Microbiology and Infection. 2013;**19**(10):902-907. DOI: 10.1111/1469-0691.12314

[69] Gosling R, von Seidlein L. The future of the RTS,S/AS01 malaria vaccine: An alternative development plan. PLoS Medicine. 2016;**13**:1001994. DOI: 10.1371/journal.pmed.1001994

[70] Fowkes FJI, Simpson JA, Beeson JG. Implications of the licensure of a partially efficacious malaria vaccine on evaluating second-generation vaccines. BMC Medicine. 2013;**11**:232. DOI: 10.1186/1741-7015-11-232

[71] WHO. Malaria vaccine: World Health Organization position paper – January 2016. Weekly Epidemiological Record. 2016;**91**:33-52. http://www.who.int/wer/2016/wer9104.pdf

[72] Sandoval-Reyes A, Bachmann MF. *Plasmodium vivax* vaccines: Why are we where we are? Human Vaccines & Immunotherapeutics. 2013;**9**:2558-2565. DOI: 10.4161/hv.26157

[73] Hoffman SL, Vekemans J, Richie TL, Duffy PE. The march toward malaria vaccines. Vaccine. 2015;**33**(Suppl 4):D13-D23. DOI: 10.1016/j.vaccine.2015.07.091

[74] Chattopadhyay R, Velmurugan S, Chakiath C, Donkor LA, Milhous W, Barnwell JW, Collins WE, Hoffman SL. Establishment of an *in vitro* assay for assessing the effects of drugs on the liver stages of *Plasmodium vivax* malaria. PLoS One. 2010;**5**:14275. DOI: 10.1371/journal.pone.0014275

[75] Sedegah M, Weiss WW, Hoffman SL. Cross-protection between attenuated *Plasmodium berghei* and *P. yoelii* sporozoites. Parasite Immunology. 2007;**29**:559-565. DOI: 10.1111/j.1365-3024.2007.00976.x

Chapter 5

Human and Simian Malaria in the Greater Mekong Subregion and Challenges for Elimination

Jeffrey Hii, Indra Vythilingam and
Arantxa Roca-Feltrer

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76901

Abstract

In recent years malaria initiatives have increasingly shifted from malaria control to a focus on achieving malaria elimination in the Southeast Asia region. However, this region experiences unique challenges in this transition due to its distinctive malaria ecosystem (mainly related to forests) and high volume of population movement (both within and between countries). These bioenvironmental factors increase the exposure of populations at higher risk due to their close association with forest, and contributes to outdoor and residual transmission. Given that this region has also historically been the source of resistance to anti-malarial drugs, the potential spread of artemisinin resistance via global transportation routes would pose a major threat to malaria control and elimination efforts worldwide. In addition, other factors also hinder the malaria elimination goal such as importation of parasite infection, uncontrolled monkey malaria (*Plasmodium knowlesi*), or the fact that many countries in this region experience mixed infections where *P. vivax* becomes a more predominant species as overall malaria transmission decreases. This chapter addresses these challenges in detail and provide recommendations and key priorities to overcome these obstacles to accelerate efforts for achieving malaria elimination.

Keywords: malaria, elimination, Greater Mekong Subregion, drug-resistance, *Plasmodium knowlesi*, vivax malaria, residual transmission

1. Introduction

In the Greater Mekong Subregion (GMS)[1], malaria is still a substantial public health problem, especially along international borders and forested areas, adversely putting populations such as migrants, refugees, and forest workers most at risk. In 2013, there were 447,800 malaria cases and 342 deaths in the GMS, with close to 700 million people living in risk areas [1]. Between 2012 and 2016, the reported number of malaria cases in the GMS fell by 74% (**Figure 1**) and malaria deaths by 91% in the same period (**Figure 2**).

Mid-year estimates for 2017 point to a further decline in cases [2]. Contributing to these impressive results, all six countries of the Subregion are making significant headway towards a common target: eliminating malaria by the year 2030 at the latest.

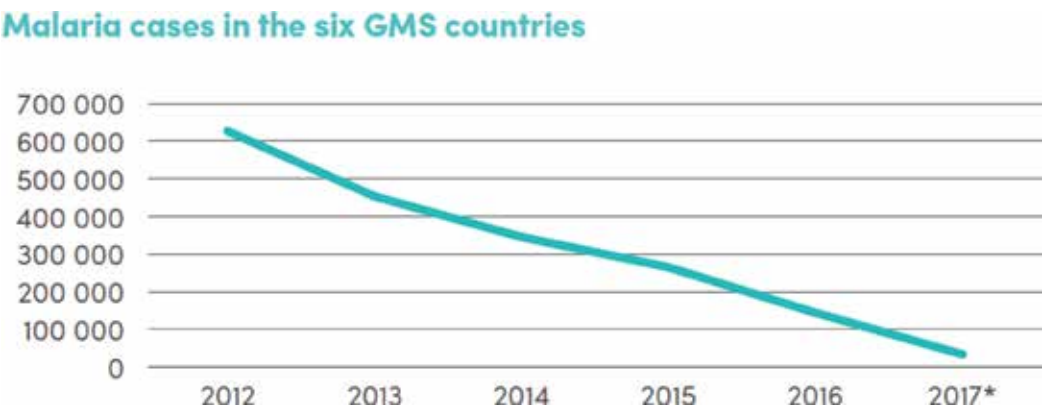


Figure 1. Declining trends of malaria transmission in the Greater Mekong Subregion (GMS) since 2012 (source: [2]).

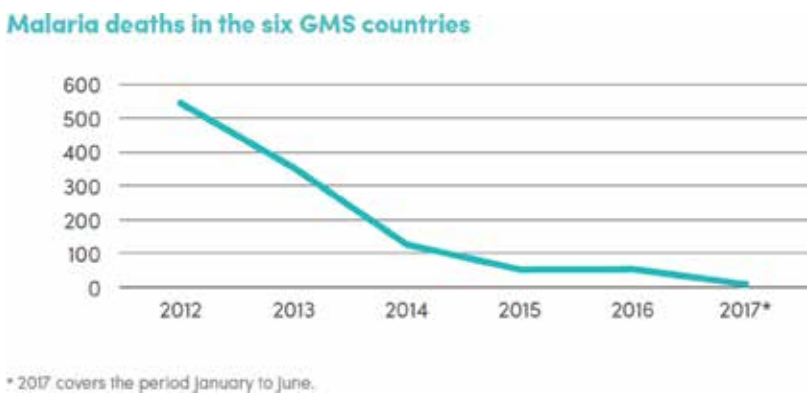


Figure 2. Declining trend of malaria deaths in the Greater Mekong Subregion (GMS) since 2012 (source: [2]).

[1]Cambodia, The People's Republic of China (specifically Yunnan Province), the Lao People's Democratic Republic (Lao PDR), Myanmar, Thailand, and Vietnam.

http://dx.doi.org/10.5772/intechopen.76901

These goals will not be easy to achieve. Despite these reductions and the subsequent move towards elimination, malaria remains an important cause of morbidity for an estimated 32 million inhabitants, especially in remote areas with low population densities and limited healthcare services and infrastructure, located in and near forested areas, which often lie close to international borders [3, 4]. In many places, the population groups most affected are ethnic minorities and forest-goers who are rapidly becoming the most important source of transmission in areas where main vectors are present. Within these groups, cultural and linguistic barriers often constrain malaria control efforts due to their high mobility and low access to interventions to prevent, diagnose and treat malaria.

In some areas the malaria situation has deteriorated by armed conflict affecting access to malaria control services. Population movements are a key feature in the GMS and are largely occupationally/economically driven; occur within borders and across borders; involve multiple factors and complex dynamics of movement; and affect different subsets of moving populations [5], thus further complicating the epidemiology and control of the disease [6]. The rapid increase in the number of large infrastructure and agricultural development projects in the region is also having a significant impact on the epidemiology of communicable diseases in general, and malaria in particular [7]. This chapter addresses several key challenges faced by elimination programmes to contain the unacceptably high disease burden against the background of rapidly declining incidence.

2. Resistance to artemisinin and ACT: current and future approaches

Antimalarial drug resistance is not a new biological phenomenon. In the 1970s and 1980s, *Plasmodium falciparum*—the parasite species responsible for the most common and deadliest form of malaria—developed widespread resistance to previous antimalarial medicines, such as chloroquine and sulfadoxine-pyrimethamine (SP) [8]. Artemisinin based combination therapies (ACTs), introduced in the 1990s, are currently the most effective antimalarial drugs [9] and represent the first line-treatment for uncomplicated falciparum malaria in all endemic countries.

Although artemisinin usually kills all malaria parasites, the use of a combination of drugs—as opposed to monotherapy—helps ensure that any remaining parasites will be killed by the partner drug before the resistant parasites can spread. According to the World Health Organisation (WHO), clinical artemisinin (and its derivatives) resistance is defined as delayed parasite clearance and represents a partial/relative resistance that has thus far only affected ring-stage malaria parasites [10]. In Southeast Asia, however, some malaria parasites have already developed resistance to artemisinin-based drugs; a recent report of a single multi-drug resistant malaria parasite lineage (PfPailin) with associated piperaquine resistance in Vietnam and its implications of subsequent transnational spread is of international concern [11]. Artemisinin resistance was first reported along the Thailand-Cambodia border in 2008 [12, 13] and has continued to spread in all Greater Mekong Subregion countries [14–18]. In addition, artemisinin resistance has been involved in selecting for resistance to ACT partner drugs, resulting in high late treatment failure rates with dihydroartemisinin-piperaquine in Cambodia [14, 19–25] and with artesunate-mefloquine on the Thai-Myanmar border [26].

There are many factors that are thought to have contributed to the emergence and spread of artemisinin resistance in the GMS. One important factor is thought to be the use of oral artemisinin monotherapy (AMT) in place of WHO-recommended ACTs (as unregulated artemisinin or artesunate monotherapy has been available since mid-1970s in the region). In Myanmar, private healthcare facilities and healthcare providers who prioritize consumers' demand instead of recommended practices were more likely to stock oral AMT [26, 27]. Malaria elimination strategies should include targeted interventions to effectively reach these outlets. Fortunately, a major achievement during the resistance containment (and more recently elimination) activities has ceased the use of artemisinin monotherapies. ACT watch methods are monitoring displacement of oral AMTs, a major objective of the resistance containment strategy [28], and data will feed into regional score cards such as the Asia Pacific Leaders Malaria Alliance Access to Quality Medicines Task Force and the World Health Organisation (WHO) Emergency Response to Artemisinin Resistance (ERAR), which are vested in supporting national programs in tracking progress towards halting the availability and use of oral AMTs [28]. In Southeast Asia, where malaria transmission is generally low and emergence of resistance has been documented in multiple independent locations [29]; containment programmes have been converted into elimination of *P. falciparum* strategies to ensure halting the spread of resistance entirely.

Other contributing factors are the use of substandard and counterfeit anti-malarial drugs and the difficulty of controlling malaria within migrant and hard-to-reach populations [30]. Given the transnational nature of this problem, the establishment of effective mechanisms for cross-country surveillance, information exchange and coordinated action is also necessary. This includes reinforcing existing institutional frameworks for regional health cooperation, particularly the Association of Southeast Asian Nations, and their potential to support enhanced capacities and cooperation to address this challenge [31]. Lastly, selection pressure—genetic mutations of wild-type genes in the parasite render them insusceptible to antimalarial drug treatment—is also thought to be important. The use of antimalarial drugs in patients with parasites containing mutations can eliminate susceptible parasites but leave resistant mutants to survive and reproduce [32].

More recently another potential contributing factor has been hypothesized. Given that there are parasite isolates that do not infect some *Anopheles* species, it is thought that artemisinin-resistant parasites are spreading so fast in Southeast Asia because they infect most or all native *Anopheles* species (e.g., *Anopheles dirus* and *An. minimus*), including African vector counterparts such as *An. coluzzii* (formerly *Anopheles gambiae* M form) [33]. The ability of artemisinin-resistant parasite clones to infect three highly genetically diverse vectors suggests that these resistant parasites have enhanced their transmission in the region and could effectively spread in sub-Saharan Africa, where most of the world's malaria mortality, morbidity, and transmission occurs [33, 34].

Since there are no equally effective alternative drugs to treat malaria, the spread of artemisinin resistance through India (Asia) to Africa and beyond could be a catastrophic setback to global efforts to control and eliminate the disease. Infection and mortality rates could dramatically increase in both regions, reversing the progress made towards malaria control and elimination efforts. The spread of artemisinin resistance would in turn expose the partner drugs in ACTs to greater selection pressure for the development of resistance and increased failure rates for the treatment of uncomplicated malaria. For severe malaria, the recent change in recommended treatment from quinine to artesunate [35] increased survival by 25%, and

http://dx.doi.org/10.5772/intechopen.76901

many endemic countries have adopted (or are adopting) this policy [36, 37]. Reverting back to quinine because of artemisinin resistance would also jeopardise all these gains achieved in the management of severe malaria.

The spread of ACT resistance requires constant and comprehensive monitoring across regions. Continuous monitoring of drug resistance in malaria-endemic countries along with contributing factors is a key and will enable health authorities and practitioners to prevent drug resistance from spreading. WHO issues regular reports about the status of artemisinin resistance in malaria endemic countries [38], provides updates on the status of resistance to artemisinins and ACT, and maintains a network of sentinel sites performing therapeutic efficacy studies of first and second-line antimalarial drugs [38, 39].

3. Targeting interventions in hard-to-reach population groups

Although most of malaria endemic countries in Southeast Asia have incorporated malaria elimination goals in their national strategic plans, yet this region experiences high volume of population movement (both within and between countries) causing a great hindrance in achieving their elimination targets given the increased risk of importation of infection, spread of drug resistance, and challenges in providing healthcare services to mobile populations at higher risk of malaria [40, 41].

It is the movement of populations that results in importation of new infections leading to a source of local transmission [42, 43]. Cross-border movement of populations has contributed to establishment of "hot-spots" of high transmission along international borders [44, 45], and spread of drug resistance [6], because mobile populations often experience delays in receiving diagnosis and treatment, have improper health-seeking behaviour or self-medicate [88], and are subject to lower levels of surveillance [41, 46, 47]. Population mobility in the GMS is strongly associated with shifting land use, including large rural infrastructure projects and agricultural industries that attract migrant labor and influence human-vector contact. With the recent Association of Southeast Asian Nations (ASEAN) Economic Community agreement, allowing free movement of goods, services and labor between ASEAN countries [48]; population movement is expected to rise even more in the coming years [6].

In addition, the epidemiology of malaria in many parts of Southeast Asia is shifting toward migratory labor force that gets exposed to vectors in the forest, construction sites, and has variable access to healthcare services [46, 47, 49–53]. Since forested regions are concentrated along borders and much of the cross-border movement is from the migrant labor population, malaria prevalence in these pockets was hypothesized to represent foci of hotspots. Following this rationale, the increased malaria risk in these groups was recently documented in a cross-border malaria project conducted in the Thai-Cambodian, Lao-Cambodia and Vietnam-Cambodian borders. In this study [45], it was observed that the odds of infection in security/armed forces and forest-goers was 8 and 13 times higher compared to low-risk occupations (e.g., teachers, traders, salesmen, etc.). Mechanisms and risk reduction strategies should be in place to appropriately cover these special occupational high-risk groups.

Therefore, although population mobility is a key factor to take into account when addressing drug resistance, it suffers from a range of challenges that limit countries' capacity to effectively engage and deliver interventions to migrant and mobile populations (MMPs). In addition, outdoor biting mosquitoes represent a major challenge for vector control for MMPs working during the night or sleeping outdoors, as well as forest-fringe communities.

Another challenge is the large proportion of asymptomatic infections within geographical clusters of high malaria transmission (hot-spots), where infections with low and submicroscopic parasite densities are highly prevalent in MMP and other risk groups [54]. Asymptomatic carriers can repeatedly fuel transmission to surrounding areas as the vector population expands during the wet season [55–57]. Whilst groups of homesteads consisting of asymptomatic carriers can act as stable clusters over several years [7], it is likely that the flight range of 800 m for *An. dirus* may account for increased probability of repeated mosquito feeding in the same house and clustering of cases over the dry season in Southeastern Thailand [58]. Recent clusters of malaria infection among the parasite reservoir responsible for preserving malaria over the dry season in Ratanakiri Province (northeastern Cambodia) may also explain recurrent transmission at the onset of the rainy season when the vector populations expand [59]. This reservoir is often not (completely) covered by control strategies [60] and parasite specific approaches are non-existent [61]. Programmatic interventions to interrupt transmission in "hidden" asymptomatic reservoir must focus on individuals with malaria infection at early stage, as asexual parasitemia left untreated will eventually produce gametocytes, and diagnostics for the sexual stage are limited [62].

This represents an important hindrance to malaria elimination as these infections are unlikely to be detected by passive surveillance and conventional diagnostic tools, and therefore require additional approaches to effectively reach all infections [63]. A combination of methods, or new diagnostics, may be required to detect infections in these asymptomatic parasite reservoirs. Also, a cross-sectoral response, involving non-health government agencies and the private sector addressing the links between malaria transmission, mobility and labor, will play an important role in responding to drug resistance and achieving elimination in the Southeast Asia region. Preliminary studies of the use of peer outreach workers to conduct screening of suspected cases, providing health education, and distributing nets in hot-spot areas in or near the forest, suggest that it is feasible to target high-risk populations in a culturally appropriate and evidence-based manner to reach the goal of elimination in Pursat Province, Cambodia [64]. Mobile Malaria Workers or peer outreach activities often face logistic challenges including muddy roads, river crossings, and transportation difficulties that make it hard to quickly respond to all infections. The recent President Malaria Initiative (PMI) studies show this is a potential resource that can be piloted or replicated across GMS countries (John Hustedt, personal communication).

Lastly, persisting low health-care coverage and access in remote locations remains an important challenge for mobile populations and migrant workers in some Southeast Asian countries, limiting the ability of malaria programmes to effectively capture these groups through the routine surveillance system, but most importantly to adequately provide the necessary preventive measures and care needed [65]. It is encouraging, however, to see that malaria infection rates in people who had sought treatment, or blood-smear examined in a previous malaria episode, and/or who knew how to prevent malaria (e.g., sleeping under a mosquito net), tend to be lower than those that did not seek treatment or had inadequate malaria knowledge [45]. This highlights the

http://dx.doi.org/10.5772/intechopen.76901

importance of scaling up and expanding the reach of point of access care and dissemination of information, such as through border posts or at large development or construction areas that are likely to host high-risk malaria occupational groups. These posts can potentially be used as effective channels to target and deliver specific interventions such as Behavioral Change Communication (BCC) materials, insecticide-treated uniforms or hammock nets.

Therefore, there is an urgent need to develop appropriate and sustainable malaria services for MMPs in different settings, in the context of the spread of artemisinin resistance and malaria elimination in the GMS. Different types of mobility require different malaria control interventions and therefore elimination strategies that should be based on an in-depth understanding of malaria risk in each group [66]. A population movement framework can assist in improved targeting of malaria (and other public health interventions) by going beyond a simple labeling of risk groups to develop a better understanding of risk behaviour and vulnerabilities. The implementation of the framework should be carefully evaluated to identify the changes in coverage, access, and effectiveness of the programme efforts to serve MMPs [67].

4. Residual and outdoor transmission: how much and where?

In 2012, global malaria transmission was reported as mainly attributable to 51 *Anopheles* species, with an average of about 3 major species per country [68]. Biological factors that determine whether a species becomes a major local threat are its competence for transmitting human malaria parasites, its anthropophilic *versus* zoophilic preference, and its abundance in relation to its ability to multiply, survive, and compete for resources with other *Anopheles* species. The third of these factors is regulated by the ecosystem's carrying capacity for potent vectors depending on their ecological niches [69]. Species of several *Anopheles* complexes are either major or secondary malaria vectors depending on their geographical range of distribution [70]. The peculiarity of these sibling species within a complex is that they cannot be distinguished using morphological criteria. However, several Asian malaria vectors within the Dirus, Leucosphyrus, Minimus, Maculatus, Culicifacies, Sundaicus, Subpictus complexes or groups show similar morphological characteristics, different ecological traits and vector competencies and overlapping geographical distribution with other vectors and non-vectors [70, 85, 92]. As some of these sibling species occur sympatrically and differ in their ability to transmit malaria and in their behaviour, the use of molecular tools to differentiate the vectors from the non-vectors is essential to target the correct species in vector control programs.

Malaria vector control relies largely on Long-Lasting Insecticidal Nets (LLINs) and Indoor Residual Spraying (IRS), along with Larval Source Management (LSM) as a supplementary measure appropriate in certain settings. These core interventions are highly efficacious for control of susceptible malaria vectors when implemented at universal coverage; LLINs and IRS contributed to a 48% reduction in malaria infection prevalence and 47% reduction in mortality worldwide between 2000 and 2013 [71]. However, malaria transmission can persist even when LLINs and/or IRS are effectively implemented and malaria vectors are susceptible to the insecticides used. This may be due to a combination of vector and human behaviour and bionomical characteristics, which compromise inadequate control measures against early and/or outdoor biting mosquitoes, and human activity away from protected houses or places

at peak biting times. With current efforts focusing on malaria elimination [72], there is considerable interest in vector behaviour that is not influenced by application of core interventions (i.e., conventional IRS and Insecticide Treated Nets (ITNs)), such as feeding earlier and resting outdoors when humans are not protected. For example, an unprecedented malaria outbreak, related to illegal rosewood logging, occurred in 2014 with a seven-fold increase of cases in 1 year in Ubon Ratchathani Province, Northeastern Thailand [73]. Insecticide-susceptible and exophilic *An. dirus s.l.* were collected from a forested area in Ban Pakla and Chong Ta Ou Thai border control station, including *An. maculatus s.l.* collected remote villages with potentially low insecticide pressure [73]. These susceptible vector species are less amenable to control interventions due to their behaviour and their interactions with humans contribute to persistent residual transmission and represent barrier to success [74, 75].

From a geographical perspective, residual malaria parasite transmission has been reported across numerous transmission settings, even with good access and usage of LLINs or well-implemented IRS [76–80]. From the programmatic perspective, residual malaria transmission (RMT) is defined as the persistent malaria transmission that occurs once universal coverage of LLINs and maximal coverage of IRS have been achieved. Identification and elucidation of RMT requires the following pre-conditions: (a) comprehensive and up-to-date LLIN and IRS coverage data, where coverage is defined as 100% access and usage of ITN/LLIN or IRS [81]; (b) outdoor human activity or behaviour to allow identification of outdoor sites and "gaps" in protection, not only before sleeping time, but also for people that remain outdoors during the night. In many countries of the GMS, LLIN and IRS distribution data are sparse or not readily available. Where these data are available, it is often compiled at a relatively high administrative level, such as district or province. Malaria transmission at the community level can vary considerably within a small area and thus to investigate RMT at this level, LLIN and IRS coverage data by village are necessary. Furthermore, LLIN coverage figures quoted at the province or district level often do not match the actual situation at the community level, perhaps due to inequities in distribution, inaccurate population estimates, and calculation of procurement need, limited replacement of outdated and damaged LLIN; the outcome of which could lead to an underestimation of the magnitude of RMT.

As malaria is becoming more and more restricted to hard-to-reach population groups, alternative or adapted control strategies are required who are somehow marginalised, poor, on the fringes of the public health system, living in dwellings that are either very close to the forest or harbour people who are exposed to the forest through their occupation (e.g., development sites and seasonal labour areas) or mobility behaviour. As shown in **Table 1**, the risk of RMT in the malaria foci is spread over the entire night, from dusk-to-dawn, requiring a combination of complementary vector control measures, such as long-lasting insecticide hammock nets (LLIHN) that can be used during different periods of the night. However, the use of LLIHN, single LLIN/ITN or topical repellents in the field may not be acceptable due to cultural and linguistic barriers of ethnic minorities and MMPs for which specific acceptability studies should be conducted to guide the feasibility of these vector control tools.

Another driver of RMT is mega and micro-development projects impacting the forest or creating new conditions suitable for vector species, and often attracting a substantial workforce from various horizons across borders and cultural boundaries [6]. Their sleeping or residential places can have additional vulnerabilities if they are remote, comprising mainly ethnic

minorities, or in conflict areas, all of which can hinder access to the public health system. Another key concern is to restrict or mitigate the widespread dispersal of parasites by these elusive population groups.

Just as forest workers often stay in the forests for several days and sleep exposed to vectors [84], rubber tappers also work in plantations at night with higher likelihoods of being bitten by *Anopheles* mosquitoes, in particular vector species of the Dirus Complex [3, 85]; they all have poor access to healthcare services [86]. Plantation work is seasonal, and manpower is often composed of highly mobile seasonal migrants, but little is known about their patterns of movement. More malaria infections were observed in people with temporary labour positions and plantation workers at the Thailand-Myanmar border [87], but this was not confirmed due to a very high proportion of the study participants opting to perform forest or field activities, and a very low number opting to work in rubber plantation [59]. Many migrants that arrive for rubber tapping settle beyond the harvest season [87] and go on to work on other cash crops (e.g., rice,

District, province, country	Ecotype	% Access to LLIN	% Use of LLIN	Proportion of *Anopheles* bites or infective bites in relation to sleeping time	Reference
Eastern region: Borkeo & O'Chum districts, Rattanakiri Province; Western region: Pailin & Pursat Provinces; Cambodia	Forest plots & villages	68.4% (Ratanakiri)	70.7% (forest workers)	After 22:00 h 71%	[53, 102]
	Forest plots	69.2% (Pailin); 81.8% (Pursat)	66.3% (forest workers)	Before 22:00 h 29%	
Ma Noi and Phuoc Binh communes, Ninh Thuan Province, south-central Vietnam	Village	NA	85%	Before 22:00 h 45% (bites only)	[83]
	Way to the forest	NA	NA	Before 19:00 h 13% (bites of *An maculatus)*	
	Forest plots	NA	53%	Before 21:00 h 64% (bites only)	
Tha Song Yang, Tak Province, Thailand	Village[a]	78%	80%	Before 21:00 h and 05:00 h	[82]
	Hamlets[b]	100%	75–95%	20.0% Suan Oi	
	Farm huts	NA	NA	33.7% Pha Man	
				37.6% farm huts	
Son Thai commune, Khan Hoa Province, central Vietnam	Village	78%	95%	Before 20:00 and 05:00 h	
	Farm huts	NA	62.7%[c]	26% farm huts	
	Forest	NA	25%[c]	37% forest	

NA: not available.

[a]Suan Oi village.

[b]Pha Man & Komonae hamlets, Thailand [82].

[c]Regular use of LLIN.

Table 1. Overview of residual malaria parasite transmission (RMT) in various ecological settings in Greater Mekong Subregion.

cassava, fruit orchards). On return to their usual settlements, they contribute to the spread of malaria within and across international borders [41, 43]. By creating hot-spots of malaria and disproportionately affecting people with certain high-risk occupations [86, 89], residual transmission under these circumstances has so far hindered progress towards elimination.

5. Correct identification of malaria vectors and *Plasmodium* detection

High levels of malaria transmission occurring in forest-fringe areas of Southeast Asia is explained by movements of people in search of forest products and exposure to many highly efficient vector species that have adapted to forest ecotypes [66, 85, 102, 103]. The wide diversity of both the deep-forest (e.g., Leucosphyrus Group of mosquitoes), forest-fringe and deforested area main vectors (e.g., *An. minimus*, *An. maculatus s.l.*, *An. culicifacies s.l.*, *An. fluviatilis s.l.*, *An. letifer*, *An. donaldi*), as well as their great potential to adapt to habitat changes, means that the consequences of deforestation on malaria transmission in Southeast Asia are difficult to predict and unlikely to be unidirectional [104]. Whilst *An. dirus* and *An. baimaii*, main vectors of the Dirus complex, can find tree-crop plantations suitable for breeding, a close association between malaria and rubber plantations has been demonstrated [4, 105–108], contributing to high larval and pupal density during the rainy season [90, 91] and low numbers during the cool-dry season [92, 109], or provide conditions that are similar to this vector's natural habitat [110]. This ecological adaptation in human settlements and shaded plantations contributes to outdoor transmission among rubber tappers.

The identification of secondary or incidental vector species poses new challenges as shown by mixed results of sporozoite-positivity using nested Polymerase chain reaction (PCR) and routine circumsporozoite enzyme-linked immunosorbent assay (CSP-ELISA) (**Table 2**). Confirmation of all positive CSP-ELISA results by a second CSP-ELISA test on the heated ELISA lysate, especially in zoophilic species showed a relatively high proportion of false positives (40%) [93]. On the other hand, PCR analysis of Deoxyribonucleic acid (DNA) extracted from the head and thorax alone, along with sequence data, revealed five *Anopheles* species (*An. hyrcanus*, *An. barbirostris s.s.*, *An. barbirostris* clade III, *An. nivipes*, and *An. peditaeniatus*) infected with *Plasmodium falciparum,* which are not considered major vectors in the GMS [94]. Similarly, out of 11 *P. falciparum* CSP positive samples from Bangladesh, seven turned out to be positive by PCR suggesting that *An. maculatus*, *An. jeyporiensis* and *An. nivipes* play important roles in malaria transmission in Kuhalong District [95]. In Vietnam, the role of a secondary vector, *An. pampanai* infected with *P. vivax*, was also reported in the Binh Phuoc Province [96]. Morphological misidentification of the closely related sympatric species, such as *An. aconitus*, *An. pampanai* and *An. varuna* are common [99, 100]. Morphological identification of *Anopheles* specimens prior to PCR assays allows them to be sort out at the group or complex level but does not permit species identification [85]. PCR assays must be applied for a reliable identification to the species level, which ensures that data received by malaria vector control programmes are suitable for targeting the correct vector species [101]. Given the low infection rates among many of these species especially in elimination phase, it is important for field entomologists to assess various

Morphological *Anopheles* species*	Nested PCR, Cambodia [93]		Circumsporozoite ELISA, Thailand [97]		Prior heating of eluate and circumsporozoite ELISA, Bangladesh [98]		PCR confirmation of ELISA-positives Bangladesh [95]	
	Total collection (%)	Positive/ total	Total collection (%)	Positive /total	Total collection (%)	Positive /total	Total collection (%)	Positive / total
An. maculatus s.l			21.43	4/640			4.3	2/97
An. annularis s.l.			14.43	3/431	0.78	1/19		
An. kochi					0.93	1/44		
An. barbirostris s.s.	6.6	3/55	3.52	1/105	2.9	1/140	7.4	1/186
An. peditaeniatus					5.08	3/139		
An. hyrcanus	0.09	2/2						
An. nigerrimus	0.87	1/21					4.1	1/104
An. philippinensis		3/219			24.7	25/1169		
An. vagus					41.9	25/1978		
An. nivipes							10.8	1/264
An. jeyporiensis					3.1	1/142	18.9	2/479
An. karwari					5.16	11/244	1.7	

*Molecular identification was specifically conducted on *Anopheles barbirostris s.s.* and *An. barbirostris* clade III; *An. hyrcanus* and *An. hyrcanus s.s*; *An. peditaeniatus* and *An. nivipes*, and morphological identification for the other *Anopheles* species.

Table 2. Sporozoite infectivity rates of less known (secondary) vectors along the Bangladesh-Thailand-Cambodia corridor.

species' role in malaria transmission in the eco-epidemiological context. When changing objectives from control to elimination of malaria in Southeast Asia, the need to focus not only in the so-called main vector species, but also on secondary vectors is increasingly important.

Deforestation may deplete the populations of deep-forest vectors and so initially reduce malaria transmission; in some localities this depletion may be followed by the invasion of other efficient vector species resulting in increased transmission. With the exception of two longitudinal studies examining the effects of progressive land use changes from pre-development forest to oil palm cultivation on the distribution of disease vectors and malaria incidence [111], there is a striking lack of primary research directly measuring the impact of deforestation on malaria in Southeast Asia [104]. Recent studies showed that *An. dirus s.l.* was abundant in rubber plantations in Myanmar [109] and *An. baimaii* (molecularly identified) adults were caught from human landing collections in Wae Kha Mi, Mon State, the site of an acceptability study of permethrin-treated clothing [110]. In Lao PDR, a total of 46 *An. dirus s.l.* were collected, of which 31 were

from immature rubber plantations, nine from mature rubber plantations, five from secondary forests and one from the rural village [105] (Tangena Julie-Ann, personal communication).

6. *Plasmodium knowlesi*: an additional challenge to malaria elimination

Plasmodium knowlesi, a simian malaria parasite, is now considered the 5th parasite affecting humans [112]. All countries in Southeast Asia have reported cases of *P. knowlesi* with the exception of Lao PDR and Timor Leste [113]. Since most countries are now working towards malaria elimination, it is pertinent to pay serious attention to malaria cases especially in areas where malaria has been reduced to very low levels. A good example is Sabah, Malaysian Borneo where large numbers of *P. knowlesi* were diagnosed in areas where *P. falciparum* and *P. vivax* were occurring in very low numbers [114]. Malaysia is working towards malaria elimination by 2020 and currently more than 60% of the malaria cases are due to *P. knowlesi* (MOH personal communication).

Recently, an increasing number of cases of *P. knowlesi* were reported from Kalimantan and Ache in Indonesia [115, 116] where malaria was in process of being eliminated. In Northern Sumatra, Indonesia where they are working towards malaria elimination, they recorded only 614 (16.5%) positive malaria cases by microscopy out of 3731 people examined [117]. However, PCR detected malaria parasites in 1169 (31.3%) individuals. Of these, 74.9% were mono-infection and 25.1% were multiple infection. *P. falciparum* constituted 24.8%, *P. vivax* 33.9%, *P. malariae* 9.3%, and *P. knowlesi* 32% [114] of the cases. It was also found that the primers developed from the SICAvar gene were more sensitive than the SSU rRNA gene [117]. It is obvious that parasite species are being mis-identified and many people who are asymptomatic are also missed by conventional microscopy [117, 118]. Thus, it is important to develop Rapid Diagnostic Tests (RDTs) that can be used by field workers to detect accurately malaria parasite species, especially *P. knowlesi*, and also additional laboratories should be established to conduct molecular assays for malaria diagnosis in the context of malaria elimination.

Deforestation and changes in the environment are the key factors leading to a surge of *P. knowlesi* malaria [119]. This parasite occurs in *Macaca fascicularis* (long-tailed) and *Macaca nemestrina* (pig-tailed) monkeys and its distribution is limited by some species of the Leucosphyrus Group of *Anopheles* mosquitoes [120]. These species are found biting in greater abundance in forest and farms compared to villages [121, 122]. However, in Sabah, Malaysian Borneo, it was found that *An. balabacensis* was abundant in villages as well [123], and sporozoite-positive specimens were reported in addition to farms and forest [123], while infective mosquitoes were found only in the forested sites and farms in Sarawak (Borneo) and Pahang (Peninsular), Malaysia [121, 122]. In addition, vector studies have also been conducted in Vietnam [124, 125] where the species *An. dirus* has been incriminated as the simian malaria vector in Khanh Phu—South Central Vietnam. Studies were conducted in the forest and forest-fringe areas near Nga Hai village where both human malaria parasites, *P. falciparum* and *P. vivax*, were found along with *P. knowlesi* in order to determine the potential role of *An. dirus* as bridge vectors of *Plasmodium* parasites from monkeys to humans [126]. Based on these studies, it was possible for *An. dirus* to pick up infection from humans and macaques during the mosquito's lifespan. However, since there have been no reports of epidemics of *P. knowlesi*, it is believed that humans are infected by mosquitoes

which acquired infection from the macaques. Perhaps even likely given that confirmed vectors of human plasmodia in Southeast Asia also become naturally infected by the monkey malaria species [127]. A recent case control study conducted in Sabah revealed that the age group >15, predominantly males, working in farms, plantations, forested areas, and with travel history, were independently associated with the risk of acquiring knowlesi malaria [128]. It also highlighted that IRS was associated with decrease of risk [128].

There are only few investigations on record in understanding bionomics of vectors transmitting *P. knowlesi* malaria. In order to implement vector control activities, the bionomics of the vectors must be understood. Based on few studies, it has been shown that the vectors are biting in the early part of the night from 18:00 h to 21:00 h and mostly outdoors [121–123, 129]. In these rural areas, people go to bed by 22:00 h and they are up by 05:00 h. The results showed that only 39.79% of *An. balabacensis* [123], 43.8% of *An. latens* [121] and 12.8% of *An. cracens* [122] were found biting during this sleeping time. Thus, current vector control measures like IRS and ITNs are not appropriate for the exophagic and exophilic vectors. The forests in Southeast Asia is providing a favorable environment with high percentage of macaques being positive for *P. knowlesi* [130–132], and with the presence of the vectors, it is going to be a daunting task to eliminate malaria. On a global scale, malaria has been reduced to low levels due to the scaling up of ITNs, IRS, ACTs, and intermittent preventive treatment to infants and pregnant women [133]. Thus, it is obvious that new tools are urgently required for successful malaria elimination.

It is known that the two human malaria species (*P. falciparum* and *P. vivax*), which infects millions of people actually were of zoonotic origin (from the African apes), which evolved thousands of years ago [134, 135]. Thus, there is always a possibility that in the future *P. knowlesi* and other simian malarias may become established in humans, especially when human malaria is eliminated. However, currently human-to-human transmission of knowlesi malaria by mosquitoes has not been established. This is crucial in the light of malaria elimination and more focused research is needed on this topic if we are to succeed with malaria elimination.

Changing landscape affects *Anopheles* distribution, mosquito density and diversity in Malaysia, and more globally Southeast Asia [105, 111, 136–138]. It has been shown that with loss of forest cover, cases of *P. knowlesi* have increased in Sabah [119]. Land use change has also led to increase of malaria cases due to various factors such as increase of macaques in small forest patches along with the colonization of the main vectors [119, 136]. It is interesting to note that *An. balabacensis*, the predominant vector of human and simian malaria, was found in great abundance in logged forest, followed by thinly logged virgin jungle reserve and was lowest in primary forest [136]. This vector was also found to be biting humans more at ground level compared to canopy level [136]. It is therefore important to include both the public health and agro-forestry sectors in controlling malaria vectors in the country. Studies from Thailand also indicate that if landscape management should be used for malaria control in northern Thailand, large-scale reduction and fragmentation of forest cover would be needed [139, 140]. Such drastic actions, however, do not align with current global objectives concerning forest and biodiversity conservation.

The vectors of simian malaria described to date were *An. hackeri* (Leucosphyrus Group) [141] recorded biting mainly the macaques and large numbers were collected resting on Nipah palm trees in Selangor in 1960s; *An. cracens* (Dirus Complex) [122] biting both macaques and humans and found mainly in the forest and farms; *An. latens* (Leucosphyrus Complex) [121] was the

predominant mosquito in the forest compared to farm and village, and was biting macaques at ground level and at six meters in the canopy compared to three meters. The biting ratio of monkey *versus* human for *An. latens* was 1:1.3 [121]. *An. introlatus* (Leucosphyrus Complex) [142] was biting in the early part of the night from 19:00 h to 21:00 h and was the predominant mosquito in Hulu Selangor where cases of *P. knowlesi* were reported. Most recently, *An. balabacensis* (Leucosphyrus Complex) has been incriminated as vector of *P. knowlesi* in Sabah [123], as well as human malaria and Bancroftian lymphatic filariasis due to *Wuchereria bancrofti* [143–145].

Although an increased number of countries are successfully eliminating human malaria in recent years, no country has yet eliminated non-human malaria, which adds another layer of complexity to be addressed. The complex situation of malaria is Southeast Asia is very unique from the rest of the tropical countries. More effort is needed to study the host switching mechanisms between the parasites in humans, macaques and vectors. A series of review papers have been published over the years and all these have indicated the importance of addressing the problem caused by *P. knowlesi,* if malaria elimination is to be successful in the region [113, 146–151].

7. Targeting vivax malaria: a bottleneck to malaria elimination

As opposed to *P. falciparum* infection, which does not have latency (dormant), *P. vivax* causes two distinct infection syndromes, one that actively proliferates and the other latent due to hypnozoites. Each of these *P. vivax* forms requires distinct therapeutic treatments and the latent form cannot be diagnosed [152]. Most acute attacks of *P. vivax* in endemic areas originate from hypnozoites, and unless that reservoir is aggressively attacked, elimination of transmission may be an unrealistic goal.

Treatment of latent vivax represents an important challenge as the only known therapies are 8-aminoquinoline drugs, which results in acute hemolytic anemia in patients deficient in glucose-6-phosphate dehydrogenase (G6PD)—a highly polymorphic inherited disorder affecting 1–30% of residents of malaria-endemic nations [153]. The single low dose of primaquine against gametocytes of *P. falciparum* does not threaten the G6PD deficient subjects [154]. Another challenge is that the parasitemia of vivax malaria patients is typically an order of magnitude lower than falciparum malaria, causing larger proportions of parasitemia to fall below diagnostics detection thresholds [152]. In addition, vivax malaria patients may exhibit very low parasitemia, and yet become severely ill. These fundamental distinctions between the two dominant human malarias explain why *P. vivax* is relatively unaffected by interventions tailored to control *P. falciparum* calling for new strategies needed for combatting vivax malaria [155].

In addition, *P. vivax* has the ability to develop at lower temperature than *P. falciparum* and has a shorter sporogonic cycle in the vector, which results in *P. vivax* extending beyond tropical climates into temperate regions. This ability, combined with its early-biting, outdoor-feeding and outdoor-resting behavior of vector mosquito species, also makes them less susceptible to vector control measures such as IRS, which have proven effective against transmission of *P. falciparum* [156]. Also having dormant forms in the liver (hypnozoites) mean that one successful infection will generate a number of parasitological and clinical episodes without reinfection. Therefore, recurrent cases cannot be prevented via vector control, though, paradoxically, successful transmission control of

vivax malaria could reduce the disease burden more than that of *P. falciparum*, because avoiding one infection will result in preventing a number of clinical episodes over several years [155].

Vivax malaria is diagnosed late, because infected people get ill with low parasite densities, which cannot be detected with current diagnostics, such as RDTs and microscopy. Delayed diagnosis means not only delayed treatment (hence prolonged morbidity, especially anemia) but also ability to transmit over an extended period. This is further amplified by the fact that mature gametocytes appear simultaneously with asexual forms—hence transmission occurs before diagnosis and treatment [157, 158].

As recently described [156], an effective *P. vivax* control and elimination toolbox should include:

- **i.** Practical point-of-care G6PD deficiency diagnostics allowing wider access to safe primaquine therapy or with tafenoquine—a related single dose hypnozoitocide recently developed by GSK and Medicines for Malaria Venture (MMV); the latter has been submitted to the United States Food and Drug Administration (FDA) seeking approval of single-dose tafenoquine for the radical cure (prevention of relapse) of vivax malaria in patients 16 years of age and older [159];
- **ii.** More sensitive point-of-care diagnostics for detecting intrinsically lower parasitemia, including sub-patent and asymptomatic infections;
- **iii.** Validated strategies for relapse prevention in special population groups, i.e., pregnant women, young infants, G6PD deficient and G6PD unknowns in which 8-aminoquinoline is contraindicated;
- **iv.** Clinical care algorithms acknowledging risk of severe and threatening syndromes despite seemingly non-threatening levels of parasitemia; and
- **v.** Interventions of proven efficacy to minimize human contact with often zoophilic, exophagic and exophilic *Anopheles* species of great diversity.

In conclusion, the malaria community needs to address these challenges and create a viable strategy to achieve vivax elimination goals, providing novel solutions for overcoming critical bottlenecks. This process needs to begin now to enhance treatment practice for 8-aminoquinoline drugs based radical cure. Highlighting the benefits of radical cure for the patient and community will improve prescription practice and patient adherence [160]. Coupling this with improved access to adequate G6PD testing will pave the way for the introduction of tafenoquine, with huge potential to accelerate the elimination of *P. vivax*.

8. Socio-ecological and adaptive management of malaria ecosystem in areas approaching malaria elimination

WHO has recently proposed sustainable prevention and control of diseases emerging within complex, dynamic, adaptive systems, such as malaria, based on interdisciplinary and approaches addressing environmental and social health determinants holistically [161]. More

insights into transmission dynamics and the possibility of intersectoral ecosystem management programs for malaria elimination and control are urgently needed. An ecosystem approach to successful reduction of vector-borne disease burden [162, 163] can lead to considerable health gains [Available at: http://www.maweb.org/documents/document.317.aspx.pdf].

Once local entomological inoculation rates (EIRs) have been reduced to a level of unstable transmission the infectious reservoir can be eliminated via several approaches without a threat of malaria re-emergence from reintroduction of parasites. At this point, use of time-limited mass drug administrations (MDA) campaign at high coverage should be sufficient to effectively clear the majority of remaining *P. falciparum* cases, and may be considered for epidemic control as part of the initial response, along with the urgent introduction of other interventions [164]. This can be supplemented by screening and treatment programmes based on WHO Global Malaria Programme's T3: Test, Treat, Track initiative supporting malaria-endemic countries in their efforts to achieve universal coverage with diagnostic testing and antimalarial treatment, as well as in strengthening their malaria surveillance system [WHO T3: Test, Treat, Track. Scaling up diagnostic testing, treatment and surveillance for malaria. World Health Organisation; 2012. http://www.who.int/malaria/publications/atoz/t3_brochure/en/]. Healthcare workers or locally trained and supervised community volunteer networks can apply this method to effectively limit reintroduction of parasites from other areas to a minimum, and apply additional active case management, e.g., the systematic detection and treatment of parasitemia using highly sensitive RDTs can reduce the risk attributed to any unscreened or asymptomatic cases.

Depending on the local situations, supplementary measures, in addition to LLINs or IRS, such as repellents or treated clothing for high-risk individuals, offer special precautionary preventive protection [1, 110, 165, 166]. Passive case management should suffice for treating any symptomatic infections as they may occur. This, however, assumes at least a periodic provision of health services at all locations, including remote ones. A transdisciplinary approach integrates different scientific perspectives [167, 168] and provides a formal platform for stakeholder participation in the research and development of new information, ideas and strategies, their testing and eventual application.

Participatory approaches that engage local communities in a complex social-ecological mapping process are a vital starting point for identifying community-applicable solutions and leveraging community capacity for local interventions [169, 170] and promoting integrative and equitable collaboration within partnership of researchers and communities [170, 171]. Ownership of continuous surveillance, monitoring, treatment and preventive efforts should be transferred to members of local communities, assuming collective responsibility for their continuous well-being.

9. Conclusions

This review attempts to consolidate the challenges of operational research for innovations in designing interventions [172], according to the current situation and progress made, for achieving malaria elimination in Southeast Asia. As the entry of artemisinin resistant parasites to India could be the first step in their spread to Africa, the current priority must be to

http://dx.doi.org/10.5772/intechopen.76901

address this problem in Southeast Asia before it can become a threat in Africa. Continuous monitoring of drug resistance in conjunction with analysis and proper interpretation is critical to guide the appropriate action for effective treatment. While *P. falciparum* elimination in the GMS is realistic, feasible and particularly urgent in the context of drug resistance, the main challenges are to ensure community participation and plan for the preservation of ACT potency so that the dosing regimens and surveillance for resistance are rigorously pursued to sustain their efficacy for as long as possible [172].

We support a priority focus on MMP and other high-risk groups to contain the spread of artemisinin resistance and new hot-spots, however, implementation challenges should be considered when planning future interventions. More efforts are needed in documenting the malaria risk among different types of MMPs, innovative tools and interventions, as well as designing implementation in a way that can be evaluated, lessons learned, and programmes adapted in an on-going process [172]. New ways of evaluating MMP interventions (including highly sensitive RDTs) are needed, as routine health information systems have limitations and might not allow capturing the information and data needed, and existing type of surveys might not be sufficient for monitoring interventions for MMP.

Malaria programmes need to heed the recent revised WHO recommendations for achieving universal coverage with LLINs or IRS for populations at risk [173]. The coverage of key interventions is critically low in some countries and sub-optimal in most others, threatening progress across the region as a whole [174]. Malaria programmes are encouraged to evaluate the magnitude (and drivers) of the residual transmission in their country, regarding both mosquito and human behavior. This information will provide a boost for industry and academic partners to develop new vector control methods and paradigms for outdoor and residual transmission.

The current precarious funding situation could undermine elimination efforts and result in a resurgence of disease. The threat posed to regional and global malaria control and elimination efforts by artemisinin resistant *P. falciparum* parasites is imminent and potentially severe. In many Asian countries, operational feasibility of *P. vivax* elimination is lower than that for *P. falciparum* [27]. Therefore, creating a viable strategy to achieve vivax elimination goals should include improvements in access to safe treatment to 8-aminoquinoline drugs based radical cure together with improved access to adequate G6PD testing in *P. vivax* endemic countries.

Whilst human *P. knowlesi* is still largely a zoonosis, all indications suggest that human-to human transmission can take place, and probably is taking place in some situations [175]. More research is required to substantiate the body of evidence for human-to-human transmission, laboratory diagnosis and clinical management, and mapping vectors of *P. knowlesi* and environmental risk factors.

The challenge for elimination programmes is dealing with dynamic, social-ecological systems for which an entirely different kind of thinking and scientific framework is required. The retooling for this next phase is more challenging this time since it requires malaria experts and managers to understand complex systems, thinking and practices. This thinking and actions are more or less contrary to conventional understandings of disease control, which tend to be top down and not guided by concepts like resilience and adaptive management developed as part of so-called ecosystem approach/management.

Acknowledgements

The authors would like to specifically thank Sergio Lopes and Allan Schapira for their comments and inputs to the manuscript. We thank Irwin Chavez for supporting EndNote citations and references. The authors thank WHO for approval to reproduce **Figures 1** and **2** taken from [2]. Data for Thailand and Vietnam in **Table 1** was supported by the Special Programme for Research and Training in Tropical Diseases (TDR), in partnership with the Global Malaria Programme of the World Health Organisation (WHO) to develop multi-country, multi-institutional research on Investigation of the magnitude and causes of residual malaria parasite transmission in selected settings.

Conflict of interest

All authors declare no conflict of interest related to the writing of this chapter.

List of acronyms

ACT	Artemisinin-based combination therapy
AMT	Artemisinin monotherapy
ASEAN	Association of Southeast Asian nations
BCC	Behavioral change communication
DNA	Deoxyribonucleic acid
CSP	Circumsporozoite
EIR	Entomological inoculation rate
ELISA	Enzyme-linked immunosorbent assay
ERAR	Emergency response to artemisinin resistance
FDA	Food and drug administration
G6PD	Glucose-6-phosphate dehydrogenase
GMS	Greater Mekong Subregion
IRS	Indoor residual spraying
ITN	Insecticide treated nets
LLIN	Long lasting insecticidal nets
LLIHN	Long lasting insecticidal hammock nets
LSM	Larval source management

MDA	Mass drug administration
MMP	Mobile and migrant populations
MMV	Medicines for malaria venture
MOH	Ministry of Health
PCR	Polymerase chain reaction
PMI	President Malaria Initiative
RDT	Rapid diagnostic test
RMT	Residual malaria transmission
SES	Socio-ecological system
SP	Sulfadoxine-pyrimethamine
WHO	World Health Organisation

Author details

Jeffrey Hii[1], Indra Vythilingam[2] and Arantxa Roca-Feltrer[3]*

*Address all correspondence to: a.roca@malariaconsortium.org

1 Malaria Consortium Asia, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

2 University of Malaya, Kuala Lumpur, Malaysia

3 Malaria Consortium, London, United Kingdom

References

[1] WHO. Strategy for Malaria Elimination in the Greater Mekong Subregion: 2015-2030. Manila: WHO Regional Office for the Western Pacific; 2015. 80 p

[2] WHO. Countries of the Greater Mekong making inroads in the race towards elimination. 2017. Available from: http://apps.who.int/iris/bitstream/10665/259602/1/WHO-HTM-GMP-2017.23-eng.pdf [Accessed: 2017-10-05]

[3] Suwonkerd W, Ritthison W, Ngo CT, Tainchum K, Bangs MJ, Chareonviriyaphap T. Vector biology and malaria transmission in Southeast Asia. In: Manguin S, editor. *Anopheles* Mosquitoes-New Insights into Malaria Vectors. Rijeka, Croatia: InTech Open Access; 2013. DOI: 10.5772/56347

[4] Bhumiratana A, Sorosjinda-Nunthawarasilp P, Kaewwaen W, Maneekan P, Pimnon S. Malaria-associated rubber plantations in Thailand. Travel Medicine and Infectious Disease. 2013;**11**:37-50. DOI: 10.1016/j.tmaid.2012.11.002

[5] WHO. Informal Consultation on Malaria and Mobile and Migrant Populations: Addressing Priority Gaps in the Context of Malaria Elimination in the GMS. Bangkok, Thailand: World Health Organization; 2016. 54 p

[6] Jitthai N. Migration and malaria. The Southeast Asian Journal of Tropical Medicine and Public Health. 2013;**44**:166-200 discussion 306-167

[7] O'Shannassy T. Towards malaria elimination in the greater Mekong subregion. The Southeast Asian Journal of Tropical Medicine and Public Health. 2013;**44**(Supp):45

[8] White NJ. Antimalarial drug resistance. The Journal of Clinical Investigation. 2004;**113**: 1084. DOI: 10.1172/JCI200421682

[9] Nosten F, White NJ. Artemisinin-based combination treatment of falciparum malaria. American Journal of Tropical Medicine and Hygiene. 2007;**77**(6_Suppl):181-192. DOI: 0.4269/ajtmh.2007.77.181

[10] Status report on artemisinin and ACT resistance. 2017. Available from: http://www.who.int/malaria/publications/atoz/artemisinin-resistance-april2017/en/ [Accessed: 2017-11-7]

[11] Imwong M, Hien TT, Thuy-Nhien NT, Dondorp AM, White NJ. Spread of a single multidrug resistant malaria parasite lineage (PfPailin) to Vietnam. The Lancet Infectious Diseases. 2017;**17**:1022-1023. DOI: 10.1016/S1473-3099(17)30524-8

[12] Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM. Evidence of artemisinin-resistant malaria in western Cambodia. New England Journal of Medicine. 2008;**359**:2619-2620. DOI: 10.1056/NEJMc0805011

[13] Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, Lwin KM, Ariey F, Hanpithakpong W, Lee SJ. Artemisinin resistance in *Plasmodium falciparum* malaria. New England Journal of Medicine. 2009;**361**:455-467. DOI: 10.1056/NEJMoa0808859

[14] Amaratunga C, Sreng S, Suon S, Phelps ES, Stepniewska K, Lim P, Zhou C, Mao S, Anderson JM, Lindegardh N. Artemisinin-resistant *Plasmodium falciparum* in Pursat province, western Cambodia: A parasite clearance rate study. The Lancet Infectious Diseases. 2012;**12**:851-858. DOI: 10.1016/S1473-3099(12)70181-0

[15] Hien TT, Thuy-Nhien NT, Phu NH, Boni MF, Thanh NV, Nha-Ca NT, Thai CQ, Van Toi P, Thuan PD, Merson L. In vivo susceptibility of *Plasmodium falciparum* to artesunate in Binh Phuoc Province, Vietnam. Malaria Journal. 2012;**11**:355. DOI: 10.1186/1475-2875-11-355

[16] Phyo AP, Nkhoma S, Stepniewska K, Ashley EA, Nair S, McGready R, ler Moo C, Al-Saai S, Dondorp AM, Lwin KM. Emergence of artemisinin-resistant malaria on the western border of Thailand: A longitudinal study. Lancet. 2012;**379**:1960-1966. DOI: 10.1016/S0140-6736(12)60484-X

[17] Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, Sreng S, Anderson JM, Mao S, Sam B. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. New England Journal of Medicine. 2014;**371**:411-423. DOI: 10.1056/NEJMoa1314981

[18] Huang F, Takala-Harrison S, Jacob CG, Liu H, Sun X, Yang H, Nyunt MM, Adams M, Zhou S, Xia Z. A single mutation in K13 predominates in southern China and is

associated with delayed clearance of *Plasmodium falciparum* following artemisinin treatment. The Journal of Infectious Diseases. 2015;**212**:1629-1635. DOI: 10.1093/infdis/jiv249

[19] Menard D, Dondorp A. Antimalarial drug resistance: A threat to malaria elimination. Cold Spring Harbor Perspectives in Medicine. 2017;**7**:a025619. DOI: 10.1101/cshperspect.a025619

[20] Leang R, Barrette A, Bouth DM, Menard D, Abdur R, Duong S, Ringwald P. Efficacy of dihydroartemisinin-piperaquine for treatment of uncomplicated *Plasmodium falciparum* and *Plasmodium vivax* in Cambodia, 2008 to 2010. Antimicrobial Agents and Chemotherapy. 2013;**57**:818-826. DOI: 10.1128/AAC.00686-12

[21] Leang R, Taylor WR, Bouth DM, Song L, Tarning J, Char MC, Kim S, Witkowski B, Duru V, Domergue A. Evidence of *Plasmodium falciparum* malaria multidrug resistance to artemisinin and piperaquine in western Cambodia: Dihydroartemisinin-piperaquine open-label multicenter clinical assessment. Antimicrobial Agents and Chemotherapy. 2015;**59**:4719-4726. DOI: 10.1128/AAC.00835-15

[22] Lon C, Manning JE, Vanachayangkul P, So M, Sea D, Se Y, Gosi P, Lanteri C, Chaorattanakawee S, Sriwichai S. Efficacy of two versus three-day regimens of dihydroartemisinin-piperaquine for uncomplicated malaria in military personnel in northern Cambodia: An open-label randomized trial. PLoS One. 2014;**9**:e93138. DOI: 10.1371/journal.pone.0093138

[23] Saunders DL, Vanachayangkul P, Lon C. Dihydroartemisinin–piperaquine failure in Cambodia. New England Journal of Medicine. 2014;**371**:484-485. DOI: 10.1056/NEJMc1403007

[24] Duru V, Khim N, Leang R, Kim S, Domergue A, Kloeung N, Ke S, Chy S, Eam R, Khean C. *Plasmodium falciparum* dihydroartemisinin-piperaquine failures in Cambodia are associated with mutant K13 parasites presenting high survival rates in novel piperaquine in vitro assays: Retrospective and prospective investigations. BMC Medicine. 2015;**13**:305. DOI: 10.1186/s12916-015-0539-5

[25] Spring MD, Lin JT, Manning JE, Vanachayangkul P, Somethy S, Bun R, Se Y, Chann S, Ittiverakul M, Sia-ngam P. Dihydroartemisinin-piperaquine failure associated with a triple mutant including kelch13 C580Y in Cambodia: An observational cohort study. The Lancet Infectious Diseases. 2015;**15**:683-691. DOI: 10.1016/S1473-3099(15)70049-6

[26] Carrara VI, Lwin KM, Phyo AP, Ashley E, Wiladphaingern J, Sriprawat K, Rijken M, Boel M, McGready R, Proux S. Malaria burden and artemisinin resistance in the mobile and migrant population on the Thai–Myanmar border, 1999-2011: An observational study. PLoS Medicine. 2013;**10**:e1001398. DOI: 10.1371/journal.pmed.1001398

[27] Thein ST, Sudhinaraset M, Khin HSS, McFarland W, Aung T. Who continues to stock oral artemisinin monotherapy? Results of a provider survey in Myanmar. Malaria Journal. 2016;**15**:334. DOI: 10.1186/s12936-016-1392-5

[28] ACTwatch G, Novotny J, Singh A, Dysoley L, Sovannaroth S, Rekol H. Evidence of successful malaria case management policy implementation in Cambodia: Results

from national ACTwatch outlet surveys. Malaria Journal. 2016;**15**:194. DOI: 10.1186/s12936-016-1200-2

[29] Ménard D, Khim N, Beghain J, Adegnika AA, Shafiul-Alam M, Amodu O, Rahim-Awab G, Barnadas C, Berry A, Boum Y. A worldwide map of *Plasmodium falciparum* K13-propeller polymorphisms. New England Journal of Medicine. 2016;**374**:2453-2464. DOI: 10.1056/NEJMoa1513137

[30] Newton PN, Dondorp A, Green M, Mayxay M, White NJ. Counterfeit artesunate antimalarials in Southeast Asia. Lancet. 2003;**362**:169. DOI: 10.1016/S0140-6736(03)13872-X

[31] Lamy M, Liverani M. Tackling substandard and falsified medicines in the Mekong: National responses and regional prospects. Asia Pacific Policy Studies. 2015;**2**:245-254. DOI: 10.1002/app5.87

[32] Krishna S, Pulcini S, Fatih F, Staines H. Artemisinins and the biological basis for the PfATP6/SERCA hypothesis. Trends in Parasitology. 2010;**26**:517-523. DOI: 10.1016/j.pt.2010.06.014

[33] Laurent BS, Miller B, Burton TA, Amaratunga C, Men S, Sovannaroth S, Fay MP, Miotto O, Gwadz RW, Anderson JM. Artemisinin-resistant *Plasmodium falciparum* clinical isolates can infect diverse mosquito vectors of Southeast Asia and Africa. Nature Communications. 2015;**6**:8614. DOI: 10.1038/ncomms9614

[34] Fairhurst R, Dondorp A. Artemisinin-resistant *Plasmodium falciparum* malaria. Microbiology Spectrum. 2016;**4**(3). DOI: 10.1128/microbiolspec.EI10-0013-2016

[35] WHO: Guidelines for the treatment of malaria—2nd edition. In: WHO Guidelines for the Treatment of Malaria. 2nd ed. Geneva, Switzerland: World Health Organisation; 2010.

[36] Dondorp A, Nosten F, Stepniewska K, Day N, White N. Artesunate versus quinine for treatment of severe falciparum malaria: A randomised trial. Lancet. 2005;**366**:717-725. DOI: 10.1016/S0140-6736(05)67176-0

[37] Dondorp AM, Fanello CI, Hendriksen IC, Gomes E, Seni A, Chhaganlal KD, Bojang K, Olaosebikan R, Anunobi N, Maitland K. Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): An open-label, randomised trial. Lancet. 2010;**376**:1647-1657. DOI: 10.1016/S0140-6736(10)61924-1

[38] WHO. Updates on Drug Resistance. 2017. Available from: http://www.who.int/malaria/areas/drug_resistance/updates/en/ [Accessed: 2017-11-7]

[39] WHO. Efficacy Database. 2017. Available from: http://www.who.int/malaria/areas/drug_resistance/drug_efficacy_database/en/ [Accessed: 2017-11-7]

[40] Prothero RM. Population movements and problems of malaria eradication in Africa. Bulletin of the World Health Organization. 1961;**24**:405

[41] Martens P, Hall L. Malaria on the move: Human population movement and malaria transmission. Emerging Infectious Diseases. 2000;**6**:103. DOI: 10.3201/eid0602.000202

[42] Prothero RM. Disease and mobility: A neglected factor in epidemiology. International Journal of Epidemiology. 1977;**6**:259-267. DOI: 10.1093/ije/6.3.259

[43] Pindolia DK, Garcia AJ, Wesolowski A, Smith DL, Buckee CO, Noor AM, Snow RW, Tatem AJ. Human movement data for malaria control and elimination strategic planning. Malaria Journal. 2012;**11**:205. DOI: 10.1186/1475-2875-11-205

[44] Delacollette C, D'Souza C, Christophel E, Thimasarn K, Abdur R, Bell D, Dai TC, Gopinath D, Lu S, Mendoza R. Malaria trends and challenges in the Greater Mekong Subregion. The Southeast Asian Journal of Tropical Medicine and Public Health. 2009;**40**:674

[45] Edwards HM, Canavati SE, Rang C, Ly P, Sovannaroth S, Canier L, Khim N, Menard D, Ashton RA, Meek SR. Novel cross-border approaches to optimise identification of asymptomatic and artemisinin-resistant *Plasmodium* infection in mobile populations crossing Cambodian borders. PLoS One. 2015;**10**:e0124300. DOI: 10.1371/journal.pone.0124300

[46] Bhumiratana A, Intarapuk A, Sorosjinda-Nunthawarasilp P, Maneekan P, Koyadun S. Border malaria associated with multidrug resistance on Thailand-Myanmar and Thailand-Cambodia borders: Transmission dynamic, vulnerability, and surveillance. BioMed Research International. 2013;**2013**:363417. DOI: 10.1155/2013/363417

[47] Lynch C, Roper C. The transit phase of migration: Circulation of malaria and its multidrug-resistant forms in Africa. PLoS Medicine. 2011;**8**:e1001040. DOI: 10.1371/journal.pmed.1001040

[48] ASEAN. Blueprint. 2008. Available from: https://archive.org/…/ASEANeconomiccommunityblueprint/ASEAN%20E [Accessed: 2017-11-9]

[49] WHO. Informal consultation on improving access to malaria control services for migrant/mobile populations in the context of the emergency response to artemisinin resistance in the GMS. 2014. Available from: http://www.searo.who.int/entity/malaria/documents/access_malaria_services…GMS/en/ [Accessed: 2017-11-12]

[50] Ministry of Health. Cambodia malaria survey. 2004. Available from: http://malariasurveys.org/documents/CMBS2004report15jul.pdf 2005 [Accessed: 2017-11-12]

[51] Ministry of Health. Cambodia malaria survey. 2007. Available from: http://malariasveys.org/documents/CMS2007%20report_22Sep10_FINAL.pdf. 2008 [Accessed: 2017-11-12]

[52] Ministry of Health. Cambodia malaria survey. 2010. Available from: http://malariasurveys.org/documents/CMS%202010%20GF%20Report%20(FINAL).pdf. 2011 [Accessed: 2017-11-12]

[53] Ministry of Health. Cambodia malaria survey. 2013 Available at: http://www.malariaconsortium.org/resources/publications/624/cambodia-malaria-survey-2013. 2014. [Accessed 2017-11-12]

[54] Okell LC, Ghani AC, Lyons E, Drakeley CJ. Submicroscopic infection in *Plasmodium falciparum*-endemic populations: A systematic review and meta-analysis. The Journal of Infectious Diseases. 2009;**200**:1509-1517. DOI: 10.1086/644781

[55] Bousema T, Griffin JT, Sauerwein RW, Smith DL, Churcher TS, Takken W, Ghani A, Drakeley C, Gosling R. Hitting hotspots: Spatial targeting of malaria for control and elimination. PLoS Medicine. 2012;**9**:e1001165. DOI: doi.org/10.1371/journal.pmed.1001165

[56] Mosha JF, Sturrock HJ, Greenwood B, Sutherland CJ, Gadalla NB, Atwal S, Hemelaar S, Brown JM, Drakeley C, Kibiki G. Hot spot or not: A comparison of spatial statistical methods to predict prospective malaria infections. Malaria Journal. 2014;**13**:53. DOI: 10.1186/1475-2875-13-53

[57] Bousema T, Stresman G, Baidjoe AY, Bradley J, Knight P, Stone W, Osoti V, Makori E, Owaga C, Odongo W. The impact of hotspot-targeted interventions on malaria transmission in Rachuonyo South District in the Western Kenyan Highlands: A cluster-randomized controlled trial. PLoS Medicine. 2016;**13**:e1001993. DOI: /10.1371/journal.pmed.1001993

[58] Rosenberg R, Andre RG, Somchit L. Highly efficient dry season transmission of malaria in Thailand. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1990;**84**:22-28

[59] Durnez L, Pareyn M, Mean V, Kim S, Khim N, Menard D, Coosemans M, Sochantha T, Sluydts V. Identification and characterization of areas of high and low risk for asymptomatic malaria infections at sub-village level in Ratanakiri, Cambodia. Malaria Journal. 2018;**17**:27. DOI: 10.1186/s12936-017-2169-1

[60] Sturrock HJ, Hsiang MS, Cohen JM, Smith DL, Greenhouse B, Bousema T, Gosling RD. Targeting asymptomatic malaria infections: Active surveillance in control and elimination. PLoS Medicine. 2013;**10**:e1001467. DOI: 10.1371/journal.pmed.1001467

[61] Moonen B, Cohen JM, Snow RW, Slutsker L, Drakeley C, Smith DL, Abeyasinghe RR, Rodriguez MH, Maharaj R, Tanner M. Operational strategies to achieve and maintain malaria elimination. The Lancet. 2010;**376**:1592-1603. DOI: 10.1016/S0140-6736(10)61269-X

[62] Lindblade KA, Steinhardt L, Samuels A, Kachur SP, Slutsker L. The silent threat: Asymptomatic parasitemia and malaria transmission. Expert Review of Anti-Infective Therapy. 2013;**11**:623-639. DOI: 10.1586/eri.13.45

[63] Harris I, Sharrock WW, Bain LM, Gray K-A, Bobogare A, Boaz L, Lilley K, Krause D, Vallely A, Johnson M-L. A large proportion of asymptomatic *Plasmodium* infections with low and sub-microscopic parasite densities in the low transmission setting of Temotu Province, Solomon Islands: Challenges for malaria diagnostics in an elimination setting. Malaria Journal. 2010;**9**:254. DOI: 10.1186/1475-2875-9-254

[64] CMEP: Response to Abnormal Increase of Malaria Cases in Pursat within Cambodia Malaria Elimination Project (CMEP). Cambodia: President's Malaria Initiative. University Research Co., LLC; 2017

[65] WPRO WHO. ERAR Dissemination Meeting to Share Findings of the Assessment of Malaria Surveillance Systems in Greater Mekong Subregion Countries. 2015. Available from: http://www.who.int/malaria/areas/greater_mekong/technical-reports/en/ [Accessed: 2017-11-12]

[66] Grietens KP, Gryseels C, Dierickx S, Bannister-Tyrrell M, Trienekens S, Uk S, Phoeuk P, Suon S, Set S, Gerrets R. Characterizing types of human mobility to inform differential and targeted malaria elimination strategies in Northeast Cambodia. Scientific Reports. 2015;**5**:16836. DOI: 10.1038/srep16837

[67] Guyant P, Canavati SE, Chea N, Ly P, Whittaker MA, Roca-Feltrer A, Yeung S. Malaria and the mobile and migrant population in Cambodia: A population movement framework to inform strategies for malaria control and elimination. Malaria Journal. 2015;**14**:252. DOI: 10.1186/s12936-015-0773-5

[68] WHO. World malaria report 2012. Global Malaria Program. 2013. Available from: http://www.who.int/malaria/publications/world_malaria_report_2013/en [Accessed: 2107-11-12]

[69] Yang G-J, Brook BW, Whelan PI, Cleland S, Bradshaw CJ. Endogenous and exogenous factors controlling temporal abundance patterns of tropical mosquitoes. Ecological Applications. 2008;**18**:2028-2040. DOI: 10.1890/07-1209.1

[70] Sinka ME, Bangs MJ, Manguin S, Chareonviriyaphap T, Patil AP, Temperley WH, Gething PW, Elyazar IR, Kabaria CW, Harbach RE. The dominant *Anopheles* vectors of human malaria in the Asia-Pacific region: Occurrence data, distribution maps and bionomic précis. Parasites & Vectors. 2011;**4**:89. DOI: 10.1186/1756-3305-4-89

[71] WHO. World Malaria Report 2013: World Health Organization; Geneva, Switzerland; 2014

[72] APMEN. Report of the fourth annual business and technical meeting of the Asia-Pacific Malaria Elimination Network (APMEN). 2012. Available from: http://apmen.org/events/annual-meeting/4/apmen-iv.html/ [Accessed: 2017-12-5]

[73] Sumarnrote A, Overgaard HJ, Marasri N, Fustec B, Thanispong K, Chareonviriyaphap T, Corbel V. Status of insecticide resistance in *Anopheles* mosquitoes in Ubon Ratchathani province, Northeastern Thailand. Malaria Journal. 2017;**16**:299. DOI: 10.1186/s12936-017-1948

[74] Lyttleton C. Deviance and resistance: Malaria elimination in the greater Mekong subregion. Social Science & Medicine. 2016;**150**:144-152. DOI: 1016

[75] Ferguson HM, Dornhaus A, Beeche A, Borgemeister C, Gottlieb M, Mulla MS, Gimnig JE, Fish D, Killeen GF. Ecology: A prerequisite for malaria elimination and eradication. PLoS Medicine. 2010;**7**:e1000303. DOI: 10.1371/journal.pmed.1000303

[76] Achee NL, Bangs MJ, Farlow R, Killeen GF, Lindsay S, Logan JG, Moore SJ, Rowland M, Sweeney K, Torr SJ. Spatial repellents: From discovery and development to evidence-based validation. Malaria Journal. 2012;**11**:164. DOI: 10.1186/1475-2875-11-164

[77] Molineaux L, Gramiccia G, Organization WH. The Garki project: research on the epidemiology and control of malaria in the Sudan savanna of West Africa. In: Molineaux L, Gramiccia G. The Garki Project: Research on the Epidemiology and Control of Malaria in the Sudan Savanna of West Africa. 1980

[78] Govella NJ, Chaki PP, Killeen GF. Entomological surveillance of behavioural resilience and resistance in residual malaria vector populations. Malaria Journal. 2013;**12**:124. DOI: 10.1186/1475-2875-12-124

[79] Durnez L, Coosemans M. Residual transmission of malaria: An old issue for new approaches. In: Manguin S, editor. *Anopheles* Mosquitoes-New Insights into Malaria Vectors. Rijeka, Croatia: InTech Open Access; 2013. DOI: 10.5772/55925

[80] Killeen GF. Characterizing, controlling and eliminating residual malaria transmission. Malaria Journal. 2014;**13**:330. DOI: 10.1186/1475-2875-13-330

[81] WHO Malaria Policy Advisory Committee (MPAC) meeting. 2017. Available from: http://www.apps.who.int/iris/bitstream/10665/259396/1/WHO-HTM-GMP-2017.21-eng.pdf [Accessed 2017-12-5]

[82] Hii J, Edwards HM. Studies to examine magnitude and identify causes (study sites in Thailand and Viet Nam). In: Workshop on Residual Malaria Transmission, Dar-Es-Salam, Tanzania, TDR/WHO, IHI and GMP/WHO. 2017

[83] Van Bortel W, Trung HD, Hoi-le-X VHN, Van-Chut N, Luu ND, Roelants P, Coosemans M. Malaria transmission and vector behaviour in a forested malaria focus in central Vietnam and the implications for vector control. Malaria Journal. 2010;**9**:373. DOI: 10.1186/1475-2875-9-373

[84] Tangena JAA, Thammavong P, Lindsay SW, Brey PT. Risk of exposure to potential vector mosquitoes for rural workers in Northern Lao PDR. PLOS Neglected Tropical Diseases. 2017;**11**:e0005802. DOI: 10.1371/journal.pntd.0005802

[85] Manguin S, Garros C, Dusfour I, Harbach R, Coosemans M. Bionomics, taxonomy, and distribution of the major malaria vector taxa of *Anopheles* subgenus *Cellia* in Southeast Asia: An updated review. Infection, Genetics and Evolution. 2008;**8**:489-503. DOI: 10.1016/j.meegid.2007.11.004

[86] Hlaing T, Wai KT, Oo T, Sint N, Min T, Myar S, Lon KN, Naing MM, Tun TT, Maung NLY. Mobility dynamics of migrant workers and their socio-behavioral parameters related to malaria in Tier II, Artemisinin Resistance Containment Zone, Myanmar. BMC Public Health. 2015;**15**:886. DOI: 10.1186/s12889-015-2241-0

[87] Parker DM, Matthews SA, Yan G, Zhou G, Lee M-C, Sirichaisinthop J, Kiattibutr K, Fan Q, Li P, Sattabongkot J. Microgeography and molecular epidemiology of malaria at the Thailand-Myanmar border in the malaria pre-elimination phase. Malaria Journal. 2015;**14**:198. DOI: 10.1186/s12936-015-0712-5

[88] Wangroongsarb P, Satimai W, Khamsiriwatchara A, Thwing J, Eliades JM, Kaewkungwal J, Delacollette C. Respondent-driven sampling on the Thailand-Cambodia border. II. Knowledge, perception, practice and treatment-seeking behaviour of migrants in malaria endemic zones. Malaria Journal. 2011;**10**:117. DOI: 10.1186/1475-2875-10-117

[89] Tipmontree R, Fungladda W, Kaewkungwal J, Tempongko MSB, Schelp FP. Migrants and malaria risk factors: A study of the Thai-Myanmar border. The Southeast Asian Journal of Tropical Medicine and Public Health. 2009;**40**:1148

[90] Rattanarithikul R, Mongkolpanya K, Noigamol C, Chanaimongkol S, Mahapibul P, Nakngen S. Dry season distribution of mosquito larvae in the bed of the Mekong River, northeastern Thailand. Journal of the American Mosquito Control Association. 1994;**10**:197-201

[91] Rattanarithikul R, Green C, Panyim S, Noigamol C, Chanaimongkol S, Mahapibul P. Larval habitats of malaria vectors and other *Anopheles* mosquitoes around a transmission focus in northwestern Thailand. Journal of the American Mosquito Control Association. 1995;**11**:428-433

[92] Hii J, Rueda L. Malaria vectors in the Greater Mekong Subregion: Overview of malaria vectors and remaining challenges. The Southeast Asian Journal of Tropical Medicine and Public Health. 2013;**44**:73-165 discussion 306-167

[93] Durnez L, Van Bortel W, Denis L, Roelants P, Veracx A, Trung HD, Sochantha T, Coosemans M. False positive circumsporozoite protein ELISA: A challenge for the estimation of the entomological inoculation rate of malaria and for vector incrimination. Malaria Journal. 2011;**0**:195. DOI: 10.1186/1475-2875-10-195

[94] Laurent BS, Oy K, Miller B, Gasteiger EB, Lee E, Sovannaroth S, Gwadz RW, Anderson JM, Fairhurst RM. Cow-baited tents are highly effective in sampling diverse *Anopheles* malaria vectors in Cambodia. Malaria Journal. 2016;**15**:440. DOI: 10.1186/s12936-016-1488

[95] Alam MS, Chakma S, Khan WA, Glass GE, Mohon AN, Elahi R, Norris LC, Podder MP, Ahmed S, Haque R. Diversity of anopheline species and their *Plasmodium* infection status in rural Bandarban, Bangladesh. Parasites & Vectors. 2012;**5**:150. DOI: 10.1186/1756-3305-5-150

[96] Ngo CT, Dubois G, Sinou V, Parzy D, Le HQ, Harbach RE, Manguin S. Diversity of *Anopheles* mosquitoes in Binh Phuoc and Dak Nong Provinces of Vietnam and their relation to disease. Parasites & Vectors. 2014;**7**:316. DOI: 10.1186/1756-3305-7-316

[97] Sriwichai P, Samung Y, Sumruayphol S, Kiattibutr K, Kumpitak C, Payakkapol A, Kaewkungwal J, Yan G, Cui L, Sattabongkot J. Natural human *Plasmodium* infections in major *Anopheles* mosquitoes in western Thailand. Parasites & Vectors. 2016;**9**:17. DOI: 10.1186/s13071-016-1295-x

[98] Bashara K, Tunob N, Ahmed TU, Howlader AJ. False positivity of circumsporozoite protein (CSP)–ELISA in zoophilic anophelines in Bangladesh. Acta Tropica. 2013;**125**:220-225

[99] Harrison BA. The Myzomyia Series of *Anopheles* (*Cellia*) in Thailand, with emphasis on intra-interspecific variations (Diptera: Culicidae). Contributions of the American Entomological Institute. 1980;**17**:1-195

[100] Van Bortel W, Harbach RE, Trung HD, Roelants P, Backeljau T, Coosemans M. Confirmation of *Anopheles varuna* in Vietnam, previously misidentified and mistargeted as the malaria vector *Anopheles minimus*. American Journal of Tropical Medicine and Hygiene. 2001;**65**:729-732

[101] Erlank E, Koekemoer LL, Coetzee M. The importance of morphological identification of African anopheline mosquitoes (Diptera: Culicidae) for malaria control programmes. Malaria Journal. 2018;**17**:43. DOI: 10.1186/s12936-018-2189-5

[102] Durnez L, Mao S, Denis L, Roelants P, Sochantha T, Coosemans M. Outdoor malaria transmission in forested villages of Cambodia. Malaria Journal. 2013;**12**:329. DOI: 10.1186/1475-2875-12-329

[103] Gryseels C, Durnez L, Gerrets R, Uk S, Suon S, Set S, Phoeuk P, Sluydts V, Heng S, Sochantha T. Re-imagining malaria: Heterogeneity of human and mosquito behaviour in relation to residual malaria transmission in Cambodia. Malaria Journal. 2015;**14**:165. DOI: 10.1186/s12936-015-0689-0

[104] Guerra C, Snow R, Hay S. A global assessment of closed forests, deforestation and malaria risk. Annals of Tropical Medicine and Parasitology. 2006;**100**:189. DOI: 10.1179/136485906X91512

[105] Tangena JAA, Thammavong P, Malaithong N, Inthavong T, Ouanesamon P, Brey PT, Lindsay SW. Diversity of mosquitoes (Diptera: Culicidae) attracted to human subjects in rubber plantations, secondary forests, and villages in Luang Prabang Province, Northern Lao PDR. Journal of Medical Entomology. 2017;**54**:1589-1604. DOI: 10.1093/jme/tjx071

[106] Tangena JAA, Thammavong P, Wilson AL, Brey PT, Lindsay SW. Risk and control of mosquito-borne diseases in Southeast Asian rubber plantations. Trends in Parasitology. 2016;**32**:402-415. DOI: 10.1016/j.pt.2016.01.009

[107] Kaewwaen W, Bhumiratana A. Landscape ecology and epidemiology of malaria associated with rubber plantations in Thailand: Integrated approaches to malaria ecotoping. Interdisciplinary perspectives. Infectious Diseases. 2015;**2015**:909106. DOI: 10.1155/2015

[108] Satitvipawee P, Wongkhang W, Pattanasin S, Hoithong P, Bhumiratana A. Predictors of malaria-association with rubber plantations in Thailand. BMC Public Health. 2012;**12**:1115. DOI: 10.1186/1471-2458-12-1115

[109] Oo T. Biology and vector competence of the anopheline mosquitoes of Myanmar with special consideration of *Anopheles dirus* [thesis]. University of Heidelber; 2003

[110] Crawshaw AF, Maung TM, Shafique M, Sint N, Nicholas S, Li MS, Roca-Feltrer A, Hii J. Acceptability of insecticide-treated clothing for malaria prevention among migrant rubber tappers in Myanmar: A cluster-randomized non-inferiority crossover trial. Malaria Journal. 2017;**16**:92. DOI: 10.1186/s12936-017-1737-8

[111] Chang MS, Hii J, Buttner P, Mansoor F. Changes in abundance and behaviour of vector mosquitoes induced by land use during the development of an oil palm plantation in Sarawak. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1997;**91**:382-386. DOI: 10.1016/S0035-9203(97)90248-0

[112] White N. *Plasmodium knowlesi*: The fifth human malaria parasite. Clinical Infectious Diseases. 2008;**46**:172. DOI: 10.1086/524889

[113] Lee KS, Vythilingam I. *Plasmodium knowlesi*: Emergent human malaria in Southeast Asia. In: Lim YAL, Vythilingam I, editors. Parasites and their Vectors A Special Focus on Southeast Asia. Vienna: Springer; 2013. pp. 5-31

[114] William T, Rahman HA, Jelip J, Ibrahim MY, Menon J, Grigg MJ, Yeo TW, Anstey NM, Barber BE. Increasing incidence of *Plasmodium knowles*i malaria following control of *P. falciparum* and *P. vivax* malaria in Sabah, Malaysia. PLOS Neglected Tropical Diseases. 2013;**7**:e2026. DOI: 10.1371/journal.pntd.0002026

[115] Setiadi W, Sudoyo H, Trimarsanto H, Sihite BA, Saragih RJ, Juliawaty R, Wangsamuda S, Asih PBS, Syafruddin D. A zoonotic human infection with simian malaria, *Plasmodium knowlesi*, in Central Kalimantan, Indonesia. Malaria Journal. 2016;**15**:218. DOI: 10.1186/s12936-016-1272-z

[116] Herdiana H, Cotter C, Coutrier FN, Zarlinda I, Zelman BW, Tirta YK, Greenhouse B, Gosling RD, Baker P, Whittaker M. Malaria risk factor assessment using active and passive surveillance data from Aceh Besar, Indonesia, a low endemic, malaria elimination setting with *Plasmodium knowlesi*, *Plasmodium vivax*, and *Plasmodium falciparum*. Malaria Journal. 2016;**15**:468. DOI: 10.1186/s12936-016-1523-z

[117] Lubis IN, Wijaya H, Lubis M, Lubis CP, Divis P, Beshir KB, Sutherland CJ. Contribution of *Plasmodium knowlesi* to multispecies human malaria infections in North Sumatera, Indonesia. The Journal of Infectious Diseases. 2017;**215**:1148-1155. DOI: 10.1093/infdis/jix091

[118] Fornace KM, Nuin NA, Betson M, Grigg MJ, William T, Anstey NM, Yeo TW, Cox J, Ying LT, Drakeley CJ. Asymptomatic and submicroscopic carriage of *Plasmodium knowlesi* malaria in household and community members of clinical cases in Sabah, Malaysia. The Journal of Infectious Diseases. 2015;**213**:784-787. DOI: 10.1093/infdis/jiv475

[119] Fornace KM, Abidin TR, Alexander N, Brock P, Grigg MJ, William T, Menon J, Drakeley C, Cox J. Association between landscape factors and spatial patterns of *Plasmodium knowlesi* infections in Sabah, Malaysia. Emerging Infectious Diseases. 2016;**22**. DOI: 10.3201/eid2202.150656

[120] Moyes CL, Henry AJ, Golding N, Huang Z, Singh B, Baird JK, Newton PN, Huffman M, Duda KA, Drakeley CJ. Defining the geographical range of the *Plasmodium knowles*i reservoir. PLOS Neglected Tropical Diseases. 2014;**8**:e2780. DOI: 10.1371/journal.pntd.0002780

[121] Tan CH, Vythilingam I, Matusop A, Chan ST, Singh B. Bionomics of *Anopheles latens* in Kapit, Sarawak, Malaysian Borneo in relation to the transmission of zoonotic simian malaria parasite *Plasmodium knowlesi*. Malaria Journal. 2008;**7**:52. DOI: 10.1186/1475-2875-7-52

[122] Jiram AI, Vythilingam I, NoorAzian YM, Yusof YM, Azahari AH, Fong MY. Entomologic investigation of *Plasmodium knowlesi* vectors in Kuala Lipis, Pahang, Malaysia. Malaria Journal. 2012;**11**:213. DOI: 10.1186/1475-2875-11-213

[123] Wong ML, Chua TH, Leong CS, Khaw LT, Fornace K, Wan-Sulaiman W-Y, William T, Drakeley C, Ferguson HM, Vythilingam I. Seasonal and spatial dynamics of the primary vector of *Plasmodium knowlesi* within a major transmission focus in Sabah, Malaysia. PLOS Neglected Tropical Diseases. 2015;**9**:e0004135. DOI: 10.1371/journal.pntd.0004135

[124] Marchand RP, Culleton R, Maeno Y, Quang NT, Nakazawa S. Co-infections of *Plasmodium knowlesi*, *P. falciparum*, and *P. vivax* among humans and *Anopheles dirus* mosquitoes, southern Vietnam. Emerging Infectious Diseases. 2011;**17**:1232-1239. DOI: 10.3201/eid1707.101551

[125] Nakazawa S, Marchand RP, Quang NT, Culleton R, Manh ND, Maeno Y. *Anopheles dirus* co-infection with human and monkey malaria parasites in Vietnam. International Journal for Parasitology. 2009;**39**:1533-1537. DOI: 10.1016/j.ijpara.2009.08.005

[126] Maeno Y, Quang NT, Culleton R, Kawai S, Masuda G, Nakazawa S, Marchand RP. Humans frequently exposed to a range of non-human primate malaria parasite species through the bites of *Anopheles dirus* mosquitoes in South-central Vietnam. Parasites & Vectors. 2015;**8**:376. DOI: 10.1186/s13071-015-0995-y

[127] Baird JK. Asia-Pacific malaria is singular, pervasive, diverse and invisible. International Journal for Parasitology. 2017;**47**:371-377. DOI: 10.1016/j.ijpara.2016.06.006

[128] Grigg MJ, Cox J, William T, Jelip J, Fornace KM, Brock PM, von Seidlein L, Barber BE, Anstey NM, Yeo TW. Individual-level factors associated with the risk of acquiring human *Plasmodium knowlesi* malaria in Malaysia: A case-control study. Lancet Planet Health. 2017;**1**:e97-e104. DOI: 10.1016/S2542-5196(17)30031-1

[129] Manin BO, Ferguson HM, Vythilingam I, Fornace K, William T, Torr SJ, Drakeley C, Chua TH. Investigating the contribution of peri-domestic transmission to risk of zoonotic malaria infection in humans. PLoS Neglected Tropical Diseases. 2016;**10**:e0005064. DOI: 10.1371/journal.pntd.0005064

[130] Akter R, Vythilingam I, Khaw LT, Qvist R, Lim YA-L, Sitam FT, Venugopalan B, Sekaran SD. Simian malaria in wild macaques: First report from Hulu Selangor district, Selangor, Malaysia. Malaria Journal. 2015;**14**:1-9. DOI: 10.1186/s12936-015-0856-3

[131] Li M. Identification and molecular characterization of simian malaria parasites in wild monkeys of Singapore. [thesis]. National University of Singapore; 2011

[132] Lee KS, Divis PCS, Zakaria SK, Matusop A, Julin RA, Conway DJ, Cox-Singh J, Singh B. *Plasmodium knowlesi:* Reservoir hosts and tracking the emergence in humans and macaques. PLoS Pathogens. 2011;**7**:e1002015. DOI: 10.1371/journal.ppat.1002015

[133] Takken W, Knols BGJ. Malaria vector control: Current and future strategies. Trends in Parasitology. 2009;**25**:101-104. DOI: 10.1016/j.pt.2008.12.002

[134] Liu W, Li Y, Learn GH, Rudicell RS, Robertson JD, Keele BF, Ndjango J-BN, Sanz CM, Morgan DB, Locatelli S. Origin of the human malaria parasite *Plasmodium falciparum* in gorillas. Nature. 2010;**467**:420-425. DOI: 10.1038/nature09442

[135] Liu W, Li Y, Shaw KS, Learn GH, Plenderleith LJ, Malenke JA, Sundararaman SA, Ramirez MA, Crystal PA, Smith AG. African origin of the malaria parasite *Plasmodium vivax*. Nature Communications. 2014;**5**:3346. DOI: 10.1038/ncomms4346

[136] Brant HL, Ewers RM, Vythilingam I, Drakeley C, Benedick S, Mumford JD. Vertical stratification of adult mosquitoes (Diptera: Culicidae) within a tropical rainforest in Sabah, Malaysia. Malaria Journal. 2016;**15**:370. DOI: 10.1186/s12936-016-1416-1

[137] Yasuoka J, Levins R. Impact of deforestation and agricultural development on anopheline ecology and malaria epidemiology. American Journal of Tropical Medicine and Hygiene. 2007;**76**:450-460. DOI: 10.4269/ajtmh.2007.76.450

[138] Singhasivanon P, Thimasarn K, Yimsamran S, Linthicum K, Nualchawee K, Dawreang D, Kongrod S, Premmanisakul N, Maneeboonyang W, Salazar N. Malaria in tree crop plantations in south-eastern and western provinces of Thailand. The Southeast Asian Journal of Tropical Medicine and Public Health. 1999;**30**:399-404

[139] Overgaard HJ, Ekbom B, Suwonkerd W, Takagi M. Effect of landscape structure on anopheline mosquito density and diversity in northern Thailand: Implications for malaria transmission and control. Landscape Ecology. 2003;**18**:605-619. DOI: 10.1023/A-102

[140] Overgaard HJ, Suwonkerd W, Hii J. The malaria landscape: Mosquitoes, transmission, landscape, insecticide resistance, and integrated control in Thailand. In: Serge-Morand DJP, Lefait-Robin R, Apiwathnasorn C, editors. Socio-Ecological Dimensions of Infectious Diseases in Southeast Asia. UK: Springer; 2015. p. 123-154

[141] Wharton RH, Eyles DE. *Anopheles hackeri*, a vector of *Plasmodium knowlesi* in Malaya. Science. 1961;**134**:279. DOI: 10.1126/science.134.3474.279

[142] Vythilingam I, Lim YA, Venugopalan B, Ngui R, Leong CS, Wong ML, Khaw L, Goh X, Yap N, Sulaiman WYW. *Plasmodium knowlesi* malaria an emerging public health problem in Hulu Selangor, Selangor, Malaysia (2009-2013): Epidemiologic and entomologic analysis. Parasites & Vectors. 2014;**7**:436. DOI: 10.1186/1756-3305-7-436

[143] Hii JKL, Kan S, Pereria M, Parmar SS, Campos RL, Chan MKC. Transmission dynamics and estimates of malaria vectorial capacity for *An. balabacensis* and *An. flavirostris* on Bangii island, Sabah, Malaysia. Annals of Tropical Medicine and Parasitology. 1988;**82**:91-101. DOI: 10.1080/00034983.1988.11812214

[144] Hii JLK, Kan S, Pereira M, Parmar SS, Campos RL, Chan MKC. Bancroftian filariasis and malaria in island and hinterland populations in Sabah, Malaysia. Tropical and Geographical Medicine. 1985;**37**:93-101

[145] Manguin S, Bangs M, Pothikasikorn J, Chareonviriyaphap T. Review on global co-transmission of human *Plasmodium* species and *Wuchereria bancrofti* by *Anopheles* mosquitoes. Infection, Genetics and Evolution. 2010;**10**:159-177

[146] Cox-Singh J. Zoonotic malaria: *Plasmodium knowlesi,* an emerging pathogen. Current Opinion in Infectious Diseases. 2012;**25**:530-536. DOI: 10.1097/QCO.0b013e3283558780

[147] Cox-Singh J, Singh B. Knowlesi malaria: Newly emergent and of public health importance? Trends in Parasitology. 2008;**24**:406-410. DOI: 10.1016/j.pt.2008.06.001

[148] Singh B, Daneshvar C. Human infections and detection of *Plasmodium knowlesi*. Clinical Microbiology Reviews. 2013;**26**:165-184. DOI: 10.1128/CMR.00079-12

[149] Vythilingam I, Hii J. Simian malaria parasites: Special emphasis on *Plasmodium knowlesi* and their *Anopheles* vectors in Southeast Asia. In: Manguin S, editor. *Anopheles* Mosquitoes—New Insights into Malaria Vectors. Rijeka, Croatia: InTech Open Access; 2013. pp. 487-510. DOI: 10.5772/54491

[150] Vythilingam I, Wong M, Wan-Yussof W. Current status of *Plasmodium knowlesi* vectors: A public health concern? Parasitology. 2018;**145**:32-40. DOI: 10.1017/S0031182016000901

[151] Barber BE, Rajahram GS, Grigg MJ, William T, Anstey NM. World malaria report: Time to acknowledge *Plasmodium knowlesi* malaria. Malaria Journal. 2017;**16**:135. DOI: 10.1186/s12936-017-1787-y

[152] Baird JK, Valecha N, Duparc S, White NJ, Price RN. Diagnosis and treatment of *Plasmodium vivax* malaria. American Journal of Tropical Medicine and Hygiene. 2016;**95**:35-51. DOI: 10.4269/ajtmh.16-0171

[153] Howes RE, Piel FB, Patil AP, Nyangiri OA, Gething PW, Dewi M, Hogg MM, Battle KE, Padilla CD, Baird JK. G6PD deficiency prevalence and estimates of affected populations in malaria endemic countries: A geostatistical model-based map. PLoS Medicine. 2012;**9**:e1001339. DOI: 10.1371/journal.pmed.1001339

[154] WHO. Single dose primaquine as a gametocytocide in *Plasmodium falciparum* malaria. 2012. Available from: http://www.who.int/malaria/publications/atoz/who_pq_policy_recommendation/en/ [Accessed: 2017-12-5]

[155] Olliaro PL, Barnwell JW, Barry A, Mendis K, Mueller I, Reeder JC, Shanks GD, Snounou G, Wongsrichanalai C. Implications of *Plasmodium vivax* biology for control, elimination, and research. American Journal of Tropical Medicine and Hygiene. 2016;**95**:4-14. DOI: 10.4269/ajtmh.16-0160

[156] WHO. Control and elimination of *Plasmodium vivax* malaria: A technical brief. World Health Organization; 2015. Available from: http://www.who.int/malaria/publications/atoz/9789241509244/en/ [Accessed: 2018-01-27]

[157] Mendis K, Sina BJ, Marchesini P, Carter R. The neglected burden of *Plasmodium vivax* malaria. American Journal of Tropical Medicine and Hygiene. 2001;**64**:97-106. DOI: 10.4269/ajtmh.2001.64.97

[158] Bousema T, Drakeley C. Epidemiology and infectivity of *Plasmodium falciparum* and *Plasmodium vivax* gametocytes in relation to malaria control and elimination. Clinical Microbiology Reviews. 2011;**24**:377-410. DOI: 10.1128/CMR.00051-10

[159] Alonso PL, Tanner M. Public health challenges and prospects for malaria control and elimination. Nature Medicine. 2013;**19**:150-155. DOI: 10.1038/nm.3077

[160] Vivax Working Group. Targeting vivax malaria in the Asia Pacific: The Asia Pacific malaria elimination network Vivax working group. Malaria Journal. 2015;**14**:484. DOI: 10.1186/s12936-015-0958-y

[161] WHO. Research priorities for the environment, agriculture and infectious diseases of poverty. 2013. Available from: http://apps.who.int/iris/bitstream/10665/78129/1/WHO_TRS_976_eng.pdf [Accessed: 2017-12-5]

[162] Campbell-Lendrum D, Molyneux D. Ecosystems and vector-borne disease control. In: Epstein P, Githeko A, Rabinovich J, Weinstein P, editors. Ecosystems and Human Well-Being: Policy Responses. Vol. 3. Washington, USA: Island Press; 2005. pp. 353-372

[163] Stewart-Ibarra M. A social-ecological systems approach to dengue-chikungunya-zika in urban coastal Ecuador. In: A social-ecological Systems Approach to Dengue-chikungunya-zika in Urban Coastal Ecuador. Vol. 2017. 2016

[164] WHO. Mass drug administration for malaria. A practical field manual. Malaria Policy Advisory Committee. 2017. Available from: http://www.who.int/malaria/mpac/mpac-mar2017-MDA-draft-manual-session7-presentation.pdf [Accessed: 2017-12-6]

[165] Wilson AL, Chen-Hussey V, Logan JG, Lindsay SW. Are topical insect repellents effective against malaria in endemic populations? A systematic review and meta-analysis. Malaria Journal. 2014;**13**:446. DOI: 10.1186/1475-2875-13-446

[166] Banks S, Murray N, WilderSmith A, Logan J. Insecticide-treated clothes for the control of vector-borne diseases: A review on effectiveness and safety. Medical and Veterinary Entomology. 2014;**28**:14-25. DOI: 10.1111/mve.12068

[167] Parkes MW, Bienen L, Breilh J, Hsu L-N, McDonald M, Patz JA, Rosenthal JP, Sahani M, Sleigh A, Waltner-Toews D. All hands on deck: Transdisciplinary approaches to emerging infectious disease. EcoHealth. 2005;**2**:258-272. DOI: 10.1007/s12275-013-2393-5

[168] Wilcox B, Kueffer C. Transdisciplinarity in EcoHealth: Status and future prospects. EcoHealth. 2008;**5**:1-3. DOI: 10.1007/s10393-008-0161-5

[169] Okello-Onen J, Mboera LE, Mugisha S. Malaria research and management need rethinking: Uganda and Tanzania case studies. In: Charron DF, editor. Ecohealth Research in Practice. New York: Springer; 2012. pp. 139-151. DOI: 10.1007/978-1-4614-0517-7

[170] Richter CH, Xu J, Wilcox BA. Opportunities and challenges of the ecosystem approach. Futures. 2015;**67**:40-51. DOI: 10.1016/j.futures.2014.12.002

[171] Minkler M, Wallerstein N. Introduction to community-based participatory research. In: Wallerstein MMN, editor. Community-Based Participatory Research for Health: From Process to Outcomes. San Francisco: John Wiley & Sons; 2008. pp. 5-23

[172] Guyant P, Corbel V, Guérin PJ, Lautissier A, Nosten F, Boyer S, Coosemans M, Dondorp AM, Sinou V, Yeung S. Past and new challenges for malaria control and elimination: The role of operational research for innovation in designing interventions. Malaria Journal. 2015;**14**:279. DOI: 10.1186/s12936-015-0802-4

[173] WHO. Revised recommendations for achieving universal coverage with long-lasting insecticidal nets in malaria control. 2017. Available from: http://www.who.int/entity/malaria/mpac/mpac-oct2017-erg-universal-coverage-session9-presentation.pdf [Accessed: 2018-01-28]

[174] Hewitt S, Delacollette C, Poirot E. Malaria control in the Greater Mekong Subregion: An overview of the current response and its limitations. The Southeast Asian Journal of Tropical Medicine and Public Health. 2013;**44**:249-305 discussion 306-247

[175] WHO. Expert Consultation on *Plasmodium knowlesi* malaria to guide malaria elimination strategies. 2017. Available from: ww.wpro.who.int/mvp/meetings/exp_con_pknowlesi_2017/en/ [Accessed: 2018-01-27]

Chapter 6

Understanding the Importance of Asymptomatic and Low-Density Infections for Malaria Elimination

Chris Drakeley, Bronner Gonçalves, Lucy Okell and Hannah Slater

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.77293

Abstract

In recent years, the use of more sensitive diagnostic techniques has demonstrated a significant number of malaria infections at densities beneath the limit of detection of conventional microscopy and rapid diagnostic tests (RDT). These low-density infections are almost always asymptomatic, found in all endemic settings, including those nearing elimination, and in all ages of the population. They typically account for a high proportion of all infections and since they have also been shown to be infectious to mosquitoes, low-density infections are thought to be important contributors to maintaining malaria transmission. However, there is currently no direct evidence that specifically targeting this low-density parasite reservoir will hasten progress towards elimination. In this chapter we review the data to date and identify knowledge gaps. We present potential scenarios for the causes of low-density infections, if and how these might drive transmission, and the likely impact of specifically targeting them.

Keywords: asymptomatic malaria, transmission, longitudinal carriage, malaria elimination, diagnostics

1. Epidemiology and relevance of asymptomatic infections

It has long been acknowledged that not all *Plasmodium falciparum* infections lead to clinical symptoms, and in the vast majority of malaria endemic settings most infections are asymptomatic. In the last two decades data on infection prevalence in endemic populations have been generated using nucleic acid amplification techniques (NAAT). The use of these sensitive diagnostic methods showed that, on average, there are approximately twice the number

of infections than those identified with more conventional diagnostics, such as microscopy [1] and rapid diagnostic tests (RDT) [2], with most of these previously undetected infections being asymptomatic. Indeed, the association between clinical disease and relatively high parasite levels [3–5] implies that most asymptomatic infections are also low-density, although parasitaemias above the microscopy detection limit are common in seemingly healthy infected individuals in endemic areas [3]. Of particular relevance, in light of the renewed interest in malaria elimination, is that in areas with low levels of transmission, often a high proportion of infections detected during prevalence surveys have sub-patent parasite densities (below the detection threshold of conventional diagnostics) [1].

For malaria elimination, a major consideration is how much the following different types of infections contribution to onwards transmission to mosquitoes: (1) clinical symptomatic and patent, (2) asymptomatic and patent, (3) asymptomatic and sub-patent, and what are the relative proportions of these infections in different endemic settings. Broadly speaking, the probability of a mosquito becoming infected after feeding on an infected human is dependent on the density of gametocytes. At an individual level, this probability of infection is higher from those with higher density symptomatic infections than those individuals with low-density asymptomatic infections [6], provided there has been enough time for gametocytes to fully mature (8–12 days). At a population level, contributions to malaria transmission from each of the three types of infections detailed above may be more balanced as there will be more individuals with low density infections than high density infections. In the context of control and elimination, symptomatic infections often have a very short duration because symptomatic individuals are more likely to seek and receive treatment and have their infections curtailed. This is more pronounced if treatment is given early after establishment of blood stage infection as gametocytes will be at relatively low densities and may not reach highly infectious levels.

Much of the data on asymptomatic infections is from community cross-sectional surveys. However, these snapshots are less informative on the dynamics of parasitaemia over time in individual infections. For example, an individual with a sub-patent infection today has several different potential infection outcomes (**Figure 1**). S/he may have been recently infected and will develop patent infection and clinical symptoms shortly afterwards, or alternatively may become a chronic parasite carrier, remaining asymptomatic for weeks or months. Conversely, individuals with sub-patent infections identified in cross-sectional surveys may also be at the tail end of an infection and will only remain minimally infectious for a short period of time (**Figure 1**). The contributions to onwards transmission from sub-patent infections that are only briefly asymptomatic [7] (all symptomatic infections invariably have a pre-clinical incubation period) and those that are chronic are likely to be very different. In community mass treatment campaigns that aim to interrupt transmission, clearing the infections of individuals who would otherwise go on develop symptoms, seek and obtain effective treatment a few days later, will reduce morbidity but is likely to have a smaller impact on reducing onwards transmission than treating infections in individuals who remain asymptomatic for several months. Estimations of the duration of malaria infections are necessary to understand the consequences of imperfect coverage during these interventions. However, quantifying infection duration in endemic settings is complicated because individuals are frequently superinfected with different falciparum clones, which means that periods of continuous parasite

http://dx.doi.org/10.5772/intechopen.77293

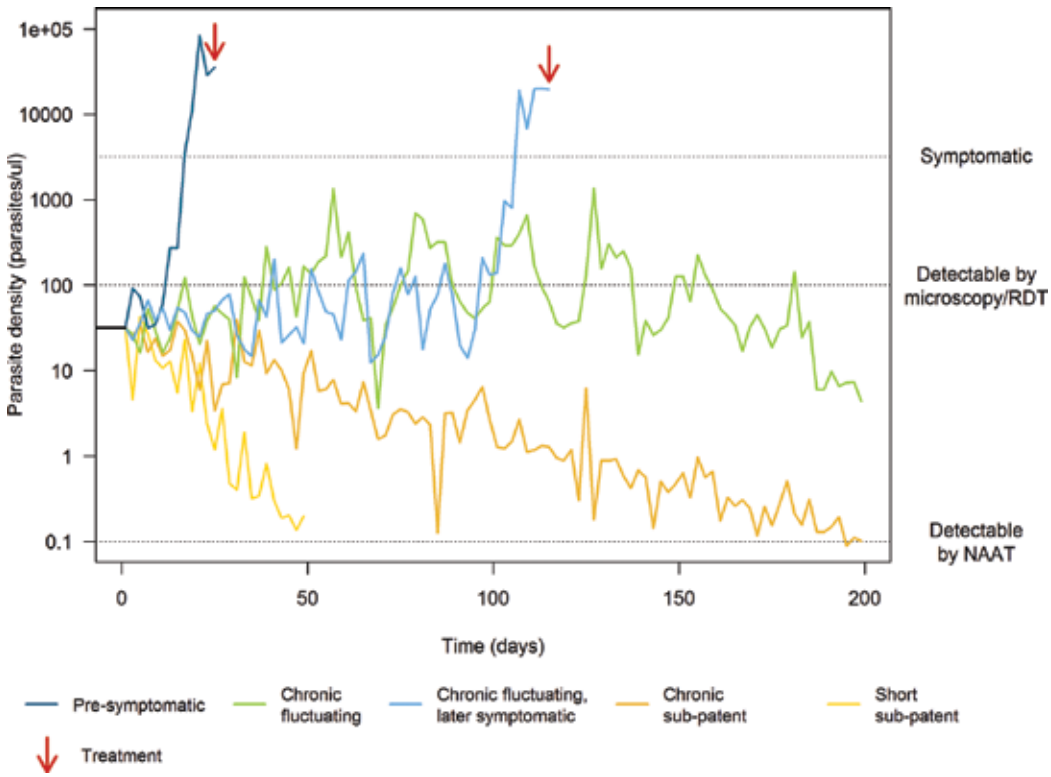


Figure 1. Hypothesised infection trajectories for sub-patent infections. An individual that has low-density asymptomatic infection on a given day can have several potential outcomes: s/he could continue to have long- (orange curve) or short-lasting (yellow curve) low-density infection, with limited infectiousness to mosquitoes and never developing symptoms or seeking treatment. The same individual could also be very recently infected and in the pre-symptomatic stage (dark blue line), or experience a slower increase in parasite density to a level where symptoms develop (light blue line). Another possible outcome is that parasitaemia fluctuates between detectable and undetectable levels by microscopy (green line). Any of these scenarios could be perturbed by re-infection, which could result in either continued asymptomatic infection or development of symptoms. RDT, rapid diagnostic test; NAAT, nucleic acid amplification techniques.

carriage often represent overlapping infections with different clones. A longitudinal study in Ghana using *msp-2* genotyping to distinguish parasite strains showed that naturally occurring infections last on average 5–6 months [8]. In Myanmar, in an area with transmission approaching elimination levels and consequently low probability of super-infection, falciparum carriage of at least 6–9 months was observed [9]. A study in Cambodia, which followed 24 adults with asymptomatic falciparum infections monthly, found that 13% carried parasites for 2–4 months, whereas the remaining 87% had cleared their parasitaemia after 1 month [10]. Finally, in a recent cohort study in Vietnam, nearly 10% of infected individuals carried parasites for 4 months or longer [11]. These studies vary in design such that a mean duration is hard to estimate, however, the data demonstrate that chronic carriage occurs in a wide range of endemicities, although its frequency and duration is likely context-specific.

Identifying the factors that moderate parasite growth to make an infection asymptomatic, (and untreated [3]) rather than symptomatic, is necessary to better understand the likelihood

of transmission from these infections. This will allow an assessment of whether specific individuals with asymptomatic infections need to be targeted and, if so, how this might be done, for example by enhanced coverage efforts or more sensitive infection detection tools targeted at those individuals who have a higher probability of being chronically infected.

In Sections 2 and 3, we consider factors that influence the establishment of asymptomatic infections and their parasite and gametocyte carriage levels. Specifically, we discuss how different factors might relate to the different archetypes of parasite dynamics described in **Figure 1**: chronic infections with fluctuating patent and sub-patent levels; chronic sub-patent infections; clinical episodes with short incubation period; clinical episodes with long incubation period; and short asymptomatic infections. Additionally, we discuss how blood sampling for parasite detection can influence estimates of prevalence of sub-microscopic infections. In Section 4, we use malariotherapy data and validated mathematical models to assess the benefits of targeting the asymptomatic reservoir of parasites.

2. Human factors influencing the duration of infection

The development of asymptomatic and low-density infections is intimately related to an individual's tolerance to parasites [12]. Several host characteristics have been linked to differential clinical expression of malaria infection, as well as to modulation of parasite levels, including genetic factors [13], acquired immunity [14, 15], co-infections with non-falciparum malaria parasites [16], iron status [17], among others. In this section, we discuss two widely prevalent factors that are likely to influence the frequencies of asymptomatic and low-density infections in various settings: haemoglobinopathies, which are genetically determined and consequently whose effects on parasites might remain unchanged with decreases in transmission, and acquired immunity, that varies with cumulative exposure to parasites and will wane as exposure drops or ceases.

Both haemoglobin S (HbS) and haemoglobin C (HbC) mutations are protective against clinical malaria [18], and evidence from a longitudinal study performed in Uganda [19] suggests that HbS reduces progression of infection to disease. This protective effect suggests that these mutations are associated with chronic infections or clinical episodes with delayed onset (**Figure 1**; light blue, green or orange line). Data also suggest that the parasite densities observed in individuals with sickle cell trait [20] are lower compared to densities in HbAA individuals and thus presumably more likely to be sub-patent and not necessarily detected in population surveys. Given the high prevalence of haemoglobinopathies in many malaria endemic countries, particularly those in Africa for HbS [21, 22], and the potential for carriage of sub-patent infections, the contribution of this group of individuals to the transmission reservoir should be considered. Determining how often parasite densities in heterozygous individuals are below the lower limit of detection of standard diagnostics would be informative. This is particularly relevant as haemoglobinopathies have been associated with increased gametocyte positivity and duration of gametocyte carriage [19, 23–25], which could amplify the infectivity of asymptomatic individuals with these mutations. Unlike naturally acquired immunity, these genetic traits will persist for several generations even after reductions in

malaria transmission and they have the potential to influence transmission phenotypes in the whole spectrum of endemicities, including in areas approaching malaria elimination.

Another cause of variation in the risk of symptoms and in parasite burden is acquired immunity against asexual blood stage parasites, which develops with cumulative exposure and consequently age. Asymptomatic adults have lower parasitaemias compared to children [4], and a higher proportion of their infections are sub-patent [26]. Adults are also less likely to develop symptoms, especially in highly endemic areas, and when they do, the parasite densities associated with fever are on average lower than the corresponding densities in children [27]. On the other hand, estimates based on clone-specific carriage show that in highly endemic areas, asymptomatic infection duration is higher in schoolchildren compared to adults [8] though the differential detectability of clones may affect observations. Together, these studies suggest that infections in adults most commonly correspond to the archetype parasite dynamics of short duration asymptomatic infection or chronic infections with sub-patent carriage (**Figure 1**, yellow and orange lines). In settings where transmission intensity approaches elimination levels, depending on how fast transmission decreases, acquired immunity in adults would still be effective against parasitaemia and symptoms, while in young children with limited cumulative exposure to falciparum parasites, this might not be the case. In this scenario, the epidemiological differences between these demographic groups could be enhanced. Interestingly, in an area of Papua New Guinea with recent declines in transmission, reductions in parasite prevalence have been associated with an increase in the proportion of infections that are sub-patent [28], indicative, perhaps of persisting immune responses that control parasitaemia in a setting where the incidence of super infection is reduced.

Short-term changes in immunity might also be relevant. For example, recent malaria infection might modulate immune responses to subsequent infections [29], which suggests that dynamics of parasitaemia might differ at the start *versus* peak of transmission season, and so might the proportion of infected individuals that remain asymptomatic. Indeed, several epidemiological studies using different methodologies have shown that the risk of clinical symptoms during infection varies during a transmission season: Mueller and colleagues [30] observed that after adjusting for the incidence of new infections, defined by molecular identification of individual clones, the risk of clinical malaria per infection was higher at the beginning of the transmission season. In Mali, the ratio of asymptomatic to symptomatic infections was higher during the low transmission season compared to the rainy season [31]. Whether this is due to modulation of host immune responses or to changes in parasite phenotype, it may result in longer infections at the end of the transmission season that would be advantageous for falciparum parasite populations to persist over the often long dry seasons. Furthermore, short-term immunological changes might also directly affect infectivity of asymptomatic infections: in Burkina Faso, experimental mosquito infections indicate that short-lived immunity that reduces transmission is boosted after season-long exposure to parasites [32].

Of note, high-density infections in the absence of symptoms have been described, in particular in young children [3, 33, 34]. The relevance of these infections to transmission is unknown, although it could be anticipated that unless commitment to sexual development is reduced, these infections will produce high numbers of gametocytes and be potentially highly infectious.

3. Parasite factors associated with infection duration

After inoculation of sporozoites and the subsequent release of merozoites from the liver, there is a period of time when parasites are present in the blood at concentrations undetectable by conventional diagnostics. In many individuals, parasites then multiply to reach detectable densities, however in other individuals, parasites may remain at low densities that are undetectable. Human challenge studies on non-immune individuals in which parasites are monitored both by molecular methods and by microscopy have estimated that infections are detectable by PCR an average of 3.7 days (range 2–4 days) [35] or 3.1 days (range 0–4) [36] before being detectable by microscopy. Controlled human infections also suggest that the parasite stages that precede blood invasion might influence asexual blood stage dynamics. For example, Churcher and colleagues [37] observed that the inoculum size (the estimated number of sporozoites injected by infected *Anopheles* mosquitoes) influences the time it takes for infections to become patent: individuals receiving five bites from mosquitoes with more than 1000 sporozoites have detectable parasitaemia at least 2 days earlier than those volunteers infected by mosquitoes with 11–101 sporozoites. Quantification of sporozoite counts in wild-caught mosquitoes is necessary to confirm the relevance of this finding in natural settings. In Papua New Guinea, it was estimated that infected malaria vectors had on average (geometric mean) 4000 sporozoites [38], which is of the same order of magnitude as sporozoite counts in mosquitoes used in controlled infections.

Microscopy has limited sensitivity to quantify low parasite densities and this will affect its utility for studying any chronicity in infection dynamics. Histidine rich protein 2 (HRP-2), a protein the parasite secretes in the plasma, is considered to be a more accurate measure of total falciparum parasite burden [39], however, this measure does not distinguish between monoclonal and multiclonal infections. Molecular tools are more sensitive and allow discrimination of different parasite genotypes. They have been used to assess the effects of super-infection and exposure to different parasite clones on clinical malaria risk. A study that involved daily blood sampling of children with initially asymptomatic infections [40] suggests that development of symptoms is often associated with appearance of a new parasite strain in the blood and increases in parasite levels. Correspondingly, recent data from Papua New Guinea [16] showed that incidence of infections by new clones correlates with clinical malaria risk. This indicates that clinical malaria is often associated with new infection, presumably by a parasite clone with a previously unencountered antigenic profile.

Consistent with this, genetic analysis of malaria parasite populations in Zambia [41] found that in some settings individuals with symptomatic infections had different parasite strains compared to asymptomatic individuals. One hypothesis for this is that symptomatic infections originated from imported or recently introduced strains and that immunity to these strains is insufficient. This indicates that the rate of importation may play a role in the proportion of infections that are asymptomatic and symptomatic, especially in areas approaching elimination. An infection with a clone to which immunity has been acquired might lead to infections with shorter duration (e.g., **Figure 1**, yellow line).

3.1. Gametocytes in asymptomatic infections

Gametocytes derive from a small percentage of asexual parasites that commit to sexual development; therefore, asymptomatic infections with low asexual levels may also have low

gametocyte densities. Data from epidemiological studies confirm that most asymptomatic infections with patent or sub-patent asexual stage parasite levels have sub-patent gametocytaemia [42], only detectable by RNA-based molecular methods. However, a few asymptomatic individuals with low-density infections have relatively high gametocyte densities, which could be related to symptomless fluctuations in parasitaemia that result in higher gametocytaemia a few days later. The rate of commitment of asexually replicating parasites to sexual development is another factor that influences gametocyte levels in malaria infections. Adults, who on average carry lower asexual stage parasite densities, have a higher sexual to asexual density ratio [43]. This could be related to an unequal increase in clearance rates of asexual and sexual parasites with age, or potentially to changes in commitment to gametocytogenesis [43]. Consistent with the latter, parasite investment in transmission stages has been shown to vary in areas with different transmission levels, being higher in settings with lower endemicity. Recent data suggest that parasite variations in commitment to gametocytes are epigenetically imprinted and higher in parasites in lower endemicity settings [44].

As articulated above, the importance of asymptomatic infections for malaria transmission does not lie in their average sexual stage parasite densities but in the durations of gametocyte carriage and infectiousness over time. A mathematical model [45] fitted to both asexual parasite and gametocyte malariotherapy data estimated infectivity over the course of an infection based on gametocyte density data. This analysis concluded that the majority of infectivity was usually concentrated early in infection, although some patients were significantly infectious later on. However, in this model, it was assumed relatively low infectivity of low gametocyte densities compared with other analyses [46]. While these data are extremely detailed, it is not known whether these dynamics are similar to those in naturally infected individuals who have immunity. Furthermore, specific *P. falciparum* strains were selected for malariotherapy because they were 'benign' and may not exhibit the same behaviour in terms of parasite multiplication rates and gametocyte commitment as parasites in endemic areas.

Although asymptomatic infections do not prompt treatment-seeking behaviour, during community mass treatment campaigns that involve treatment regardless of symptomatology (e.g., mass drug administration (MDA) or mass screening and treatment), these infections are cleared with antimalarials. In a meta-analysis of trials with gametocyte density data [47], the combinations artesunate-mefloquine and artemether-lumefantrine were more effective in preventing the appearance of gametocytes and in clearing existing sexual stage parasites compared to dihydroartemisinin-piperaquine. The choice of drugs to be used during control interventions thus may be important to limit residual transmission from these infections. In Section 4, we discuss the impact of different interventions that target asymptomatic and symptomatic infections.

3.2. Underestimations of parasitaemia linked to sampling

Two variables linked to blood sampling for parasite detection can influence prevalence and density estimates: volume and timing. Even sensitive molecular assays will not detect low parasite densities in samples if nucleic acids are isolated from small blood volumes. High-volume PCR has been used in epidemiological studies in Southeast Asia to circumvent

this problem and less than 30% of all falciparum infections are estimated to be missed by this method [48]. The timing of blood sampling in parasitological surveys might also affect parasite detection and quantification because asexual falciparum parasites do not circulate continuously; sequestration of falciparum schizonts starts 12-18 hours after merozoite invasion and during this period they might not be detectable. An intensive longitudinal study in Tanzania showed that periodic changes in parasite densities are common. The periodicity of clone-specific detectability indicates that in natural infections, synchronised sequestration of clonal parasite populations occurs [49]. A study [50] that collected samples on two consecutive days found a prevalence disparity of approximately 25% between the two samples. Periodic changes in parasite levels could have a direct impact on the selection of diagnostics, for example by favouring assays that detect more persistent markers, such as HRP-2.

The detection of either asexual or sexual stage parasites is sufficient to establish the diagnosis of infection. Although gametocytes are not known to periodically sequester, there is evidence of periodic variation in gametocyte levels [51] in peripheral blood. For several decades now [52], accumulation of mature gametocytes in the skin [53] has been hypothesised as a possible mechanism of transmission enhancement. If confirmed, this would imply that subpatent gametocytaemias in peripheral blood might be associated with higher-than-expected infectivity.

4. Contribution of low-density asymptomatic infections to transmission

In the previous sections, we discussed factors influencing the duration and average density of individual infections. In this section, our goal is to understand the significance and contribution of low-density asymptomatic infections to local transmission. This question is particularly important in areas where control efforts have pushed transmission towards near elimination levels – in this case it has been hypothesised that chronic low density asymptomatic infections could maintain local transmission.

4.1. Should we detect and treat low-density asymptomatic infections?

Since identifying the reservoir of low-density infections, there has been interest in developing more sensitive rapid diagnostics in order to detect and treat these infections. However, the benefit of treating such infections, both at the individual level and in terms of preventing onward transmission to others, remains unclear. The impact of treating a low-density infection depends not only on its current infectiousness to mosquitoes, but the future course of infection and infectiousness that is prevented (**Figures 1** and **2**). If low-density infections most commonly represent the tail end of an infection, which will clear rapidly without treatment, then the benefit of treatment would be small (**Figure 2**, yellow bar). However, if such infections commonly become chronic and lead to future periods of higher parasite densities, infectiousness, and possibly also symptoms, the benefit of treating such infections would be greater (**Figure 2**, green and light blue bars). Consistent with this second scenario, longitudinal data

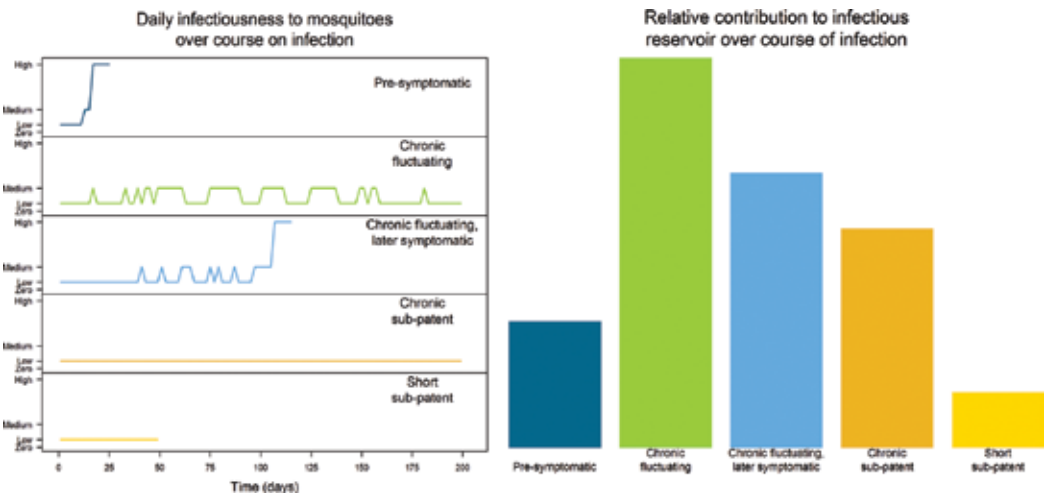


Figure 2. Estimated infectiousness to mosquitoes over course of infection and cumulative relative contribution to onwards infectiousness for each type of infection. The infectiousness of each parasite density trajectory from **Figure 1** is estimated by assuming that individuals with very high parasite densities (associated with being febrile) are three times as infectious as individuals with microscopy-detectable asymptomatic infection, who are then in turn three times as infectious as individuals with sub-microscopic asymptomatic infection [32, 56]. The cumulative infectivity of an individual is simply the area under the infectiousness curve (left panel). This area under the curve of each type of infection is compared in the right panel.

from Vietnam suggest that chronic sub-patent infections can lead to high parasitaemias, 5–6 orders of magnitude higher [11].

The relative proportions of low density infections which go on to rapidly clear *versus* those which become higher density infections are unknown and likely depends on many of the factors highlighted in Sections 2 and 3, such as age, immunity and host genetics. Studying variations in detectability over the course of a single naturally acquired infection is difficult for a few reasons: (i) super- and co-infections: in high transmission settings, most individuals are infected with more than one parasite clone [54] and standard techniques do not indicate the density of each parasite genotype (therefore the density of older *versus* newer infections cannot be distinguished); (ii) even using molecular methods, parasite densities often fluctuate below detection limits before the end of an infection and it is difficult to distinguish this from clearance of infection; (iii) long follow up is needed: even in endemic areas where individuals have immunity, specific parasite genotypes have been shown to persist for more than 6 months [55]. Here, we use a simple modelling framework to explore how the duration and infectiousness of an infection affect the impact of different intervention strategies.

4.2. Model framework

Four archetypal parasite density trajectories are identified from the malaria therapy data [57, 58] to represent broadly four potential outcomes of a new infection (**Figure 3**): (1) initially symptomatic before becoming asymptomatic and fluctuating between patent and sub-patent levels for a long time (~300 days); (2) initially symptomatic before becoming asymptomatic and fluctuating

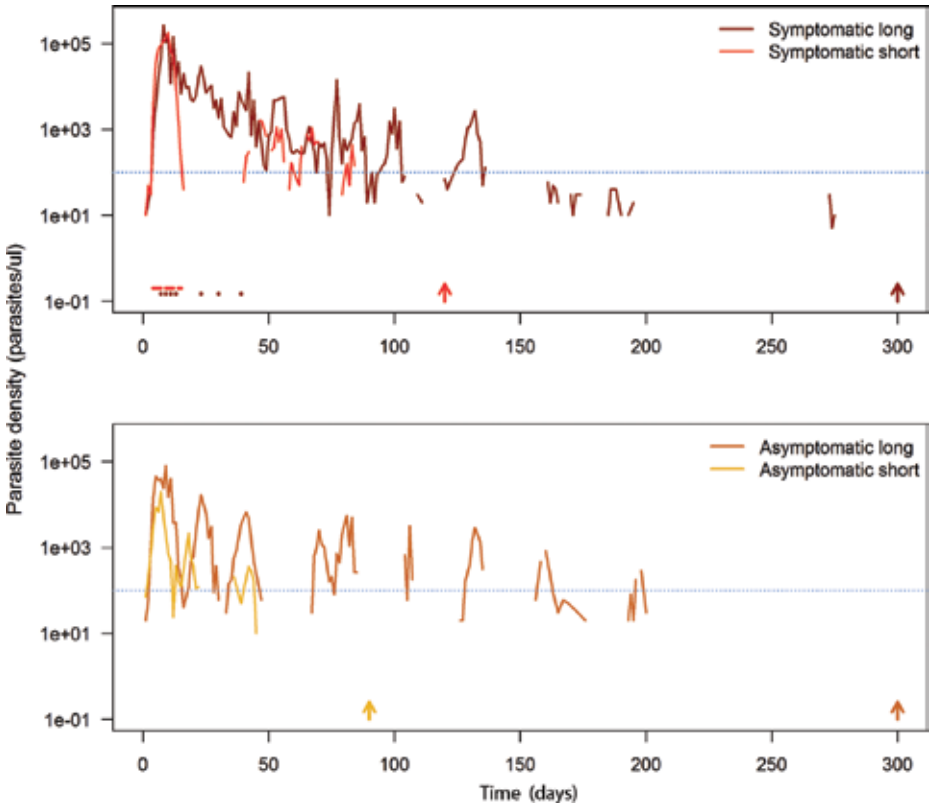


Figure 3. Parasite density trajectories from four infected individuals. The arrows represent the estimated time to clear infection (assuming a period of sub-patent infection after the last patent day of infection). The upper panel shows two symptomatic patients (red points indicate the days on which the patients were febrile) and the lower panel shows asymptomatic patients. The horizontal dotted line indicates the limit of detection of field microscopy (100 parasites/μl).

between patent and sub-patent levels for a short time (~120 days); (3) always asymptomatic and fluctuating between patent and sub-patent densities for a long time (~300 days); and (4) always asymptomatic and fluctuating between patent and sub-patent for a short time (~90 days). Note how these relate to the hypothesised profiles in **Figure 1**.

An age-structured population of individuals is simulated whereby individuals have a daily probability of acquiring a new infection. Upon being infected, an individual's probability of developing symptoms is based on their age and the intensity of transmission (fitted estimates taken from [56]) (**Table 1**). In a single simulation, infections are assumed to be either all long (300 days) or all short (120 or 90 days). Infected individuals will follow one of the parasite density trajectories shown in **Figure 3** unless they are treated or re-infected. Febrile individuals have a 50% probability of receiving treatment. Treated individuals are assumed to clear their asexual parasites after being febrile for 3 days, they then become non-infectious after 6 days. These individuals are also assumed to be protected from reinfection for 14 days after treatment.

The model is simulated with either high transmission (20% slide prevalence) or low transmission (5% slide prevalence) and the daily probability of infection is fitted to achieve these prevalence levels. Infected individuals can be reinfected (unless they received treatment in the

	Age range (in years)		
	0–5	**5–15**	**15+**
High transmission (20% slide prevalence)	66%	52%	38%
Low transmission (5% slide prevalence)	78%	70%	59%

Table 1. Probability of developing symptoms upon being successfully inoculated (estimates from [56]).

last 14 days) at any time and will start at the beginning of a new parasite density trajectory selected based on their age-specific probability of developing symptoms. The impact of two interventions is simulated: increasing treatment coverage among febrile individuals to 90% or delivering a single round of MDA to a random 80% of the population. The effect of each intervention is assessed by calculating the percentage reduction in the combined onwards infectiousness of the whole population in the following year, which depends on whether they are in a patent and symptomatic, patent and asymptomatic or sub-patent state. Parasite density is translated to infectiousness according to assumptions described in **Figure 2**. After an intervention the infection risk is reduced proportionally with the reduction in the proportion of the population that are infected to account for the population-level impact of these interventions on transmission.

MDA is predicted to be more effective at reducing the infectious reservoir than increasing treatment coverage among febrile individuals in low transmission settings with both short and long infection durations and high transmission settings with long infection durations only (**Figure 4**). In these scenarios the rebound of infection is slow, meaning over the course of a year, MDA prevents a higher number of infected/infectious days than increasing treatment coverage of febrile individuals. In high transmission settings with a short duration of infection, a higher force of infection is needed to achieve a given prevalence. Therefore, the effect of any intervention is reduced because the population become reinfected quicker. In this scenario, increasing treatment coverage is more effective because it is a sustained intervention. It is important to note that the outcome metric considered here is the reduction in the infectious reservoir—increasing treatment coverage is likely to always have the greatest impact on reducing malaria morbidity and mortality in all transmission scenarios. The model simply illustrates how our uncertainties about the duration of untreated infection affect estimates of intervention impact.

5. Conclusions

Since asymptomatically infected individuals do not actively seek antimalarial treatment, their infections may last longer than symptomatic episodes. In this chapter, we discussed human and parasite factors that influence the dynamics of parasitaemia and the duration of gametocyte circulation in these infections. These factors result in a range of infection profiles, the relative combinations of which in a population will define not only the composition of the infectious reservoir but the likelihood of success of intervention measures. For example, our calculations suggest that MDA is most effective if infections have long durations. In a high transmission setting, MDA might have been expected to be more effective than increasing

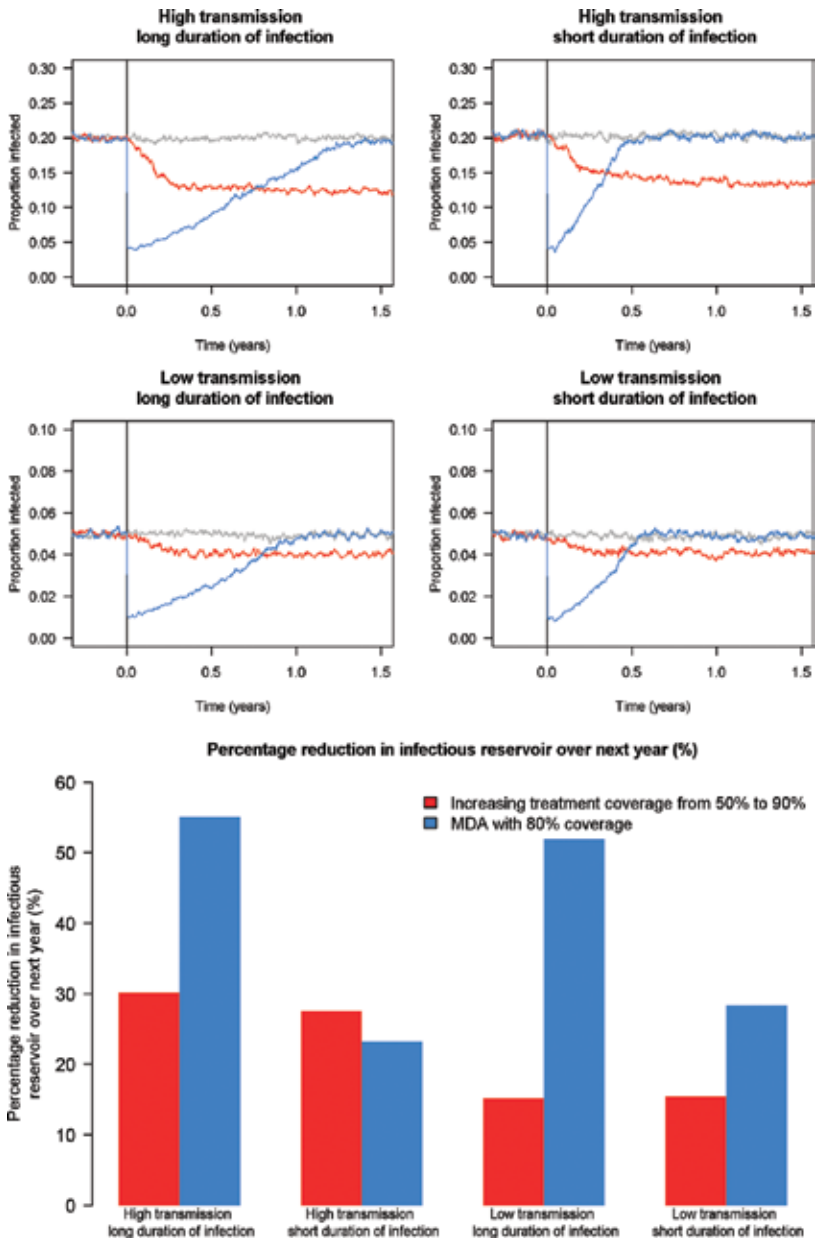


Figure 4. Simulated impact of increasing treatment coverage or mass drug administration (MDA) on the proportion of mosquitoes infected by a population: influence of transmission setting and infection duration. The grey lines represent continuing 50% treatment coverage and no MDA, and the blue and red lines as shown in the legend.

treatment coverage, because higher immunity reduces the probability of developing symptoms and the proportion of infections getting treated. However, when transmission is high, the reduction in prevalence after an MDA is temporary, due to the drug half-life and imperfect coverage levels, and individuals are likely to become reinfected quickly, therefore in the

absence of repeated rounds of MDA, increasing treatment coverage may in fact be more effective in the long term (**Figure 4**). However, the model assumes no seasonality, when in reality many malaria endemic regions transmission is highly seasonal; this could underestimate the impact of MDA. As discussed above, seasonal changes in infection duration represent another aspect of the epidemiology of asymptomatic infections that could be explored to target interventions. Indeed, where transmission is seasonal, infections persisting during the dry season correspond to long-term asymptomatic carriage since incidence of new infections is thought to be negligible. This means that during this period, infections are likely to be missed by passive surveillance, while active approaches, such as MDA, might be more efficacious.

Determining the optimal control strategy, and moreover, whether asymptomatic/sub-patent infections actually need to be identified and treated, will require careful analysis of local epidemiological data. The three key metrics that need to be determined are: (1) the proportion of individuals that develop symptoms and seek treatment, (2) the distribution of durations of asymptomatic infections, and (3) the relative infectivity of different infections. These factors are in turn driven by the complex interplay of host immunological factors, such as strain-specific immunity, intrinsic parasite growth factors and population characteristics (e.g. prevalence of HbAA *versus* HbAS, variation in demographic risk within a community). The relative high prevalence of asymptomatic and low-density infections in areas with low transmission and high treatment coverage might indicate that either these infections are contributing towards transmission and enabling malaria to persist or that they reflect the tail end of infections with transmission maintained by the few highly infectious symptomatic cases. This will vary in different settings and whilst the rapid identification and treatment of symptomatic malaria infections remains key to all control approaches, a better understanding of the nature of asymptomatic infections will determine if and what additional measures are required for malaria elimination.

Acknowledgements

CD and BG would like to acknowledge funding from the Bill & Melinda Gates Foundation (OPP1034789 & OPP1173572). LO is funded by a UK Royal Society Dorothy Hodgkin Fellowship, and also acknowledges grants from the Bill & Melinda Gates Foundation and Medicines for Malaria Venture. HS is supported by an Imperial College junior research fellowship.

List of abbreviations

HbAA	haemoglobin A (homozygous)
HbAS	haemoglobin AS (heterozygous)
HbC	haemoglobin C
HbS	haemoglobin S

HRP-2	histidine-rich protein 2
MDA	mass drug administration
msp-2	merozoite surface protein 2
NAAT	nucleic acid amplification techniques
PCR	polymerase chain reaction
RDT	rapid diagnostic test
RNA	ribonucleic acid

Author details

Chris Drakeley[1]*, Bronner Gonçalves[1], Lucy Okell[2] and Hannah Slater[2]

*Address all correspondence to: chris.drakeley@lshtm.ac.uk

1 Department of Immunology and Infection, London School of Hygiene and Tropical Medicine, UK

2 Department of Infectious Disease Epidemiology, Imperial College London, UK

References

[1] Okell LC, Ghani AC, Lyons E, Drakeley CJ. Submicroscopic infection in *Plasmodium falciparum*-endemic populations: A systematic review and meta-analysis. The Journal of infectious Diseases. 2009;**200**:1509-1517. DOI: 10.1086/644781

[2] Wu L, van den Hoogen LL, Slater H, Walker PG, Ghani AC, Drakeley CJ, et al. Comparison of diagnostics for the detection of asymptomatic *Plasmodium falciparum* infections to inform control and elimination strategies. Nature. 2015;**528**:S86-S93. DOI: 10.1038/nature16039

[3] Cox MJ, Kum DE, Tavul L, Narara A, Raiko A, Baisor M, et al. Dynamics of malaria parasitaemia associated with febrile illness in children from a rural area of Madang, Papua New Guinea. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1994;**88**:191-197

[4] Rogier C, Commenges D, Trape JF. Evidence for an age-dependent pyrogenic threshold of *Plasmodium falciparum* parasitemia in highly endemic populations. The American Journal of Tropical Medicine and Hygiene. 1996;**54**:613-619

[5] Muller I, Genton B, Rare L, Kiniboro B, Kastens W, Zimmerman P, et al. Three different *Plasmodium* species show similar patterns of clinical tolerance of malaria infection. Malaria Journal. 2009;**8**:158. DOI: 10.1186/1475-2875-8-158

[6] Vantaux A, Samreth R, Piv EP, Khim N, Kim S, Berne L, et al. Contribution to malaria transmission of symptomatic and asymptomatic parasite carriers in Cambodia. The Journal of Infectious Diseases. 2018;**217**(10):1561-1568. DOI: 10.1093/infdis/jiy060

[7] Missinou MA, Lell B, Kremsner PG. Uncommon asymptomatic *Plasmodium falciparum* infections in Gabonese children. Clinical Infectious Diseases. 2003;**36**:1198-1202. DOI: 10.1086/374555

[8] Felger I, Maire M, Bretscher MT, Falk N, Tiaden A, Sama W, et al. The dynamics of natural *Plasmodium falciparum* infections. PLoS One. 2012;**7**:e45542. DOI: 10.1371/journal.pone.0045542

[9] Landier J, Kajeechiwa L, Thwin MM, Parker DM, Chaumeau V, Wiladphaingern J, et al. Safety and effectiveness of mass drug administration to accelerate elimination of artemisinin-resistant falciparum malaria: A pilot trial in four villages of eastern Myanmar. Wellcome Open Research. 2017;**2**:81. DOI: 10.12688/wellcomeopenres.12240.1

[10] Tripura R, Peto TJ, Chalk J, Lee SJ, Sirithiranont P, Nguon C, et al. Persistent *Plasmodium falciparum* and *Plasmodium vivax* infections in a western Cambodian population: Implications for prevention, treatment and elimination strategies. Malaria Journal. 2016;**15**:181. DOI: 10.1186/s12936-016-1224-7

[11] Nguyen TN, von Seidlein L, Nguyen TV, Truong PN, Hung SD, Pham HT, et al. The persistence and oscillations of submicroscopic *Plasmodium falciparum* and *Plasmodium vivax* infections over time in Vietnam: An open cohort study. The Lancet Infectious Diseases. 2018;**18**(5):565-572. DOI: 10.1016/S1473-3099(18)30046-X

[12] Schneider DS, Ayres JS. Two ways to survive infection: What resistance and tolerance can teach us about treating infectious diseases. Nature Reviews. Immunology. 2008;**8**:889-895. DOI: 10.1038/nri2432

[13] Kwiatkowski DP, Luoni G. Host genetic factors in resistance and susceptibility to malaria. Parassitologia. 2006;**48**:450-467

[14] Carneiro I, Roca-Feltrer A, Griffin JT, Smith L, Tanner M, Schellenberg JA, et al. Age-patterns of malaria vary with severity, transmission intensity and seasonality in sub-Saharan Africa: A systematic review and pooled analysis. PLoS One. 2010;**5**:e8988. DOI: 10.1371/journal.pone.0008988

[15] Doolan DL, Dobano C, Baird JK. Acquired immunity to malaria. Clinical Microbiology Reviews. 2009;**22**:13-36. DOI: 10.1128/CMR.00025-08

[16] Hofmann NE, Karl S, Wampfler R, Kiniboro B, Teliki A, Iga J, et al. The complex relationship of exposure to new *Plasmodium* infections and incidence of clinical malaria in Papua New Guinea. eLife. 2017;**6**:e23708. DOI: 10.7554/eLife.23708

[17] Gwamaka M, Kurtis JD, Sorensen BE, Holte S, Morrison R, Mutabingwa TK, et al. Iron deficiency protects against severe *Plasmodium falciparum* malaria and death in young children. Clinical Infectious Diseases. 2012;**54**:1137-1144. DOI: 10.1093/cid/cis010

[18] Taylor SM, Parobek CM, Fairhurst RM. Haemoglobinopathies and the clinical epidemiology of malaria: A systematic review and meta-analysis. The Lancet Infectious Diseases. 2012;**12**:457-468. DOI: 10.1016/S1473-3099(12)70055-5

[19] Gong L, Maiteki-Sebuguzi C, Rosenthal PJ, Hubbard AE, Drakeley CJ, Dorsey G, et al. Evidence for both innate and acquired mechanisms of protection from *Plasmodium falciparum* in children with sickle cell trait. Blood. 2012;**119**:3808-3814. DOI: 10.1182/blood-2011-08-371062

[20] Williams TN, Mwangi TW, Wambua S, Alexander ND, Kortok M, Snow RW, et al. Sickle cell trait and the risk of *Plasmodium falciparum* malaria and other childhood diseases. The Journal of Infectious Diseases. 2005;**192**:178-186. DOI: 10.1086/430744

[21] Piel FB, Howes RE, Patil AP, Nyangiri OA, Gething PW, Bhatt S, et al. The distribution of haemoglobin C and its prevalence in newborns in Africa. Scientific Reports. 2013;**3**:1671. DOI: 10.1038/srep01671

[22] Piel FB, Patil AP, Howes RE, Nyangiri OA, Gething PW, Williams TN, et al. Global distribution of the sickle cell gene and geographical confirmation of the malaria hypothesis. Nature Communications. 2010;**1**:104. DOI: 10.1038/ncomms1104

[23] Ringelhann B, Hathorn MK, Jilly P, Grant F, Parniczky G. A new look at the protection of hemoglobin AS and AC genotypes against *Plasmodium falciparum* infection: A census tract approach. American Journal of Human Genetics. 1976;**28**:270-279

[24] Gouagna LC, Bancone G, Yao F, Yameogo B, Dabire KR, Costantini C, et al. Genetic variation in human HBB is associated with *Plasmodium falciparum* transmission. Nature Genetics. 2010;**42**:328-331. DOI: 10.1038/ng.554

[25] Goncalves BP, Sagara I, Coulibaly M, Wu Y, Assadou MH, Guindo A, et al. Hemoglobin variants shape the distribution of malaria parasites in human populations and their transmission potential. Scientific Reports. 2017;**7**:14267. DOI: 10.1038/s41598-017-14627-y

[26] Rek J, Katrak S, Obasi H, Nayebare P, Katureebe A, Kakande E, et al. Characterizing microscopic and submicroscopic malaria parasitaemia at three sites with varied transmission intensity in Uganda. Malaria Journal. 2016;**15**:470. DOI: 10.1186/s12936-016-1519-8

[27] Miller MJ. Observations on the natural history of malaria in the semi-resistant West African. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1958;**52**: 152-168

[28] Koepfli C, Ome-Kaius M, Jally S, Malau E, Maripal S, Ginny J, et al. Sustained malaria control over an eight-year period in Papua New Guinea: The challenge of low-density asymptomatic infections. The Journal of Infectious Diseases. 2017;**216**(11):1434-1443. DOI: 10.1093/infdis/jix507

[29] Portugal S, Moebius J, Skinner J, Doumbo S, Doumtabe D, Kone Y, et al. Exposure-dependent control of malaria-induced inflammation in children. PLoS Pathogens. 2014;**10**:e1004079. DOI: 10.1371/journal.ppat.1004079

[30] Mueller I, Schoepflin S, Smith TA, Benton KL, Bretscher MT, Lin E, et al. Force of infection is key to understanding the epidemiology of *Plasmodium falciparum* malaria in Papua New Guinean children. Proceedings of the National Academy of Sciences of the United States of America. 2012;**109**:10030-10035. DOI: 10.1073/pnas.1200841109

[31] Coulibaly D, Travassos MA, Tolo Y, Laurens MB, Kone AK, Traore K, et al. Spatio-temporal dynamics of asymptomatic malaria: Bridging the gap between annual malaria resurgences in a Sahelian environment. The American Journal of Tropical Medicine and Hygiene. 2017;**97**:1761-1769. DOI: 10.4269/ajtmh.17-0074

[32] Ouedraogo AL, Goncalves BP, Gneme A, Wenger EA, Guelbeogo MW, Ouedraogo A, et al. Dynamics of the human infectious reservoir for malaria determined by mosquito feeding assays and ultrasensitive malaria diagnosis in Burkina Faso. The Journal of Infectious Diseases. 2016;**213**:90-99. DOI: 10.1093/infdis/jiv370

[33] Rogier C, Tall A, Diagne N, Fontenille D, Spiegel A, Trape JF. *Plasmodium falciparum* clinical malaria: lessons from longitudinal studies in Senegal. Parassitologia. 1999;**41**:255-259

[34] Goncalves BP, Huang CY, Morrison R, Holte S, Kabyemela E, Prevots DR, et al. Parasite burden and severity of malaria in Tanzanian children. The New England Journal of Medicine. 2014;**370**:1799-1808. DOI: 10.1056/NEJMoa1303944

[35] Murphy SC, Prentice JL, Williamson K, Wallis CK, Fang FC, Fried M, et al. Real-time quantitative reverse transcription PCR for monitoring of blood-stage *Plasmodium falciparum* infections in malaria human challenge trials. The American Journal of Tropical Medicine and Hygiene. 2012;**86**:383-394. DOI: 10.4269/ajtmh.2012.10-0658

[36] Lyke KE, Laurens M, Adams M, Billingsley PF, Richman A, Loyevsky M, et al. *Plasmodium falciparum* malaria challenge by the bite of aseptic *Anopheles stephensi* mosquitoes: Results of a randomized infectivity trial. PLoS One. 2010;**5**:e13490. DOI: 10.1371/journal.pone.0013490

[37] Churcher TS, Sinden RE, Edwards NJ, Poulton ID, Rampling TW, Brock PM, et al. Probability of transmission of malaria from mosquito to human is regulated by mosquito parasite density in naive and vaccinated hosts. PLoS Pathogens. 2017;**13**:e1006108. DOI: 10.1371/journal.ppat.1006108

[38] Burkot TR, Graves PM, Cattan JA, Wirtz RA, Gibson FD. The efficiency of sporozoite transmission in the human malarias, *Plasmodium falciparum* and *P. vivax*. Bulletin of the World Health Organization. 1987;**65**:375-380

[39] Dondorp AM, Desakorn V, Pongtavornpinyo W, Sahassananda D, Silamut K, Chotivanich K, et al. Estimation of the total parasite biomass in acute falciparum malaria from plasma PfHRP2. PLoS Medicine. 2005;**2**:e204. DOI: 10.1371/journal.pmed.0020204

[40] Kun JF, Missinou MA, Lell B, Sovric M, Knoop H, Bojowald B, et al. New emerging *Plasmodium falciparum* genotypes in children during the transition phase from asymptomatic parasitemia to malaria. The American Journal of Tropical Medicine and Hygiene. 2002;**66**:653-658

[41] Searle KM, Katowa B, Kobayashi T, Siame MNS, Mharakurwa S, Carpi G, et al. Distinct parasite populations infect individuals identified through passive and active case detection in a region of declining malaria transmission in southern Zambia. Malaria Journal. 2017;**16**:154. DOI: 10.1186/s12936-017-1810-3

[42] Goncalves BP, Kapulu MC, Sawa P, Guelbeogo WM, Tiono AB, Grignard L, et al. Examining the human infectious reservoir for *Plasmodium falciparum* malaria in areas of differing transmission intensity. Nature Communications. 2017;**8**:1133. DOI: 10.1038/s41467-017-01270-4

[43] Ouedraogo AL, Bousema T, de Vlas SJ, Cuzin-Ouattara N, Verhave JP, Drakeley C, et al. The plasticity of *Plasmodium falciparum* gametocytaemia in relation to age in Burkina Faso. Malaria Journal. 2010;**9**:281. DOI: 10.1186/1475-2875-9-281

[44] Rono MK, Nyonda MA, Simam JJ, Ngoi JM, Mok S, Kortok MM, et al. Adaptation of *Plasmodium falciparum* to its transmission environment. Nature Ecology & Evolution. 2017;**2**:377-387. DOI: 10.1038/s41559-017-0419-9

[45] Johnston GL, Smith DL, Fidock DA. Malaria's missing number: Calculating the human component of R0 by a within-host mechanistic model of *Plasmodium falciparum* infection and transmission. PLoS Computational Biology. 2013;**9**:e1003025. DOI: 10.1371/journal.pcbi.1003025

[46] Churcher TS, Bousema T, Walker M, Drakeley C, Schneider P, Ouedraogo AL, et al. Predicting mosquito infection from *Plasmodium falciparum* gametocyte density and estimating the reservoir of infection. eLife. 2013;**2**:e00626. DOI: 10.7554/eLife.00626

[47] WWARN Gametocyte Study Group. Gametocyte carriage in uncomplicated *Plasmodium falciparum* malaria following treatment with artemisinin combination therapy: A systematic review and meta-analysis of individual patient data. BMC Medicine. 2016;**14**:79. DOI: 10.1186/s12916-016-0621-7

[48] Imwong M, Stepniewska K, Tripura R, Peto TJ, Lwin KM, Vihokhern B, et al. Numerical distributions of parasite densities during asymptomatic malaria. The Journal of Infectious Diseases. 2016;**213**:1322-1329. DOI: 10.1093/infdis/jiv596

[49] Farnert A, Snounou G, Rooth I, Bjorkman A. Daily dynamics of *Plasmodium falciparum* subpopulations in asymptomatic children in a holoendemic area. The American Journal of Tropical Medicine and Hygiene. 1997;**56**:538-547

[50] Koepfli C, Schoepflin S, Bretscher M, Lin E, Kiniboro B, Zimmerman PA, et al. How much remains undetected? Probability of molecular detection of human Plasmodia in the field. PLoS One. 2011;**6**:e19010. DOI: 10.1371/journal.pone.0019010

[51] Magesa SM, Mdira YK, Akida JA, Bygbjerg IC, Jakobsen PH. Observations on the periodicity of *Plasmodium falciparum* gametocytes in natural human infections. Acta Tropica. 2000;**76**:239-246

[52] Chardome M, Janssen PJ. Inquiry on malarial incidence by the dermal method in the region of Lubilash, Belgian Congo. Annales de la Société Belge de Médecine Tropicale (1920). 1952;**32**:209-211

[53] Nixon CP. *Plasmodium falciparum* gametocyte transit through the cutaneous microvasculature: A new target for malaria transmission blocking vaccines? Human Vaccines & Immunotherapeutics. 2016;**12**:3189-3195. DOI: 10.1080/21645515.2016.1183076

[54] Koepfli C, Mueller I. Malaria epidemiology at the clone level. Trends in Parasitology. 2017;**33**:974-985. DOI: 10.1016/j.pt.2017.08.013

[55] Falk N, Maire N, Sama W, Owusu-Agyei S, Smith T, Beck HP, et al. Comparison of PCR-RFLP and Genescan-based genotyping for analyzing infection dynamics of *Plasmodium falciparum*. The American Journal of Tropical Medicine and Hygiene. 2006;**74**:944-950

[56] Griffin JT, Hollingsworth TD, Reyburn H, Drakeley CJ, Riley EM, Ghani AC. Gradual acquisition of immunity to severe malaria with increasing exposure. Proceedings of the Biological Sciences. 2015;**282**:20142657. DOI: 10.1098/rspb.2014.2657

[57] Collins WE, Jeffery GM. A retrospective examination of sporozoite- and trophozoite-induced infections with *Plasmodium falciparum*: Development of parasitologic and clinical immunity during primary infection. The American Journal of Tropical Medicine and Hygiene. 1999;**61**:4-19

[58] Jeffery GM, Eyles DE. The duration in the human host of infections with a Panama strain of *Plasmodium falciparum*. The American Journal of Tropical Medicine and Hygiene. 1954;**3**:219-224

Chapter 7

Insecticide Resistance in Malaria Vectors: An Update at a Global Scale

Jacob M. Riveron, Magellan Tchouakui,
Leon Mugenzi, Benjamin D. Menze,
Mu-Chun Chiang and Charles S. Wondji

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.78375

Abstract

Malaria remains the deadliest vector-borne disease in the world. With nearly half of the world's population at risk, 216 million people suffered from malaria in 2016, with over 400,000 deaths, mainly in sub-Saharan Africa. Important global efforts have been made to eliminate malaria leading to significant reduction in malaria cases and mortality in Africa by 42% and 66%, respectively. Early diagnosis, improved drug therapies and better health infrastructure are key components, but this extraordinary success is mainly due the use of long-lasting insecticidal nets (LLINs) and indoor residual sprayings (IRS) of insecticide. Unfortunately, the emergence and spread of resistance in mosquito populations against insecticides is jeopardising the effectiveness of the most efficient malaria control interventions. To help establish suitable resistance management strategies, it is vital to better understand the distribution of resistance, its mechanisms and impact on effectiveness of control interventions and malaria transmission. In this chapter, we present the current status of insecticide resistance worldwide in main malaria vectors as well as its impact on malaria transmission, and discuss the molecular mechanisms and future perspectives.

Keywords: malaria, mosquito, insecticide resistance, pyrethroids, bed nets, metabolic resistance, cytochrome P450, knockdown resistance

1. Introduction

In 1993, Steven Spielberg produced 'Jurassic Park', one of the most internationally acclaimed movies at that time. This science-fiction story is based on the cloning of dinosaurs using its DNA from mosquitoes that had been preserved in amber. Although the idea is brilliant, the technical

limitations to get entire genome of dinosaurs from ancient DNA make it impossible [1]. However, the movie is right on one fact that mosquitoes existed at the same time as dinosaurs probably biting them as other animals before evolving to become human biters [2]. But only few mosquitoes have specialised in biting humans (anthropophily), although those that succeeded have caused devastating consequences to mankind. From all diseases that mosquitoes can transmit, malaria has been and still is the one with the greatest health and socioeconomic impact, from the ancient Egypt to present time [3]. For example, malaria has been suggested as one of the causes of the death of the great Tutankhamun [4], one of the Egypt's famous pharaoh. Malaria remains the deadliest vector-borne disease in the world. With nearly half of the world's population at risk, 216 million people suffered from malaria in 2016, with over 400,000 deaths, mainly in sub-Saharan Africa [5]. Recent global efforts have been made to control and eliminate malaria leading to significant reduction in malaria cases and mortality in Africa by 42% and 66%, respectively. Early diagnosis, improved drug therapies and better health infrastructure are key components, but this success is mainly due the use of insecticide-treated nets (ITNs), long-lasting insecticidal nets (LLINs) and indoor residual sprayings (IRS) of insecticide [6]. Unfortunately, the emergence and spread of resistance in mosquito populations against insecticides is jeopardising the effectiveness of the most efficient malaria control interventions [7]. Insecticide resistance is spreading globally. Currently, of 73 countries with ongoing malaria transmission that provided data, 60 countries reported resistance to at least one class of insecticides, while 50 reported resistance to two or more insecticide classes [5]. In this chapter, we present the current status of insecticide resistance worldwide in main malaria vectors, as well as its impact on the epidemiology, and discuss the molecular mechanisms and future perspectives.

2. Insecticide resistance in malaria vectors

The term insecticide resistance is defined as the ability of an insect to withstand the effects of an insecticide by becoming resistant to its toxic effects by means of natural selection and mutations [8]. Repeated exposure to insecticides selects individuals possessing biochemical machineries that can detoxify the insecticides more rapidly or are less sensitive to it [9]. These individual survivors could then pass the resistance mechanism to the successive generations resulting in pest populations that are more resistant.

2.1. Development of the insecticide resistance

Resistance has been observed in more than 500 insect species worldwide [10], including malaria mosquitoes. Mosquitoes are typical R-strategists (animals that reproduce fast and produce a large number of offspring), and can adapt fast to environmental changes. As a consequence of this and the widespread use of insecticides in agriculture and public health, resistance has arisen relatively rapidly in malaria vectors. Insecticide-resistant phenotypes are favoured where mosquitoes are exposed to sub-lethal doses of the insecticide. Under these conditions, resistant individuals have a better chance to survive and reproduce; this means

selection pressure towards resistant populations. Such conditions can result from vector control through insecticide decay (on treated walls or nets) or bad spraying technique. Insecticide resistance was first reported in malaria vectors in the 1950s [11], and resistance to dichlorodiphenyltrichloroethane (DDT) and pyrethroids is now widespread [12]. Resistance is predicted to impair malaria control efforts but evidences from field studies remain limited and potentially conflicting [7]. To date, malaria vectors have developed resistance to the main chemical classes used in public health, i.e., pyrethroids (PYs), organochlorines (OCs), carbamates (CAs) and organophosphates (OPs). Although public health use of insecticide has an impact on the development of resistance in mosquitoes, one key source of resistance in malaria vectors remains the massive use of insecticides for control of agricultural pests [13]. Other chemicals and factors aside from insecticides may create a selective environment, which favours build-up of resistant populations [14].

2.2. Monitoring of insecticide resistance

Surveillance to monitor the emergence and spread of resistance is an essential step in insecticide resistance management (IRM) providing baseline data for programme planning and choice of insecticide [15, 16]. Effective resistance monitoring can improve the efficacy of vector control and may also delay or prevent the onset and spread of resistance. Insecticide resistance is commonly assessed by exposing mosquitoes to a diagnostic dose using standard protocols published by WHO [17]. However, if resistance alleles are partially or fully recessive, like *kdr* [18], bioassays will only detect resistance when alleles have already reached a frequency high enough for resistant homozygotes to occur. Detection of resistance at the molecular level is more sensitive and can provide early warning of target-site and metabolic resistance.

2.3. Worldwide pattern of insecticide resistance

The worldwide distribution of the dominant malaria vectors is represented in **Figure 1**.

2.3.1. Sub-Saharan Africa

Malaria morbidity in sub-Saharan Africa represents 90% of the total cases reported worldwide [5, 19]. Many vectors play an important role in malaria transmission across Africa, notably the four major malaria vector species, i.e., *Anopheles gambiae* (including *An. gambiae sensu stricto* (s.s.) and *An. coluzzii*), *An. arabiensis* and *An. funestus* s.s. [20]. In the past decade, PY resistance in these major malaria vectors has spread across the continent being prevalent in west, central, east and southern Africa [12]. As far as we know, south-western Africa (Namibia and Botswana) remains the only region where PY-resistant *Anopheles* populations have not yet been reported (**Figure 2A**; data source: irmapper.com, 2017). The PY resistance is a great concern because PYs are the main insecticide class recommended for LLINs impregnation [21]. Resistance to LLIN exposure increases mosquito survival, which may lead to rising malaria incidence and fatality in Africa [22]. However, insecticide resistance of malaria vectors is not limited to PYs only but also exists to the other three classes of insecticides used in public health, such as CAs, OCs and, to a lesser extent, OPs [12]. However, some differences have been observed in the distribution of resistance among regions across the continent. For

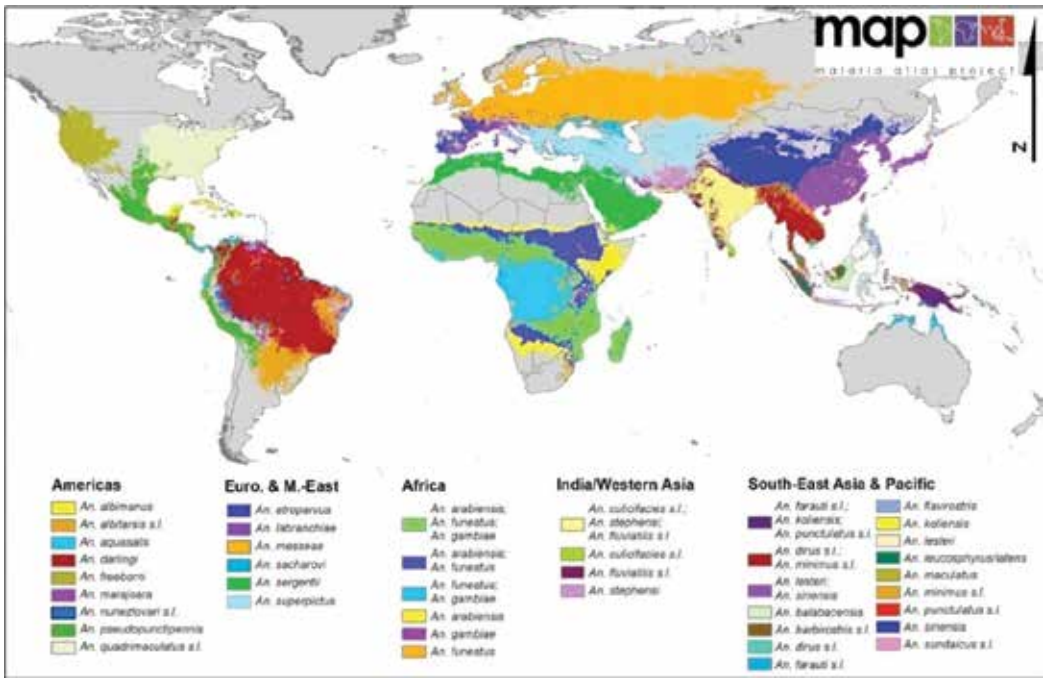


Figure 1. Global distribution of the dominant vector species of malaria [137].

example, resistance to DDT, the most common OC used in IRS, has been reported in *An. gambiae* and *An. funestus* in western, central and eastern Africa [23–28], whereas it is practically absent in southern Africa (**Figure 2B**), with the exception of an *An. funestus* s.s. population in southern Malawi [29]. DDT resistance has been also reported in *An. arabiensis* in southern Africa, specifically in Madagascar, Mozambique and South Africa [30–32]. In addition, resistance to CA, especially bendiocarb, which is commonly used in IRS, has been reported across Africa (data source: irmapper.com, 2017), although the regions with widespread CA resistance are focused in west and southern Africa (**Figure 2C**). So far, resistance to OP is less prevalent, limited to few reports in West and East Africa for *An. gambiae* s.s. and *An. arabiensis*, respectively (**Figure 2D**) [33, 34], whereas *An. funestus* s.s. populations remain fully susceptible across the continent.

2.3.2. Southeast Asia and Western Pacific Region

After Africa, Southeast Asia is the area with a higher incidence of malaria, with 7% of the cases reported [5]. A good number of vectors (belonging to complexes or groups of species that are difficult to distinguish) are involved in transmission, presenting an extraordinary biodiversity, heterogeneity in distribution, linked with a high variety in host feeding and ecological habitat preferences, as well as high differences in vector competence [35–37]. Currently in Southeast Asia, PY resistance has been detected in *An. epiroticus* in Vietnam [38], *An. minimus* in Thailand and Vietnam [35, 38], *An. sinensis* in China and Vietnam [39, 40] and *An. vagus* in Cambodia and Vietnam [38]. Similarly, DDT resistance has been detected in *An. minimus* in Cambodia

http://dx.doi.org/10.5772/intechopen.78375

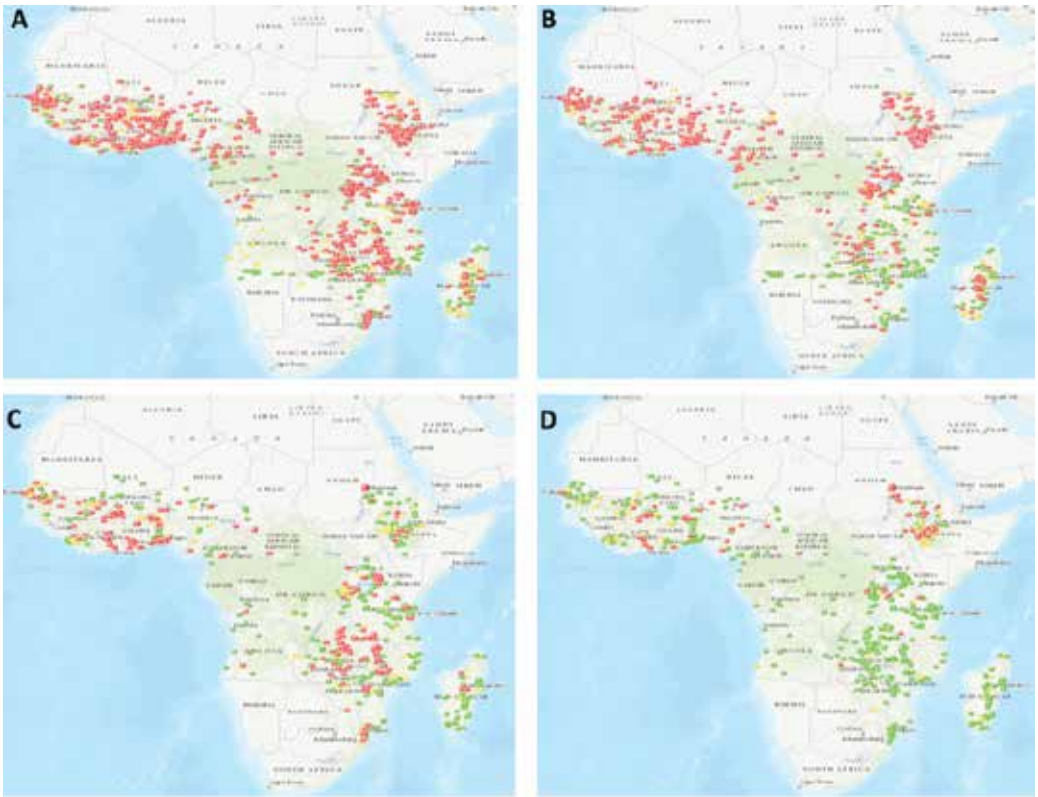


Figure 2. Distribution of resistance to all four classes of insecticides in the major malaria vectors belonging to *An. gambiae* complex and *An. funestus* group in Africa from 1985 to 2017. The green dots represent full susceptibility, orange is for suspected resistance and red for confirmed resistance. (A) Widespread resistance to pyrethroids. (B) Widespread resistance to DDT (organochlorines) although susceptibility is observed in southern Africa in *An. funestus* populations. (C) Profile of resistance to carbamate with significant areas of resistance in west and southern Africa. (D) Broad susceptibility to organophosphates across the continent but with pockets of resistance in West Africa and Ethiopia in *An. gambiae* s.l.

and Laos [40], *An. dirus* and *An. minimus* in Thailand [35] and possibly *An. epiroticus* in Vietnam [38]. Alarming, high level of multiple resistance to all classes of insecticides used in public health has been reported recently in *An. sinensis* in malaria endemic areas of China, including permethrin, deltamethrin, bendiocarb, DDT, malathion and fenitrothion, among others [41–43]. In South Asia, represented mainly by India, *An. baimaii* and *An. minimus* are also present but geographically restricted to East and Northeast regions and are fully susceptible to all classes of insecticides [44, 45]. *An. stephensi*, *An. culicifacies* species E and *An. fluviatilis* species S are the other predominant vectors responsible for malaria transmission in mainland India [36]. *An. stephensi*, prime urban vector in India, has shown resistance to PY, DDT and OPs in Goa State [46]. In addition, resistance to DDT was also detected in *An. stephensi* populations from Gujarat and Rajasthan [47]. PY-resistant populations of *An. culicifacies* s.l., present mainly in rural areas, have been reported in almost all the regions [48, 49]. In the last few years, resistance of *An. culicifacies* s.l. is increasingly being spread in many States such as Chhattisgarh, Odisha and Tamil Nadu. Also, high resistance to DDT and

OP has been reported in most districts of Odisha, a State with high prevalence of malaria, as well as in other regions with lower endemicity. *An. fluviatilis* S, the other major malaria vector in Odisha, remains fully susceptible to all insecticides [49].

2.3.3. Eastern Mediterranean

Malarial morbidity in this region accounts for only 2% of reported cases world over [5]. Afghanistan, Pakistan, Sudan, South Sudan and Yemen account for the majority of the malaria cases. In Afghanistan, a 2016 study done in five different locations reported that *An. stephensi* is the main vector, followed by *An. culicifacies* s.l. and *An. superpictus*, and other marginal species such as *An. subpictus*, *An. splendidus* and *An. nigerrimus* [50]. Different populations of three most abundant vectors, *An. culicifacies* s.l., *An. superpictus* and *An. subpictus*, showed resistance to the PY class II, deltamethrin. However, only populations of *An. culicifacies* s.l. and *An. superpictus* showed resistance to the PY class I permethrin and the OC insecticide DDT, while *An. subpictus* remained susceptible to both insecticides. Furthermore, *An. stephensi* showed resistance to the OP insecticide malathion, whereas *An. culicifacies* s.l. and *An. superpictus* were susceptible to this insecticide. Finally, these three species remained susceptible to the CA insecticide bendiocarb. Similarly, in Pakistan, a neighbouring country of Afghanistan, *An. stephensi* and *An. subpictus* are the main malaria vectors [51]. Populations of *An. subpictus* and *An. stephensi* showed resistance to the PY insecticide class I permethrin, the PYs class II deltamethrin and lambda-cyhalothrin and the OC insecticide DDT, while susceptible to the OP insecticide malathion, with the only exception of the populations of southern districts of the Punjab, resistant to malathion [52–54]. In Sudan, *An. arabiensis* is the major malaria vector reported from all parts of the country, coexisting sympatrically with *An. gambiae* s.s. and *An. funestus* [55]. *An. arabiensis* populations in Sudan are resistant to all the insecticides used in public health: PYs [56–60], CAs [58], OCs [57, 59–61] and OP [57, 58]. Limited data, however, are available in South Sudan, where resistance to the PY deltamethrin has been reported in *An. arabiensis* in two localities, Juba Payam and Northern Bari Payam [62].

2.3.4. Latin America

Malaria cases have declined considerably in this region in the past two decades, with many of the countries going into pre-elimination phase [63]. However, with 562,000 cases reported during 2015–2016, malaria is still a high burden, especially in countries in the Amazonia region such as Brazil, Colombia, Peru and most recently Venezuela [5], that showed an alarming increase over 76% of the reported cases (from 136,402 to 240,613) between 2015 and 2016, displaying an unprecedented 365% increase in malaria cases between 2000 and 2015 [5]. This country now encompasses Brazil as the larger contributor to the malaria burden in the Americas. *An. darlingi* is the primary malaria vector in the Amazonia region [64]. Fortunately, *An. darlingi* has shown susceptibility to all the insecticides across most of its distribution range, with exception of one population in western Colombia, which showed resistance to PY and DDT, but susceptibility to OPs [65–67]. However, studies to track the insecticide resistance and the available data are scanty. Thus, we cannot discard that resistance to insecticides in *An. darlingi*, as well as other malaria vectors, does not exist but rather could be more widespread in the Amazon region [63]. Similarly, the insecticide resistance of the secondary malaria vectors, often zoophilic but occasionally anthropophilic, is likely induced by the insecticide selection

http://dx.doi.org/10.5772/intechopen.78375

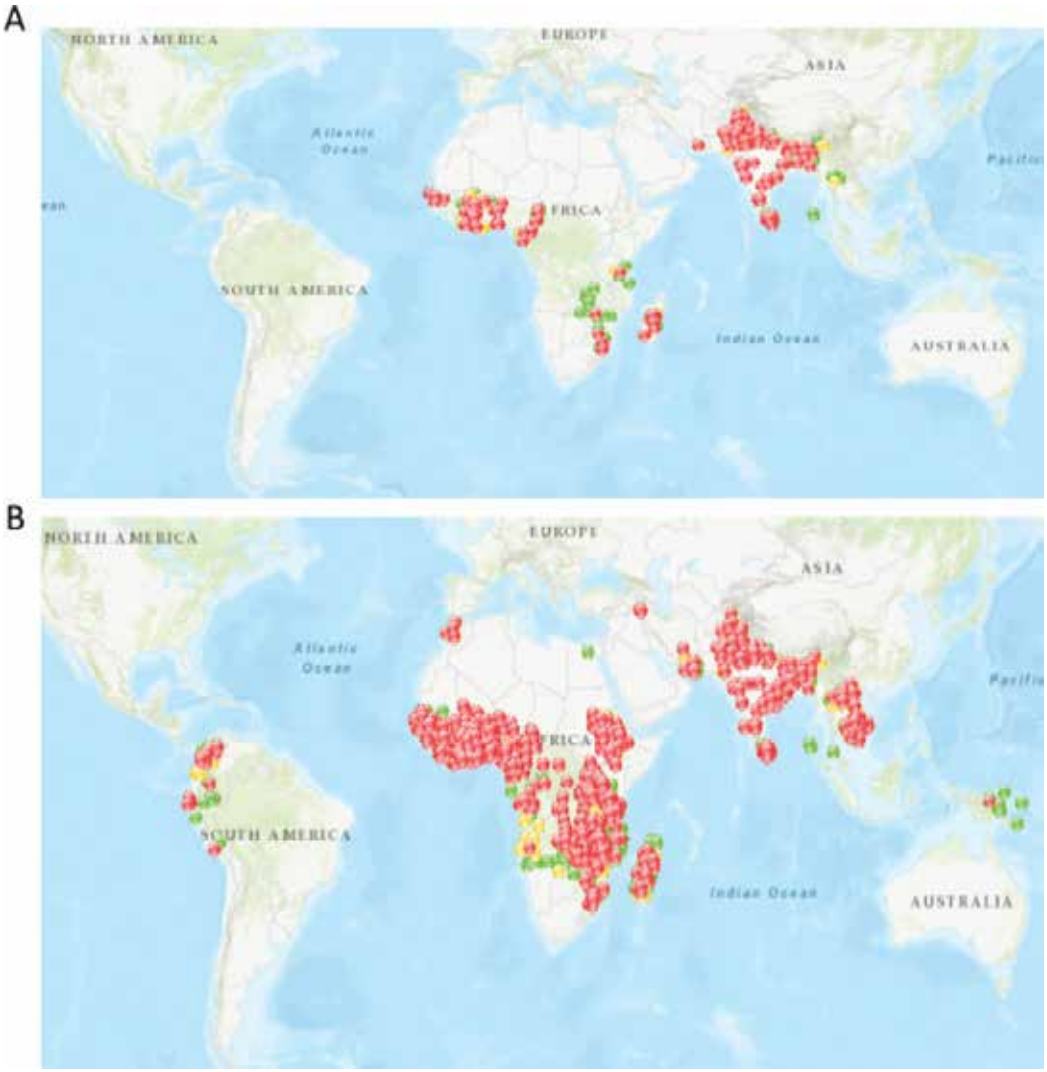


Figure 3. Worldwide view of the escalation of insecticide resistance in malaria vectors: (A) resistance profile in malaria vectors between 1985 and 2000 with limited resistance reported in West Africa and Southeast Asia; (B) significant increase of resistance in African and in other regions from 2000 to 2017.

pressure from agriculture activities. Field populations of *An. albimanus* have been reported resistant to PY in Colombia, Panama and Peru [67–69]. *An. albimanus* population in the north-western coast of Peru have shown cross-resistance to all classes of insecticides used in public health for being resistant to PYs, CAs, OCs and OPs [70]. Nevertheless, this is not the only case of cross-resistance reported for *An. albimanus*. Another population in southern Mexico has shown low resistance to PY and OP, linked with high resistance to DDT [71]. Similarly, a population of *An. nuneztovari*, secondary malaria vector distributed mainly in Colombia and Venezuela, showed cross-resistance to DDT and OPs in one specific location of Colombia, close to the border with Venezuela [72]. Other malaria vectors, such as *An. benarrochi* and *An.*

pseudopunctipennis, have shown susceptibility to PY across most range of their distribution with only two exceptions: one *An. benarrochi* population at the border between Peru and Brazil resistant to permethrin, and one *An. pseudopunctipennis* population in the northwest Peru resistant to permethrin, cypermethrin, deltamethrin and lambda-cyhalothrin [36], the latter population also showed cross-resistance to OP (malathion).

In conclusion, resistance to insecticide is steadily spreading worldwide in most vectors as shown by the comparison of resistance profile between 1985 and 2000 (**Figure 3A**) and 1985 to 2017 (**Figure 3B**) from IR mapper (http://www.irmapper.com/). This represents a serious challenge to malaria control, which relies heavily on insecticide-based tools.

3. Insecticide resistance mechanisms

A proportion of insect populations can tolerate doses of insecticides which have been proved lethal to the majority of the individuals in a normal population of the same species through various mechanisms such as: (i) insecticide can be broken down or detoxified much faster in the resistant mosquitoes than in the susceptible ones, hence quickly eliminated from their body (metabolic resistance); (ii) the target of the insecticide can be genetically altered to prevent the insecticide from binding thereby reducing the insecticide effect (target-site resistance); or (iii) resistant mosquitoes may absorb the toxin slower than susceptible insects (penetration resistance). An illustration of these mechanisms is represented in **Figure 4**.

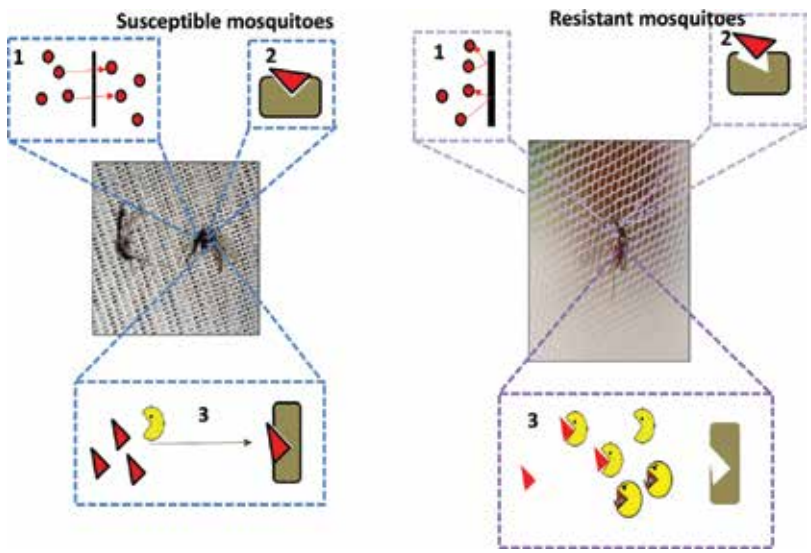


Figure 4. Illustration of the physiological insecticide resistance mechanisms in mosquitoes. (1) Reduced penetration: physiological changes to the cuticle of the mosquitoes prevent the absorption or penetration of insecticide. (2) Target-site resistance: insecticides have a target site within the mosquito. This site can become modified so that the insecticide no longer binds to it. (3) Metabolic resistance: enhanced enzyme systems break down insecticides before they can have a toxic effect on the mosquito.

3.1. Methods used to study resistance mechanisms

Insecticide resistance monitoring is essential to understand the actual threat and how resistance is spreading among malaria vectors [7]. Once resistance has reached very high levels (fixed in the population), most insecticide resistance management strategies, which are based to restore susceptibility, would not work. Thus, regular monitoring is crucial. Three detection methods (**Table 1**) can be used to monitor insecticide resistance, each method providing different information. Bioassays are the most popular way to monitor resistance where mosquitoes are exposed fixed doses of insecticides for a fixed time and the percentage mortality is recorded 24 h post-exposure [73]. Even though they are simple to perform, bioassays have several disadvantages such as requiring a large number of mosquitoes, affected by variations in humidity, temperature and time of the day [74]. Some authors argue that bioassays should be supplemented with DNA markers or even partially replaced by these DNA markers [75]. It should be noted that DNA markers are usually specific to certain mechanisms hence the need to perform them is to avoid unknown mechanisms going undetected. Until now, no assay has been developed that is suitable to monitor cuticular or behavioural resistance.

3.2. Target-site resistance

One of the mechanisms mosquito becomes resistant is by altering the target site of the insecticide thereby preventing it from binding effectively hence the insecticide has little or no effect on the insect. Most insecticide targets are found within the nervous system and mutations in these target sites (mainly receptors) lead to reduced sensitivity. For example, PYs and DDT act on the voltage-gated sodium channels (VGSCs) and mutation in the amino sequence of this gene results in reduced sensitivity of the channels preventing PYs and DDT from binding [76]. Insects with this mutation can withstand prolong exposure to insecticide without being knocked down, hence the name "knockdown resistance" (*kdr*) [77]. The replacement of the *leucine* residue for a *phenylalanine* or a *serine* at position 1014 in the VGSC is one of the most common amino acid substitution associated with PY resistance in malaria vector [77]. Also an *alanine* to *serine* substitution at position 302 (or 296) of the γ-amino-butyric acid (GABA) receptor is found in the dieldrin-resistant (*rdl*) insect species including *An. gambiae* [78] and *An. funestus* [79]. Similarly, mutations in the gene coding for the neurotransmitter acetylcholinesterase (*ace-1*), the target site of OPs and CAs, have been found [80], which reduces the inhibition effect of the insecticide on the enzyme [81, 82]. Substitution of *glycine* to *serine* at position 119 has been reported in *An. albimanus* and *An. gambiae*, and this mutation confers resistance to OPs and CAs [83]. Duplication of the *ace-1* gene has been reported in the *An. gambiae* and *An. coluzzii* [84]. However, in species such as *An. funestus,* other mutations were detected in *ace-1* including the N485I shown to be associated with bendiocarb resistance in southern African populations [85].

3.3. Metabolic resistance

Metabolic resistance is the most common and challenging of all insecticide resistance mechanisms. Mosquitoes have enzyme systems that protect them from xenobiotic compounds and

Susceptibility bioassay tests	**Biochemical assays**	**Molecular assays**
Description	Description	Description
Vectors are exposed to fixed insecticide concentrations, and the level of vector mortality is subsequently recorded. The results are expressed as the percentage of vectors knocked down, alive or dead. Susceptibility testing requires samples of at least 100 live mosquitoes per testing site. These susceptibility tests are generally used for routine monitoring, as they can be applied in the field. They provide standardised data that are relatively easily interpreted. Either WHO paper bioassays or CDC bottle bioassays can be used. The results obtained with the two methods are not comparable. In order to observe longitudinal or temporal patterns in resistance, countries and academic institutions in all regions must therefore use the same method consistently over time.	Biochemical assays detect the presence of a particular resistance mechanism or an increase in enzyme activity. They require fresh mosquitoes, but much fewer than for bioassays. Unlike bioassays, biochemical assays can identify some specific resistance mechanisms and indicate an increase in metabolic enzyme activity. Biochemical assays are normally used in conjunction with synergist and molecular assays.	Molecular tests are used on the actual gene, allowing detailed and direct analysis of resistance genes. Testing can be done with straightforward polymerase chain reaction techniques (30) with DNA or in more elaborate microarray tests with RNA. More advanced molecular methods can provide complex genetic information including whether the mutation is unique or has spread. These are the most accurate tests for measuring resistance frequency in vector populations. Molecular tests must, however, be correlated with susceptibility testing.
Limitations	Limitations	Limitations
Susceptibility tests identify the existence of resistance once it is at a detectable level but do not establish the resistance mechanism involved. They may also not identify resistance if the frequency is too low. Several countries have reported shortages in the supply of testing materials and have switched between the WHO and CDC tests, making results difficult to compare. In some cases, they have limited their testing.	The method is more difficult to use in the field as it requires sophisticated equipment, and interpretation of the results requires strong technical skills. Further, the correlation between chemical reactions in these tests and increased ability to metabolise insecticides is not yet well defined.	The method requires sophisticated equipment and entomological capacity. It can be used to detect target site resistance and a few identified metabolic mechanisms. Therefore, susceptibility tests should be used to complement molecular results, as the absence of identifiable genotypic resistance does not necessarily mean that resistance does not exist.

Table 1. Different methods for monitoring insecticide resistance in mosquito vectors and their limitations.

some of these enzyme systems can break down insecticide before it can reach its site of action. In metabolic resistance, enzymes that detoxify the insecticide can be overexpressed or alter the affinity of the enzyme for the insecticide through amino acid substitutions [86]. Overexpression of insecticide resistance genes is the most frequent mechanism in resistant mosquitoes. This increased expression of insecticide resistance genes can be due to *cis*- or *trans*-acting elements in the promoter or gene amplification [87, 88]. This overexpression results in the high level of enzyme production in the resistant mosquitoes that enables them to break down the insecticide at a faster rate before it reaches the target site. Cytochrome P450 monooxygenases,

glutathione S-transferases (GSTs) and esterases are the three major enzyme families that are involved in breaking down of insecticides.

3.3.1. Cytochrome P450 monooxygenases

Of the six families of P450s, genes belonging to the CYP4, CYP6 and CYP9 have been observed in resistant mosquitoes with increased transcriptional level [89], with the majority of those implicated in resistance belonging to the CYP6 family. For a P450 to be involved in resistance, it does not only have to be overexpressed but also must be able to metabolise/sequester the insecticide to which the insect is resistant and also be better metaboliser than those for the susceptible strain [90]. In *An. gambiae*, CYP6P3 and CYP6M2 have been shown to metabolise type I and type II PYs [91], and CYP6M2 can metabolise DDT [92]. In *An. funestus*, the duplicated P450 CYP6P9a and CYP6P9b and CYP6M7 have been shown to metabolise PYs [93, 94], whereas CYP6Z1 confers cross-resistance to both pyrethroids and carbamates [85]. Furthermore, allelic variation of P450 genes, such as CYP6P9a/b, has been shown to drive pyrethroid resistance in field populations of *An. funestus* [95] with signature of selective sweep associated with scale-up of bed nets [96].

3.3.2. Glutathione S-transferases

The GSTs are involved in the phase two of the detoxification of xenobiotic compounds where they conjugate the substrate with glutathione enhancing solubility thus facilitating the excretion. In insect, six classes of GSTs, i.e., delta, sigma, epsilon, omega, theta and zeta have been identified [97]. Insects resistant to major classes of insecticide show elevated levels of GSTs activities. For example, GSTs confer resistance to DDT in mosquitoes including *An. gambiae* [98], *An. dirus* [99], *Aedes aegypti* [100] and *An. funestus* [26]. A single amino acid change in GSTe2 (L119F) has been shown to confer a cross-resistance to DDT and PYs in *An. funestus* [26], whereas a similar change is also reported in *An. gambiae* (I114T) [101].

3.3.3. Esterases

CAs and OPs are the main insecticides that are metabolised or sequestered by esterase-mediated insecticide resistance. Esterase levels in the resistant mosquitoes can either be elevated like in *Culex* or non-elevated like in *Anopheles* species (*An. arabiensis, An. stephensi* and *An. culicifacies*). Esterase-mediated insecticide resistance in *Anopheles* spp. was associated with allelic variants that can metabolise the insecticide at a faster rate than those of the susceptible and shown to confer resistance to malathion [102, 103]. The role of esterases in PY resistance still needs to be investigated.

3.4. Cuticular or reduced penetration resistance

Cuticular resistance occurs when mosquitoes reduce the absorption of insecticide into their bodies by altering the structure or composition of the cuticle. A wide range of insecticides are threatened by this mechanism as for their lethal effect to occur, most insecticides must cross the cuticle in order to reach their site of action. Cuticular resistance enhances the resistance

conferred by other mechanisms. This mechanism has not been extensively studied as compared to the other mechanisms because there are very few examples. Recently, Yahouédo et al. [104] studied the role of the cuticular resistance in PY-resistant strain of *An. gambiae* called MRS, free of *kdr* mutations. They succeeded to show that lower amount of insecticide was absorbed in the MRS strain than in the susceptible strain and also that the MRS strain had a significantly thicker cuticle layers than those of the susceptible strain. *CPLCG3* gene, which codes for a structural protein contributing to the cuticle thickness, was found to be constitutively upregulated. Similar evidences of cuticular resistance were shown for *An. funestus* with proofs of cuticle thickening in PY-resistant mosquitoes [105].

4. Impact of current insecticide resistance in parasite transmission: a global warning based on reported level of resistance?

4.1. Fitness cost of resistant lab and field *Anopheles* populations

The use of insecticide selects small proportion of individuals possessing resistance genes allowing them to resist and survive the effects of the insecticide, transferring the genetic modifications conferring resistance to the progeny. This should most likely increase the proportion of resistant individuals within the population. However, mutations or genes conferring resistance are usually associated with a fitness cost and may disrupt normal physiological functions [106, 107]. For example, resistant vectors may have lower mating success [108, 109], lower fecundity and fertility, higher developmental time and lower longevity. Resistant individuals may be also more susceptible to natural predators [110] or more prone to mortality during overwintering. Most insecticide resistance management strategies rely on the fact that fitness cost may impact the spread and persistence of resistance alleles in the vector populations [7].

4.2. Impact of resistance on life traits: longevity, fecundity and mating male competitiveness

Resistance caused by overproduction of metabolic enzymes generally shows lower fitness cost than target site resistance, most probably because the primary function of the enzyme is not disrupted [111]. But to date, little is known about the effective impact of metabolic resistance on the life traits of the vector due to the absence of DNA-based molecular marker. Nevertheless, many studies demonstrated that resistant strains of arthropods often present lower fitness compared to their susceptible counterparts [112]. For example, it was shown that resistance strains may be associated with relatively slower larval development, reduced survival rates among larvae and adults, reduced fecundity in females and reduced fertility [106, 113, 114]. It was shown that target-site resistance due to *kdr* and *rdl* mutations is able to impact negatively the male mating competitiveness in the malaria vector *An. gambiae,* whereas metabolic resistance had no effect [109]. Among all the parameter elucidated above, vector longevity is an essential parameter in disease transmission because it increases the potential for infective bites to hosts. Furthermore, the effect of longevity on disease transmission is crucial for parasites like *Plasmodium* that need a minimum incubation period in the vector before being transmitted to a new host. Insecticide resistance is generally thought to increase longevity of resistant

vectors, thereby increasing infectiousness of parasites and threatening vector control. However, the development of resistance in a mosquito often comes with a price subsequently affecting the fitness of the vector [115]. As a consequence of the fitness cost of insecticide resistance on the life traits (mentioned above) of the vectors, reversion to susceptibility is expected. A good example of reversal to susceptibility occurred in *An. arabiensis* in Sudan. In this country, after antimalarial house spraying in the early 1980s, resistance to malathion was noticed. This prompted a switch of insecticide treatment to fenitrothion (OP insecticide), and susceptibility to malathion was restored in the following years [10]. However, reversal rates are variable and may be very slow, particularly when an insecticide has been used for many years. For example, the same *rdl* gene has been reported to be maintained in field populations of Sri Lanka despite the withdrawal of cyclodiene insecticides for mosquito control for more than 30 years [116]. Before implementing any resistance management strategy in the field, knowledge of the reversal rate is crucial.

4.3. Epidemiological consequences of the insecticide resistance on malaria incidence

4.3.1. Past and current evidences

There are large number of confounding factors threatening the assessment of epidemiological consequences of the insecticide resistance on malaria incidence and data interpretation [117]. For this reason, only few studies have assessed the epidemiological impact of insecticide resistance. Impact of PY resistance on control failure was reported from the borders of Mozambique and South Africa. In 1996, the malaria control programme in KwaZulu-Natal (South Africa) switched from using DDT to deltamethrin for indoor spraying [118]. After four years of deltamethrin spraying, reported malaria cases increased approximately fourfold. *An. funestus*, previously eradicated, had reappeared and was observed emerging alive from PY-sprayed houses. Bioassays showed that this species was resistant to PYs but susceptible to DDT [119]. The decision to revert to IRS with DDT was accompanied by a decline in malaria cases by 91% [120]. On the Bioko Island on the West African coast, increased density of PY-resistant *An. gambiae* was also reported after IRS campaign with lambda-cyhalothrin, although a significant reduction in transmission index and malaria reported cases was observed [121, 122]. High frequencies of the L1014F *kdr* allele were observed in the local *An. gambiae* population. When PYs were replaced by CAs (bendiocarb), mosquito population declined [122]. Nevertheless, in an operational scale programme such as this, the possible contribution of other factors to the failure of PY IRS to control mosquito population density cannot be overlooked; thus, the direct consequence of the high *kdr* frequency is uncertain. After initiation of interventions combining IRS with PYs and ITNs in the highland provinces of Burundi in 2002, significant reduction was recorded in *Anopheles* density by 82% [123]. Consequently, transmission intensity was reduced by 90% and occurrence of malaria cases by 43% in children, despite high frequencies of the L1014S *kdr* allele in the main vector *An. gambiae* s.s. [123]. Many interventions took place in Africa in order to investigate the efficacy of ITNs for malaria prevention [124]. However, the extent to which PY resistance might affect the effectiveness of such interventions is not well elucidated. In Korhogo area, north of Côte d'Ivoire where the 1014F *kdr* allele frequency in *An. gambiae* is up to 80% [125], and malaria is endemic, lambda-cyhalothrin-treated nets had a significant impact on the entomological inoculation rate with around 55% reduction. Malaria

incidence in children <5 years of age decreased also (56% reduction of clinical attacks) compared to a control group having no nets [126]. This was the first clear-cut evidence of ITNs continuing to provide effective personal protection against malaria in an area with a high frequency of *kdr* in the vector populations. However, absence of a physical barrier in the control group might have overestimated the impact of PY-treated nets against *kdr* mosquitoes in this study. In southern Benin, a randomised controlled trial was carried out in a mesoendemic area to assess the impact of LLINs scale-up on malaria morbidity in children <5 years of age [127]. In this area, where the *kdr* frequency is around 50–60% in *An. gambiae* s.s., transmission increased during the rainy season but was not followed by a seasonal variation in parasite infection and clinical incidence. The evidence is clear that implementation of vector control tools (ITNs and/or IRS) has significantly decreased malaria incidence and parasite infection prevalence in children in endemic countries across Africa, despite moderate-to-high PY resistance observed in local malaria vectors.

5. Behavioural resistance to insecticides used in public health

As we have mentioned previously, the extraordinary success of malaria reduction in Africa is largely due the use of insecticides applied indoors through LLINs and IRS [6]. This malaria control approach takes advantage of the strong human preference, as well as the indoor feeding and resting behaviour of African malaria-transmitting mosquitoes [128]. As we have shown in this chapter, progress has been made in understanding the genetic basis of the ability of mosquitoes to survive insecticide entering the body. However, little is known about the causes of increasingly reported changes in blood-feeding behaviour developed by certain species of malaria-transmitting mosquitoes to avoid exposure to insecticides [7]. This phenomenon is known as behavioural resistance and it is defined as any modification in insect behaviour that helps to circumvent the lethal effects of insecticides. Thus, through intraspecific behavioural shifts in biting time, location and host preference, malaria-transmitting mosquitoes avoid exposure to insecticides, feeding on humans when most people are not protected [129], jeopardising the current control strategy in Africa primarily based on indoor application of insecticides [130–132]. Recent studies conducted in West and East Africa have shown that indoor application of insecticides may induce intraspecific behavioural shifts towards early biting, exophagic biting and exophilic resting behaviour in malaria-transmitting mosquitoes [130, 131, 133]. Similarly, current studies conducted in Central Africa showed a comparable shift towards exophilic resting behaviour [134]. Mathematical modelling and field evidences have proved that these shifts in blood-feeding behaviour could threaten and impact on the current control programmes [132, 135]. The mechanisms driving these shifts have not yet been elucidated, although some studies have shown that both genetic and environmental factors play a key role [135, 136].

6. Conclusion and perspectives

Insecticide resistance is undoubtedly a major challenge to the control of malaria vectors worldwide as it limits the tools available to achieve the goal of controlling and eliminating this

debilitating disease. It is therefore of the utmost importance that novel insecticides and new control tools be designed to help manage and mitigate the impact of resistance. Through the work of various partners such as Innovative Vector Control Consortium (IVCC), UNITAID and several manufacturers, the challenge of producing new insecticides and tools is beginning to be met. This is exemplified by the recent prequalification by the WHO of the new insecticide Sumishield (clothianidin, a neonicotinoid) in October 2017. This new insecticide together with the organophosphate Actellic (pirimiphos-methyl) could now allow countries to effectively design and implement suitable resistance management strategies for IRS interventions according to WHO's Global Plan for Insecticide Resistance Management (GPIRM). With other new insecticides expected to enter the market in the near future, resistance management strategies such as rotation of insecticides could become more realistic to implement. However, even with new insecticides available, the community should avoid being complacent as the mosquitoes will surely develop resistance with time if consideration is not given to how to use such new insecticides including between public health and agriculture sectors. Detection of resistance markers notably for metabolic resistance is also urgently needed to not only track the spread of resistance but to better assess its impact on control interventions or mosquito fitness and malaria transmission. The recent detection of markers such as L119F-GSTe2 in *An. funestus* shows that this is possible, but more efforts are needed focusing importantly on cytochrome P450s, the key metabolizers. It will be important to take advantage of the advances in genomics with the power of next-generation sequencing tools to detect potential resistance markers early enough to allow control programmes to track resistance when it is still at early stage when it could easily be managed. This will allow avoiding repeating the situation observed with PY resistance and ensure a continued effectiveness of current and future insecticide-based interventions.

Acknowledgements

This work was supported by a Wellcome Trust Senior Fellowship in Biomedical Sciences (WT101893MA) to CSW.

Conflict of interest

No conflict of interest.

Acronyms

CA	carbamate
CDC	Centers for Disease Control and Prevention
DDT	dichlorodiphenyltrichloroethane
DNA	deoxyribonucleic acid

GABA	gamma-aminobutyric acid
GPIRM	Global Plan for Insecticide Resistance Management
GST	glutathione S-transferase
IRM	insecticide resistance management
IRS	indoor residual spraying
ITN	insecticide-treated nets
IVCC	Innovative Vector Control Consortium
Kdr	knockdown resistance
LLIN	long-lasting insecticidal net
OC	organochlorines
OP	organophosphates
PY	pyrethroids
RNA	ribonucleic acid
VGSC	voltage-gated sodium channel
WHO	World Health Organization

Author details

Jacob M. Riveron[1,2], Magellan Tchouakui[2,3], Leon Mugenzi[2,4], Benjamin D. Menze[1,2], Mu-Chun Chiang[1] and Charles S. Wondji[1,2]*

*Address all correspondence to: charles.wondji@lstmed.ac.uk

1 Department of Vector Biology, Liverpool School of Tropical Medicine, Liverpool, UK

2 Centre for Research in Infectious Diseases (CRID), Yaoundé, Cameroon

3 Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé 1, Cameroon

4 Department of Biochemistry and Molecular Biology, University of Buea, Buea, Cameroon

References

[1] Penney D, Wadsworth C, Fox G, Kennedy SL, Preziosi RF, Brown TA. Absence of ancient DNA in sub-fossil insect inclusions preserved in “Anthropocene” Colombian copal. PLoS One. 2013;**8**:e73150. DOI: 10.1371/journal.pone.0073150

[2] Bertone MA, Courtney GW, Wiegmann BM. Phylogenetics and temporal diversification of the earliest true flies (Insecta: Diptera) based on multiple nuclear genes. Systematic Entomology. 2008;**33**:668-687

[3] Worrall E, Basu S, Hanson K. Is malaria a disease of poverty? A review of the literature. Tropical Medicine & International Health. 2005;**10**:1047-1059

[4] Hawass Z, Gad YZ, Ismail S, Khairat R, Fathalla D, Hasan N, et al. Ancestry and pathology in King Tutankhamun's family. JAMA. 2010;**303**:638-647. DOI: 10.1001/jama.2010.121

[5] WHO. World Malaria Report 2017. Geneva, Switzerland: World Health Organization; 2017

[6] Bhatt S, Weiss D, Cameron E, Bisanzio D, Mappin B, Dalrymple U, et al. The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. Nature. 2015; **526**:207-211

[7] WHO. Global Plan for Insecticide Resistance Management in Malaria Vectors. Geneva, Switzerland: World Health Organization; 2012. 132 pp. Available from: www.who.int/malaria/vector_control/ivm/gpirm/en/

[8] Davidson G. Insecticide resistance in *Anopheles sundaicus*. Nature. 1957;**4598**:1333-1335

[9] Gilbert LI, Gill SS. Insect Control: Biological and Synthetic Agents. London, United Kingdom: Academic Press; 2010. 470 p

[10] Corbel V, N'Guessan R. Distribution, mechanisms, impact and management of insecticide resistance in malaria vectors: A pragmatic review. In: Manguin S, editor. *Anopheles* Mosquitoes—New Insights into Malaria Vectors. Rijeka: InTech Open Access; 2013

[11] Elliott R, Ramakrishna V. Insecticide resistance in *Anopheles gambiae* Giles. Nature. 1956; **177**:532-533

[12] Ranson H, Lissenden N. Insecticide resistance in African *Anopheles* mosquitoes: A worsening situation that needs urgent action to maintain malaria control. Trends in Parasitology. 2016;**32**:187-196

[13] Diabate A, Baldet T, Chandre F, Akoobeto M, Guiguemde TR, Darriet F, et al. The role of agricultural use of insecticides in resistance to pyrethroids in *Anopheles gambiae* sl in Burkina Faso. The American Journal of Tropical Medicine and Hygiene. 2002;**67**:617-622

[14] Poupardin R, Reynaud S, Strode C, Ranson H, Vontas J, David J-P. Cross-induction of detoxification genes by environmental xenobiotics and insecticides in the mosquito *Aedes aegypti*: Impact on larval tolerance to chemical insecticides. Insect Biochemistry and Molecular Biology. 2008;**38**:540-551

[15] Black WC, Gorrochetegui-Escalante N, Randle NP, Donnelly MJ. The Yin and Yang of linkage disequilibrium: Mapping of genes and nucleotides conferring insecticide resistance in insect disease vectors. In: Transgenesis and the Management of Vector-Borne Disease. New York, USA: Springer; 2008. pp. 71-83

[16] Black 4th WC, Gorrochetegui-Escalante N, Randle NP, Donnelly MJ. The Yin and Yang of linkage disequilibrium. Advances in Experimental Medicine and Biology. 2008;**627**:71-83. DOI: 10.1007/978-0-387-78225-6_6

[17] WHO. Test Procedures for Insecticide Resistance Monitoring in Malaria Vector Mosquitoes. Geneva: World Health Organization; 2016

[18] Chandre F, Darriet F, Duchon S, Finot L, Manguin S, Carnevale P, et al. Modifications of pyrethroid effects associated with *kdr* mutation in *Anopheles gambia*e. Medical and Veterinary Entomology. 2000;**14**:81-88

[19] Weill M, Chandre F, Brengues C, Manguin S, Akogbeto M, Pasteur N, et al. The kdr mutation occurs in the Mopti form of *Anopheles gambiae* s.s. through introgression. Insect Molecular Biology. 2000;**9**:451-455

[20] Sinka ME, Bangs MJ, Manguin S, Coetzee M, Mbogo CM, Hemingway J, et al. The dominant *Anopheles* vectors of human malaria in Africa, Europe and the Middle East: Occurrence data, distribution maps and bionomic précis. Parasites & Vectors. 2010;**3**:117

[21] Hougard J-M, Duchon S, Darriet F, Zaim M, Rogier C, Guillet P. Comparative performances, under laboratory conditions, of seven pyrethroid insecticides used for impregnation of mosquito nets. Bulletin of the World Health Organization. 2003;**81**:324-333

[22] Hemingway J, Ranson H, Magill A, Kolaczinski J, Fornadel C, Gimnig J, et al. Averting a malaria disaster: Will insecticide resistance derail malaria control? The Lancet. 2016;**387**: 1785-1788

[23] Kawada H, Dida GO, Ohashi K, Komagata O, Kasai S, Tomita T, et al. Multimodal pyrethroid resistance in malaria vectors, *Anopheles gambiae* ss, *Anopheles arabiensis*, and *Anopheles funestus* ss in western Kenya. PLoS One. 2011;**6**:e22574

[24] Bobanga T, Ayieko W, Zanga M, Umesumbu S, Landela A, Fataki O, et al. Field efficacy and acceptability of PermaNet® 3.0 and OlysetNet® in Kinshasa, Democratic Republic of the Congo. Journal of Vector Borne Diseases. 2013;**50**:206

[25] Mulamba C, Riveron JM, Ibrahim SS, Irving H, Barnes KG, Mukwaya LG, et al. Widespread pyrethroid and DDT resistance in the major malaria vector *Anopheles funestus* in East Africa is driven by metabolic resistance mechanisms. PLoS One. 2014;**9**:e110058. DOI: 10.1371/journal.pone.0110058

[26] Riveron JM, Yunta C, Ibrahim SS, Djouaka R, Irving H, Menze BD, et al. A single mutation in the *GSTe2* gene allows tracking of metabolically based insecticide resistance in a major malaria vector. Genome Biology. 2014;**15**:R27. DOI: 10.1186/gb-2014-15-2-r27

[27] Riveron JM, Osae M, Egyir-Yawson A, Irving H, Ibrahim SS, Wondji CS. Multiple insecticide resistance in the major malaria vector *Anopheles funestus* in southern Ghana: Implications for malaria control. Parasites & Vectors. 2016;**9**:504

[28] Menze BD, Riveron JM, Ibrahim SS, Irving H, Antonio-Nkondjio C, Awono-Ambene PH, et al. Multiple insecticide resistance in the malaria vector *Anopheles funestus* from

Northern Cameroon is mediated by metabolic resistance alongside potential target site insensitivity mutations. PLoS One. 2016;**11**:e0163261

[29] Riveron JM, Chiumia M, Menze BD, Barnes KG, Irving H, Ibrahim SS, et al. Rise of multiple insecticide resistance in *Anopheles funestus* in Malawi: A major concern for malaria vector control. Malaria Journal. 2015;**14**:344. DOI: 10.1186/s12936-015-0877-y

[30] Ratovonjato J, Le Goff G, Rajaonarivelo E, Rakotondraibe E, Robert V. Données récentes sur la sensibilité d'*Anopheles arabiensis* et d'*Anopheles funestus* aux pyréthrinoïdes et au DDT sur les Hautes Terres Centrales de Madagascar: Résultats préliminaires montrant une absence de la mutation *kdr* chez *An. arabiensis*. Archives de l'Institut Pasteur de Madagascar. 2003;**69**:63-69

[31] Hargreaves K, Hunt R, Brooke B, Mthembu J, Weeto M, Awolola T, et al. *Anopheles arabiensis* and *An. quadriannulatus* resistance to DDT in South Africa. Medical and Veterinary Entomology. 2003;**17**:417-422

[32] Casimiro S, Coleman M, Hemingway J, Sharp B. Insecticide resistance in *Anopheles arabiensis* and *Anopheles gambiae* from Mozambique. Journal of Medical Entomology. 2006;**43**:276-282

[33] Fettene M, Olana D, Christian R, Koekemoer L, Coetzee M. Insecticide resistance in *Anopheles arabiensis* from Ethiopia. African Entomology: Journal of the Entomological Society of Southern Africa. 2013;**21**:89-94

[34] Edi CV, Djogbenou L, Jenkins AM, Regna K, Muskavitch MA, Poupardin R, et al. CYP6 P450 enzymes and ACE-1 duplication produce extreme and multiple insecticide resistance in the malaria mosquito *Anopheles gambiae*. PLoS Genetics. 2014;**10**:e1004236

[35] Chareonviriyaphap T, Bangs MJ, Ratanatham S. Status of malaria in Thailand. The Southeast Asian Journal of Tropical Medicine and Public Health. 2000;**31**:225-237

[36] Quiñones ML, Norris DE, Conn JE, Moreno M, Burkot TR, Bugoro H, et al. Insecticide resistance in areas under investigation by the International Centers of Excellence for malaria research: A challenge for malaria control and elimination. The American Journal of Tropical Medicine and Hygiene. 2015;**93**:69-78

[37] Manguin S, Garros C, Dusfour I, Harbach R, Coosemans M. Bionomics, taxonomy, and distribution of the major malaria vector taxa of *Anopheles* subgenus *Cellia* in Southeast Asia: An updated review. Infection, Genetics and Evolution. 2008;**8**:489-503

[38] Van Bortel W, Trung HD, Sochantha T, Socheat D, Sumrandee C, Baimai V, et al. The insecticide resistance status of malaria vectors in the Mekong region. Malaria Journal. 2008;**7**:102

[39] Cui F, Raymond M, Qiao CL. Insecticide resistance in vector mosquitoes in China. Pest Management Science. 2006;**62**:1013-1022

[40] Verhaeghen K, Van Bortel W, Trung HD, Sochantha T, Keokenchanh K, Coosemans M. Knockdown resistance in *Anopheles vagus*, *An. sinensis*, *An. paraliae* and *An. peditaeniatus* populations of the Mekong region. Parasites & Vectors. 2010;**3**:59

[41] Wang D-Q, Xia Z-G, Zhou S-S, Zhou X-N, Wang R-B, Zhang Q-F. A potential threat to malaria elimination: Extensive deltamethrin and DDT resistance to *Anopheles sinensis* from the malaria-endemic areas in China. Malaria Journal. 2013;**12**:164

[42] Chang X, Zhong D, Fang Q, Hartsel J, Zhou G, Shi L, et al. Multiple resistances and complex mechanisms of *Anopheles sinensis* mosquito: A major obstacle to mosquito-borne diseases control and elimination in China. PLoS Neglected Tropical Diseases. 2014;**8**:e2889

[43] Zhang S, Guo S, Feng X, Afelt A, Frutos R, Zhou S, et al. *Anopheles* vectors in mainland China while approaching malaria elimination. Trends in Parasitology. 2017;**33**(11):889-900

[44] Dev V, Sharma VP. The dominant mosquito vectors of human malaria in India. In: Manguin S, editor. *Anopheles* Mosquitoes—New Insights into Malaria Vectors. Rijeka: InTech Open Access; 2013

[45] Dev V, Manguin S. Biology, distribution and control of *Anopheles* (*Cellia*) *minimus* in the context of malaria transmission in Northeastern India. Parasites & Vectors. 2016;**9**:585. DOI: 10.1186/s13071-016-1878-6

[46] Mishra A, Chand S, Barik T, Dua V, Raghavendra K. Insecticide resistance status in *Anopheles culicifacies* in Madhya Pradesh, Central India. Journal of Vector Borne Diseases. 2012;**49**:39

[47] Bhatt R, Sharma S, Barik T, Raghavendra K. Status of insecticide resistance in malaria vector, *Anopheles culicifacies* in Chhattisgarh state, India. Journal of Vector Borne Diseases. 2012;**49**:36

[48] Raghavendra K, Barik T, Sharma S, Das M, Dua V, Pandey A, et al. A note on the insecticide susceptibility status of principal malaria vector *Anopheles culicifacies* in four states of India. Journal of Vector Borne Diseases. 2014;**51**:230

[49] Sahu S, Gunasekaran K, Raju H, Vanamail P, Pradhan M, Jambulingam P. Response of malaria vectors to conventional insecticides in the southern districts of Odisha state, India. The Indian Journal of Medical Research. 2014;**139**:294

[50] Ahmad M, Buhler C, Pignatelli P, Ranson H, Nahzat SM, Naseem M, et al. Status of insecticide resistance in high-risk malaria provinces in Afghanistan. Malaria Journal. 2016;**15**:98

[51] Ashfaq M, Hebert PD, Mirza JH, Khan AM, Zafar Y, Mirza MS. Analyzing mosquito (Diptera: Culicidae) diversity in Pakistan by DNA barcoding. PLoS One. 2014;**9**:e97268

[52] Rathor HR, Nadeem G, Khan IA. Pesticide susceptibility status of *Anopheles* mosquitoes in four flood-affected districts of South Punjab, Pakistan. Vector Borne and Zoonotic Diseases. 2013;**13**:60-66

[53] Naeem H, Ashraf K, Rashid MI, Oneeb M, Akbar H. Insecticide resistance status of *Anopheles subpictus* from district Kasur, Punjab, Pakistan. Science International. 2014;**26**: 1205-1208

[54] Hammad M, Bhatti A, Mukhtar MU, Arslan A, Mushtaq S. Susceptibility/resistance of selected insecticides in *Anopheles* mosquitoes of district Mirpur Khas, Sindh, Pakistan. Journal of Entomology and Zoology Studies. 2015;**3**(6):321

[55] Petrarca V, Nugud A, Ahmed M, Haridi A, Di Deco M, Coluzzi M. Cytogenetics of the *Anopheles gambiae* complex in Sudan, with special reference to *An. arabiensis*: Relationships with East and West African populations. Medical and Veterinary Entomology. 2000;**14**:149-164

[56] Ranson H, Abdallah H, Badolo A, Guelbeogo WM, Kerah-Hinzoumbé C, Yangalbé-Kalnoné E, et al. Insecticide resistance in *Anopheles gambiae*: Data from the first year of a multi-country study highlight the extent of the problem. Malaria Journal. 2009;**8**:299

[57] Abdalla H, Matambo T, Koekemoer L, Mnzava A, Hunt R, Coetzee M. Insecticide susceptibility and vector status of natural populations of *Anopheles arabiensis* from Sudan. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2008;**102**:263-271

[58] Matambo T, Abdalla H, Brooke B, Koekemoer L, Mnzava A, Hunt R, et al. Insecticide resistance in the malarial mosquito *Anopheles arabiensis* and association with the *kdr* mutation. Medical and Veterinary Entomology. 2007;**21**:97-102

[59] Himeidan YE, Hamid MMA, Jones CM, Ranson H. Extensive permethrin and DDT resistance in *Anopheles arabiensis* from eastern and Central Sudan. Parasites & Vectors. 2011;**4**:154

[60] Abdalla H, Wilding CS, Nardini L, Pignatelli P, Koekemoer LL, Ranson H, et al. Insecticide resistance in *Anopheles arabiensis* in Sudan: Temporal trends and underlying mechanisms. Parasites & Vectors. 2014;**7**:213

[61] Bashir NH, Adam YA, Nour BY. Monitoring susceptibility of *Anopheles arabiensis* Patton, 1905 (Diptera: Culicidae) to the recommended insecticides in Gedarif state, Sudan. International Journal of Mosquito Research. 2017;**4**(3):21-26

[62] Remijo C, Assad Y, Bala A. Susceptibility of *Anopheles* species populations collected from four localities in Juba County to DDT and deltamethrin, Central Equatoria state, southern Sudan. Resistant Pest Management Newsletter. 2010;**19**:2-8

[63] Alimi TO, Fuller DO, Quinones ML, Xue R-D, Herrera SV, Arevalo-Herrera M, et al. Prospects and recommendations for risk mapping to improve strategies for effective malaria vector control interventions in Latin America. Malaria Journal. 2015;**14**:519

[64] Tadei WP, Dutary Thatcher B. Malaria vectors in the Brazilian Amazon: *Anopheles* of the subgenus *Nyssorhynchus*. Revista do Instituto de Medicina Tropical de São Paulo. 2000; **42**:87-94

[65] Suarez MF, Quiñones M, Palacios J, Carrillo A. First record of DDT resistance in *Anopheles darlingi*. Journal of the American Mosquito Control Association. 1990;**6**:72-74

[66] Quinones ML, Suarez MF. Irritability to DDT of natural populations of the primary malaria vectors in Colombia. Journal of the American Mosquito Control Association. 1989;**5**:56-59

[67] Fonseca-González I. Estatus de la resistencia a insecticidas de los vectores primarios de malaria y dengue en Antioquia, Chocó, Norte de Santander y Putumayo, Colombia. Medellín: Universidad de Antioquia; 2008. p. 183

[68] Cáceres L, Rovira J, García A, Torres R. Determinación de la resistencia a insecticidas organofosforados, carbamatos y piretroides en tres poblaciones de *Anopheles albimanus* (Diptera: Culicidae) de Panamá. Biomédica. 2011;**31**:419-427

[69] Perea EZ, León RB, Salcedo MP, Brogdon WG, Devine GJ. Adaptation and evaluation of the bottle assay for monitoring insecticide resistance in disease vector mosquitoes in the Peruvian Amazon. Malaria Journal. 2009;**8**:208

[70] Vargas F, Córdova O, Alvarado A. Determinación de la resistencia a insecticidas en *Aedes aegypti*, *Anopheles albimanus* y *Lutzomyia peruensis* procedentes del norte peruano. Revista Peruana de Medicina Experimental y Salud Pública. 2006;**23**:259-264

[71] Hemingway J, Penilla RP, Rodriguez AD, James BM, Edge W, Rogers H, et al. Resistance management strategies in malaria vector mosquito control. A large-scale field trial in Southern Mexico. Pest Management Science. 1997;**51**:375-382

[72] Fonseca-González I, Cárdenas R, Quiñones ML, McAllister J, Brogdon WG. Pyrethroid and organophosphates resistance in *Anopheles* (*N.*) *nuneztovari* Gabaldón populations from malaria endemic areas in Colombia. Parasitology Research. 2009;**105**:1399

[73] Williams J, Pinto J. In: USAID, editor.Training Manual on Malaria Entomology; for Entomology and Vector Control Technicians (Basic Level). RTI International for USAID, President Malaria Initiative, PAHO, WHO. 2012. p. 78

[74] Tainchum K, Ritthison W, Sathantriphop S, Tanasilchayakul S, Manguin S, Bangs MJ, et al. Influence of time of assay on behavioral responses of laboratory and field populations *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae) to DEET. Journal of Medical Entomology. 2014;**51**:1227-1236. DOI: 10.1603/ME14004

[75] Weetman D, Donnelly MJ. Evolution of insecticide resistance diagnostics in malaria vectors. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2015;**109**: 291-293. DOI: 10.1093/trstmh/trv017

[76] Davies TG, Field LM, Usherwood PN, Williamson MS. DDT, pyrethrins, pyrethroids and insect sodium channels. IUBMB Life. 2007;**59**:151-162. DOI: 10.1080/15216540701352042

[77] Donnelly MJ, Corbel V, Weetman D, Wilding CS, Williamson MS, WCt B. Does *kdr* genotype predict insecticide-resistance phenotype in mosquitoes? Trends in Parasitology. 2009;**25**:213-219. DOI: 10.1016/j.pt.2009.02.007

[78] Du W, Awolola TS, Howell P, Koekemoer LL, Brooke BD, Benedict MQ, et al. Independent mutations in the Rdl locus confer dieldrin resistance to *Anopheles gambiae* and *An. arabiensis*. Insect Molecular Biology. 2005;**14**:179-183. IMB544 [pii]. DOI: 10.1111/j.1365-2583.2005.00544.x

[79] Wondji CS, Dabire RK, Tukur Z, Irving H, Djouaka R, Morgan JC. Identification and distribution of a GABA receptor mutation conferring dieldrin resistance in the malaria vector *Anopheles funestus* in Africa. Insect Biochemistry and Molecular Biology. 2011;**41**: 484-491. S0965-1748(11)00080-4 [pii]. DOI: 10.1016/j.ibmb.2011.03.012

[80] Fournier D. Mutations of acetylcholinesterase which confer insecticide resistance in insect populations. Chemico-Biological Interactions. 2005;**157-158**:257-261. DOI: 10.1016/j.cbi.2005.10.040

[81] Weill M, Lutfalla G, Mogensen K, Chandre F, Berthomieu A, Berticat C, et al. Insecticide resistance in mosquito vectors. Nature. 2003;**423**:136-137

[82] Alout H. Evolution of insecticide resistance at the *ace-1* locus in mosquito species [PhD thesis]. France: University of Montpellier 2; 2009

[83] Weill M, Malcolm C, Chandre F, Mogensen K, Berthomieu A, Marquine M, et al. The unique mutation in ace-1 giving high insecticide resistance is easily detectable in mosquito vectors. Insect Molecular Biology. 2004;**13**:1-7

[84] Djogbenou L, Chandre F, Berthomieu A, Dabire R, Koffi A, Alout H, et al. Evidence of introgression of the *ace-1*(R) mutation and of the ace-1 duplication in West African *Anopheles gambiae* s. s. PLoS One. 2008;**3**:e2172. DOI: 10.1371/journal.pone.0002172

[85] Ibrahim SS, Ndula M, Riveron JM, Irving H, Wondji CS. The P450 CYP6Z1 confers carbamate/pyrethroid cross-resistance in a major African malaria vector beside a novel carbamate-insensitive N485I acetylcholinesterase-1 mutation. Molecular Ecology. 2016; **25**:3436-3452. DOI: 10.1111/mec.13673

[86] Hemingway J, Hawkes NJ, McCarroll L, Ranson H. The molecular basis of insecticide resistance in mosquitoes. Insect Biochemistry and Molecular Biology. 2004;**34**:653-665

[87] Guillemaud T, Makate N, Raymond M, Hirst B, Callaghan A. Esterase gene amplification in *Culex pipiens*. Insect Molecular Biology. 1997;**6**:319-327

[88] Hawkes NJ, Hemingway J. Analysis of the promoters for the beta-esterase genes associated with insecticide resistance in the mosquito *Culex quinquefasciatus*. Biochimica et Biophysica Acta. 2002;**1574**:51-62

[89] Feyereisen R. Evolution of insect P450. Biochemical Society Transactions. 2006;**34**:1252-1255. DOI: 10.1042/bst0341252

[90] Scott JG, Liu N, Wen Z. Insect cytochromes P450: Diversity, insecticide resistance and tolerance to plant toxins. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology. 1998;**121**:147-155

[91] Stevenson BJ, Bibby J, Pignatelli P, Muangnoicharoen S, O'Neill PM, Lian LY, et al. Cytochrome P450 6M2 from the malaria vector *Anopheles gambiae* metabolizes pyrethroids: Sequential metabolism of deltamethrin revealed. Insect Biochemistry and Molecular Biology. 2011;**41**:492-502. DOI: 10.1016/j.ibmb.2011.02.003

[92] Mitchell SN, Stevenson BJ, Muller P, Wilding CS, Egyir-Yawson A, Field SG, et al. Identification and validation of a gene causing cross-resistance between insecticide classes in *Anopheles gambiae* from Ghana. Proceedings of the National Academy of Sciences of the United States of America. 2012;**109**:6147-6152. DOI: 10.1073/pnas.1203452109

[93] Riveron JM, Irving H, Ndula M, Barnes KG, Ibrahim SS, Paine MJ, et al. Directionally selected cytochrome P450 alleles are driving the spread of pyrethroid resistance in the major malaria vector *Anopheles funestus*. Proceedings of the National Academy of Sciences of the United States of America. 2013;**110**:252-257. DOI: 10.1073/pnas.1216705110

[94] Riveron JM, Ibrahim SS, Chanda E, Mzilahowa T, Cuamba N, Irving H, et al. The highly polymorphic CYP6M7 cytochrome P450 gene partners with the directionally selected CYP6P9a and CYP6P9b genes to expand the pyrethroid resistance front in the malaria vector *Anopheles funestus* in Africa. BMC Genomics. 2014;**15**:817. DOI: 10.1186/1471-2164-15-817

[95] Ibrahim SS, Riveron JM, Bibby J, Irving H, Yunta C, Paine MJ, et al. Allelic variation of cytochrome P450s drives resistance to bednet insecticides in a major malaria vector. PLoS Genetics. 2015;**11**:e1005618. DOI: 10.1371/journal.pgen.1005618

[96] Barnes KG, Weedall GD, Ndula M, Irving H, Mzihalowa T, Hemingway J, et al. Genomic footprints of selective sweeps from metabolic resistance to pyrethroids in African malaria vectors are driven by scale up of insecticide-based vector control. PLoS Genetics. 2017;**13**: e1006539. DOI: 10.1371/journal.pgen.1006539

[97] Enayati AA, Ranson H, Hemingway J. Insect glutathione transferases and insecticide resistance. Insect Molecular Biology. 2005;**14**:3-8

[98] Prapanthadara LA, Hemingway J, Ketterman AJ. Partial purification and characterization of glutathione S-transferases involved in DDT resistance from the mosquito *Anopheles gambiae*. Pesticide Biochemistry and Physiology. 1993;**47**:119-133. DOI: 10.1006/pest.1993.1070

[99] Prapanthadara L-A, Koottathep S, Promtet N, Hemingway J, Ketterman AJ. Purification and characterization of a major glutathione S-transferase from the mosquito *Anopheles dirus* (species B). Insect Biochemistry and Molecular Biology. 1996;**26**:277-285. DOI: 10.1016/0965-1748(95)00090-9

[100] Grant DF, Dietze EC, Hammock BD. Glutathione S-transferase isozymes in *Aedes aegypti*: Purification, characterization, and isozyme-specific regulation. Insect Biochemistry. 1991; **21**:421-433. DOI: 10.1016/0020-1790(91)90009-4

[101] Mitchell SN, Rigden DJ, Dowd AJ, Lu F, Wilding CS, Weetman D, et al. Metabolic and target-site mechanisms combine to confer strong DDT resistance in *Anopheles gambiae*. PLoS One. 2014;**9**:e92662. DOI: 10.1371/journal.pone.0092662

[102] Herath PR, Miles SJ, Davidson G. Fenitrothion (OMS 43) resistance in the taxon *Anopheles culicifacies* Giles. The Journal of Tropical Medicine and Hygiene. 1981;**84**:87-88

[103] Hemingway J. The genetics of malathion resistance in *Anopheles stephensi* from Pakistan. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1983;**77**:106-108. DOI: 10.1016/0035-9203(83)90030-5

[104] Yahouédo GA, Chandre F, Rossignol M, Ginibre C, Balabanidou V, Mendez NGA, et al. Contributions of cuticle permeability and enzyme detoxification to pyrethroid resistance in the major malaria vector *Anopheles gambiae*. Scientific Reports. 2017;**7**:11091. DOI: 10.1038/s41598-017-11357-z

[105] Wood OR, Hanrahan S, Coetzee M, Koekemoer LL, Brooke BD. Cuticle thickening associated with pyrethroid resistance in the major malaria vector *Anopheles funestus*. Parasites & Vectors. 2010;**3**:67. DOI: 10.1186/1756-3305-3-67

[106] Rowland M. Behaviour and fitness of γHCH/dieldrin resistant and susceptible female *Anopheles gambiae* and *An. stephensi* mosquitoes in the absence of insecticide. Medical and Veterinary Entomology. 1991;**5**:193-206. DOI: 10.1111/j.1365-2915.1991.tb00542.x

[107] Rowland M. Activity and mating competitiveness of gamma HCH/dieldrin resistant and susceptible male and virgin female *Anopheles gambiae* and *An. stephensi* mosquitoes, with assessment of an insecticide-rotation strategy. Medical and Veterinary Entomology. 1991; **5**:207-222

[108] Berticat C, Boquien G, Raymond M, Chevillon C. Insecticide resistance genes induce a mating competition cost in *Culex pipiens* mosquitoes. Genetical Research. 2002;**79**:41-47

[109] Platt N, Kwiatkowska RM, Irving H, Diabate A, Dabire R, Wondji CS. Target-site resistance mutations (*kdr* and *RDL*), but not metabolic resistance, negatively impact male mating competiveness in the malaria vector *Anopheles gambiae*. Heredity (Edinb). 2015; **115**:243-252. DOI: 10.1038/hdy.2015.33 hdy201533 [pii]

[110] Agnew P, Berticat C, Bedhomme S, Sidobre C, Michalakis Y. Parasitism increases and decreases the costs of insecticide resistance in mosquitoes. International Journal of Organic Evolution. 2004;**58**:579-586

[111] Shi MA, Lougarre A, Alies C, Fremaux I, Tang ZH, Stojan J, et al. Acetylcholinesterase alterations reveal the fitness cost of mutations conferring insecticide resistance. BMC Evolutionary Biology. 2004;**4**:5. DOI: 10.1186/1471-2148-4-5 1471-2148-4-5 [pii]

[112] Georghiou GP, Taylor CE. Factors influencing the evolution of resistance. In: Pesticide Resistance: Strategies and Tactics for Management. Washington, DC: National Academy Press; 1986. pp. 157-169

[113] Brito LP, Linss JG, Lima-Camara TN, Belinato TA, Peixoto AA, Lima JB, et al. Assessing the effects of *Aedes aegypti kdr* mutations on pyrethroid resistance and its fitness cost. PLoS One. 2013;**8**:e60878. DOI: 10.1371/journal.pone.0060878

[114] Martins AJ, Ribeiro CD, Bellinato DF, Peixoto AA, Valle D, Lima JB. Effect of insecticide resistance on development, longevity and reproduction of field or laboratory selected

Aedes aegypti populations. PLoS One. 2012;**7**:e31889. DOI: 10.1371/journal.pone.0031889 PONE-D-11-16359 [pii]

[115] Rivero A, Vézilier J, Weill M, Read AF, Gandon S. Insecticide control of vector-borne diseases: When is insecticide resistance a problem? PLoS Pathogens. 2010;**6**:e1001000. DOI: 10.1371/journal.ppat.1001000

[116] Mahande AM, Dusfour I, Matias JR, Kweka EJ. Knockdown resistance, *rdl* alleles, and the annual entomological inoculation rate of wild mosquito populations from Lower Moshi, northern Tanzania. Journal of Global Infectious Diseases. 2012;**4**:114-119. DOI: 10.4103/0974-777X.96776 JGID-4-114 [pii]

[117] Ranson H, N'Guessan R, Lines J, Moiroux N, Nkuni Z, Corbel V. Pyrethroid resistance in African anopheline mosquitoes: What are the implications for malaria control? Trends in Parasitology. 2011;**27**:91-98. DOI: 10.1016/j.pt.2010.08.004

[118] Brooke BD, Kloke G, Hunt RH, Koekemoer LL, Temu EA, Taylor ME, et al. Bioassay and biochemical analyses of insecticide resistance in southern African *Anopheles funestus* (Diptera: Culicidae). Bulletin of Entomological Research. 2001;**91**:265-272

[119] Hargreaves K, Koekemoer LL, Brooke BD, Hunt RH, Mthembu J, Coetzee M. *Anopheles funestus* resistant to pyrethroid insecticides in South Africa. Medical and Veterinary Entomology. 2000;**14**:181-189

[120] Maharaj R, Mthembu DJ, Sharp BL. Impact of DDT re-introduction on malaria transmission in KwaZulu-Natal. South African Medical Journal. 2005;**95**:871-874

[121] Kleinschmidt I, Sharp B, Benavente LE, Schwabe C, Torrez M, Kuklinski J, et al. Reduction in infection with *Plasmodium falciparum* one year after the introduction of malaria control interventions on Bioko Island, Equatorial Guinea. The American Journal of Tropical Medicine and Hygiene. 2006;**74**:972-978. 74/6/972 [pii]

[122] Sharp BL, Ridl FC, Govender D, Kuklinski J, Kleinschmidt I. Malaria vector control by indoor residual insecticide spraying on the tropical island of Bioko, Equatorial Guinea. Malaria Journal. 2007;**6**:52. 1475-2875-6-52 [pii]. DOI: 10.1186/1475-2875-6-52

[123] Protopopoff N, Van Bortel W, Marcotty T, Van Herp M, Maes P, Baza D, et al. Spatial targeted vector control in the highlands of Burundi and its impact on malaria transmission. Malaria Journal. 2007;**6**:158. 1475-2875-6-158 [pii]. DOI: 10.1186/1475-2875-6-158

[124] Lengeler C. Insecticide-treated bednets and curtains for preventing malaria. Cochrane Database of Systematic Reviews. 2000;(2):CD000363 [pii]. DOI: 10.1002/14651858.CD000363

[125] Martinez-Torres D, Chandre F, Williamson MS, Darriet F, Berge JB, Devonshire AL, et al. Molecular characterization of pyrethroid knockdown resistance (*kdr*) in the major malaria vector *Anopheles gambiae* s.s. Insect Molecular Biology. 1998;**7**:179-184

[126] Henry MC, Assi SB, Rogier C, Dossou-Yovo J, Chandre F, Guillet P, et al. Protective efficacy of lambda-cyhalothrin treated nets in *Anopheles gambiae* pyrethroid resistance areas of Cote d'Ivoire. The American Journal of Tropical Medicine and Hygiene. 2005;**73**: 859-864. 73/5/859 [pii]

[127] Corbel V, Akogbeto M, Damien GB, Djenontin A, Chandre F, Rogier C, et al. Combination of malaria vector control interventions in pyrethroid resistance area in Benin: A cluster randomised controlled trial. The Lancet Infectious Diseases. 2012;**12**:617-626

[128] Gillies MT, De Meillon B: The Anophelinae of Africa South of the Sahara (Ethiopian Zoogeographical Region). 1968;**54**:1-343

[129] Govella NJ, Chaki PP, Killeen GF. Entomological surveillance of behavioural resilience and resistance in residual malaria vector populations. Malaria Journal. 2013;**12**:124. DOI: 10.1186/1475-2875-12-124

[130] Moiroux N, Gomez MB, Pennetier C, Elanga E, Djenontin A, Chandre F, et al. Changes in *Anopheles funestus* biting behavior following universal coverage of long-lasting insecticidal nets in Benin. The Journal of Infectious Diseases. 2012;**206**:1622-1629. DOI: 10.1093/infdis/jis565

[131] Ojuka P, Boum Y 2nd, Denoeud-Ndam L, Nabasumba C, Muller Y, Okia M, et al. Early biting and insecticide resistance in the malaria vector *Anopheles* might compromise the effectiveness of vector control intervention in Southwestern Uganda. Malaria Journal. 2015;**14**:148. DOI: 10.1186/s12936-015-0653-z

[132] Thomsen EK, Koimbu G, Pulford J, Jamea-Maiasa S, Ura Y, Keven JB, et al. Mosquito behaviour change after distribution of bednets results in decreased protection against malaria exposure. Journal of Infectious Diseases. 2017;**215**(5):790-797. DOI: 10.1093/infdis/jiw615

[133] Meyers JI, Pathikonda S, Popkin-Hall ZR, Medeiros MC, Fuseini G, Matias A, et al. Increasing outdoor host-seeking in *Anopheles gambiae* over 6 years of vector control on Bioko Island. Malaria Journal. 2016;**15**:239. DOI: 10.1186/s12936-016-1286-6

[134] Etang J, Nono BF, Awono-Ambene P, Bigoga J, Eyisap WE, Piameu M, et al. Resting behaviour of deltamethrin-resistant malaria vectors, *Anopheles arabiensis* and *Anopheles coluzzii*, from North Cameroon: Upshots from a two-level ordinary logit model. In: Current Topics in Malaria. Rijeka: InTech Open Access; 2016

[135] Killeen GF, Chitnis N. Potential causes and consequences of behavioural resilience and resistance in malaria vector populations: A mathematical modelling analysis. Malaria Journal. 2014;**13**:97. DOI: 10.1186/1475-2875-13-97

[136] Main BJ, Lee Y, Ferguson HM, Kreppel KS, Kihonda A, Govella NJ, et al. The genetic basis of host preference and resting behavior in the major African malaria vector, *Anopheles arabiensis*. PLoS Genetics. 2016;**12**:e1006303

[137] Sinka ME, Bangs MJ, Manguin S, Rubio-Palis Y, Chareonviriyaphap T, Coetzee M, Mbogo CM, Hemingway J, Patil AP, Temperley WH, Gething PW, Kabaria CW, Okara RM, Burkot TR, Harbach RE, Hay SI. A global map of dominant malaria vectors. Parasites & Vectors. 2012;**5**:69

Section 3

Current Status and Advances in Specific Regions

Chapter 8

Malaria Elimination in the Greater Mekong Subregion: Challenges and Prospects

Liwang Cui, Yaming Cao, Jaranit Kaewkungwal, Amnat Khamsiriwatchara, Saranath Lawpoolsri, Than Naing Soe, Myat Phone Kyaw and Jetsumon Sattabongkot

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76337

Abstract

Malaria is a significant public health problem and impediment to socioeconomic development in countries of the Greater Mekong Subregion (GMS), which comprises Cambodia, China's Yunnan Province, Lao People's Democratic Republic, Myanmar, Thailand, and Vietnam. Over the past decade, intensified malaria control has greatly reduced the regional malaria burden. Driven by increasing political commitment, motivated by recent achievements in malaria control, and urged by the imminent threat of emerging artemisinin resistance, the GMS countries have endorsed a regional malaria elimination plan with a goal of eliminating malaria by 2030. However, this ambitious, but laudable, goal faces a daunting array of challenges and requires integrated strategies tailored to the region, which should be based on a mechanistic understanding of the human, parasite, and vector factors sustaining continued malaria transmission along international borders. Malaria epidemiology in the GMS is complex and rapidly evolving. Spatial heterogeneity requires targeted use of the limited resources. Border malaria accounts for continued malaria transmission and represents sources of parasite introduction through porous borders by highly mobile human populations. Asymptomatic infections constitute huge parasite reservoir requiring interventions in time and place to pave the way for malaria elimination. Of the two most predominant malaria parasites, *Plasmodium falciparum* and *P. vivax*, the prevalence of the latter is increasing in most member GMS countries. This parasite requires the use of 8-aminoquinoline drugs to prevent relapses from liver hypnozoites, but high prevalence of glucose-6-phosphate dehydrogenase deficiency in the endemic human populations makes it difficult to adopt this treatment regimen. The recent emergence of resistance to artemisinins and partner drugs in *P. falciparum* has raised both regional and global concerns, and elimination efforts are invariably prioritized against this parasite to avert spread. Moreover, the effectiveness of the two core vector control interventions—insecticide-treated nets and indoor residual spraying—has been declining due to insecticide

resistance and increased outdoor biting activity of mosquito vectors. These technical challenges, though varying from country to country, require integrated approaches and better understanding of the malaria epidemiology enabling targeted control of the parasites and vectors. Understanding the mechanism and distribution of drug-resistant parasites will allow effective drug treatment and prevent, or slow down, the spread of drug resistance. Coordination among the GMS countries is essential to prevent parasite reintroduction across the international borders to achieve regional malaria elimination.

Keywords: malaria elimination, Greater Mekong Subregion, epidemiology, drug resistance, migration, insecticide resistance

1. Introduction

With steady gains in the fight against malaria over the past decade, the international malaria community once again is embracing the global goal of malaria eradication. Meanwhile, the World Health Organization (WHO) has launched a new Global Technical Strategy for Malaria (http://www.who.int/malaria/areas/global_technical_strategy/en/) as the operational framework guiding malarious nations and regions in their pursuit of malaria elimination. In the Greater Mekong Subregion (GMS) of Southeast Asia (SEA), which comprises Cambodia, China's Yunnan Province, Lao People's Democratic Republic (Laos), Myanmar, Thailand, and Vietnam, malaria has been a significant public health problem and impediment to socioeconomic development [1, 2]. Intensified malaria control in recent years, fueled by increased international funding and local bustling economic development, has greatly reduced the regional malaria burden. Compared with confirmed malaria cases in 2010, the number of malaria cases in the GMS was reduced by ~50% in 2014. Driven by increasing political will and financial support and motivated by recent achievements in malaria control, the six GMS nations have endorsed a regional malaria elimination plan with an ultimate goal of eliminating *Plasmodium falciparum* malaria by 2025 and all malaria by 2030 [3]. Emerging artemisinin resistance in this region further escalated urgency for National Malaria Control Programmes (NMCPs) to make such a transition of their aims [4, 5]. However, this ambitious goal faces numerous technical challenges [6] and requires integrated strategies tailored to the whole region and individual countries. In the malaria elimination settings, control strategies need to align with the changing malaria epidemiology. Control measures such as long-lasting insecticide-treated bed nets (LLINs), indoor residual insecticide spraying (IRS), rapid diagnostic tests (RDTs), and artemisinin combined therapies (ACTs) used to effectively reduce malaria burden in hyperendemic regions may not be enough for the malaria elimination task. Additional tools such as mass drug administration (MDA) and innovative vector control programs may be needed. Here, we attempt to provide an updated view of the changing malaria epidemiology, the challenges, and prospect of malaria elimination in the GMS.

2. Border malaria

Malaria epidemiology in the GMS is complex and rapidly evolving. There is immense spatial heterogeneity in both regional and countrywide disease distribution (**Figure 1** and **Table 1**). Within the GMS, Myanmar has the heaviest malaria burden and accounts for more than 53%

of regionally confirmed malaria cases. Within each country, the pattern of malaria distribution remains similar, but transmission is still concentrated along international borders—the so-called border malaria. In border areas, there is poor accessibility to healthcare services, and surveillance for malaria is far less than optimal [8]. Given that these border regions represent probable malaria reservoirs and that importation and dispersal by migratory human populations are extremely difficult to monitor, border malaria constitutes one of the biggest obstacles for malaria elimination. Highly mobile populations crossing porous borders are a major contributor to parasite introduction and continued transmission [9]. Border areas also are home to ethnic minorities, hill tribes, temporary and seasonal migrants, refugees, and internally displaced people; many have poor educational level, limited access to healthcare services, and reduced legal rights. Geographical and cultural isolation leaves these groups at a high risk for infection and poor access to treatment [1, 2, 10, 11]. In Thailand, malaria makes up ~31% of communicable diseases diagnosed in migrants, as compared to 3% in Thai natives [12]. Heavy population flow along the extremely porous borders makes neighboring countries very vulnerable to malaria introduction and reintroduction [13, 14]. As a result, malaria prevalence on both sides of the border is often highly correlated [15]. In Yunnan Province of China, although autochthonous *P. vivax* malaria was still detected, *P. falciparum* infections were mostly associated with travel history to Myanmar [16]. There is also genetic evidence of asymmetric parasite flow from the more endemic to the less endemic side of the border [17, 18]. On a smaller geographical scale in a border village in Western Thailand, malaria incidence was clustered and significantly associated with citizen status indicating recent migration [19]. Moreover, there is a high probability that frequent border crossings by migrants will spread artemisinin-resistant *P. falciparum* [20, 21] beyond the "containment zone" [22, 23]. More sophisticated surveillance

Figure 1. The geographical proximity of countries and reported malaria cases for data based on 2016 in the Greater Mekong Subregion (GMS). *Note*: The majority of malaria cases in Yunnan Province of China were imported.

Country/drug policy*	Year	No. of malaria cases	% of confirmed cases°			No. of death cases
			Pf	Pv	Others	
China	2011	3000	41.9	56.6	1.5	≤100
Uncomplicated Pf: ART + NQ; AS + AQ; D-P Severe malaria: AM; AS; pyronaridine *P. vivax:* CQ + PQ (8d)	2012	240	8.2	91.8	—	0
	2013	≤100	64.1	35.9	—	0
	2014	≤100	10.7	89.3	—	0
	2015	≤100	3.0	78.8	18.2	0
	2016	≤10	0.0	100.0	—	0
Cambodia	2011	203,600	62.6	37.4	—	400
Uncomplicated Pf: AS + MQ, D-P Severe malaria: AM; AS; QN *P. vivax:* D-P + PQ (14d)	2012	146,000	50.4	49.6	—	220
	2013	76,500	45.8	54.2	—	110
	2014	89,700	58.8	41.2	—	150
	2015	120,300	61.3	38.7	—	210
	2016	83,300	58.2	41.8	—	140
Laos	2011	42,800	92.7	7.1	0.2	≤100
Uncomplicated Pf: AL Severe malaria: AS + AL *P. vivax:* CQ + PQ (14d)	2012	112,700	83.4	16.6	—	250
	2013	93,500	67.0	33.0	—	170
	2014	117,300	52.9	47.1	—	180
	2015	87,900	42.3	57.7	—	120
	2016	27,390	39.5	60.5	—	≤100
Myanmar	2011	1,506,000	68.4	31.6	—	2800
Uncomplicated Pf: AL; AM; AS + MQ; D-P; PQ Severe malaria: AM; AS; QN *P. vivax:* CQ + PQ (14d)	2012	1,974,000	71.8	28.2	—	4000
	2013	585,000	70.4	29.6	—	1100
	2014	360,000	69.9	30.1	—	700
	2015	236,500	64.1	35.9	—	400
	2016	142,600	60.3	39.7	—	240
Thailand	2011	24,900	40.5	59.5	0.1	≤100
Uncomplicated Pf: D-P Severe malaria: QN + doxycycline *P. vivax:* CQ + PQ (14d)	2012	32,600	39.8	60.2	—	≤100
	2013	33,300	44.0	46.8	9.3	≤100
	2014	37,900	37.8	54.1	8.1	≤100
	2015	8000	41.7	58.0	0.2	≤100
	2016	11,520	32.5	46.1	21.5	≤100

Country/drug policy*	Year	No. of malaria cases	% of confirmed cases°			No. of death cases
			Pf	Pv	Others	
Vietnam	2011	22,630	64.3	35.7	—	≤100
Uncomplicated Pf:	2012	26,610	61.3	38.7	—	≤100
D-P	2013	23,140	58.0	42.0	—	≤100
Severe malaria:	2014	21,200	54.2	45.8	—	≤100
AS; QN	2015	12,560	48.9	51.0	0.2	≤100
P. vivax: CQ + PQ (14d)	2016	6000	57.6	42.1	0.4	≤10

*AL, artemether + lumefantrine; AM, artemether; AQ, amodiaquine; ART, artemisinin; AS, artesunate; CQ, chloroquine; D-P, dihydroartemisinin + piperaquine; MQ, mefloquine; NQ, naphthoquine; PQ, primaquine; QN, quinine.
°Pf: *Plasmodium falciparum*; Pv: *Plasmodium vivax*.

Table 1. Antimalarial drug policy and malaria transmission trends in the Greater Mekong Subregion (GMS) countries during 2011–2016 [7].

tools are needed to provide a clear picture of border malaria transmission so that targeted control measures are implemented to curb the spread of resistance and to prevent the reintroduction of parasites into populations where they have been eliminated. Thus, malaria elimination is a multinational, multipronged issue, with cross-border migration posing one of the largest threats to its success [24]. In recognition of this issue, the GMS countries have initiated bi- and multilateral coordination between the NMCPs. While the healthcare systems in the GMS countries are improving, further bolstering is needed to meet the malaria elimination challenge.

3. Asymptomatic malaria as an important reservoir

It has long been held as conventional wisdom that asymptomatic infections would be much less frequent in low-endemicity settings because the level of exposure-related immunity to malaria in human populations may be low [25]. However, asymptomatic infections represent the vast majority of infections in all endemic settings [26]. The use of molecular tools is essential for identifying submicroscopic infections. For both *P. falciparum* and *P. vivax*, microscopy detects only 1/3–1/2 of the infections detected by regular PCR [27, 28]. As the sensitivity of detection methods increases (e.g., with the use of a larger blood volume or reverse transcriptase-PCR targeting the parasite 18S rRNA), greater proportions of asymptomatic infections are discovered, revealing larger pools of infections [29, 30]. In Western Thailand and other GMS regions, qPCR and large-volume ultrasensitive qPCR could detect as much as 20% of the villagers harboring malaria infections as compared to ~5% detected by microscopy [31, 32]. Although we still do not have a clear picture about how much these asymptomatic infections actually contribute to malaria transmission in these areas [33], studies in Western Thailand have clearly demonstrated mosquito infectivity of submicroscopic *P. falciparum* and *P. vivax* [34], albeit the asymptomatic parasite carriers were found to be much less infective to mosquitoes than acute cases [35]. Since asymptomatic individuals are unlikely to seek treatment, they are missed by passive case detection, and submicroscopic infections also are missed by microscopy-based active case detection. It is highly possible that these asymptomatic infections act as important silent reservoirs of transmission. Even under such

low-endemicity settings, it is estimated that submicroscopic carriers may be the source of 20–50% of all human-to-mosquito transmission [36], underlining the significance of managing this population in the malaria elimination phase. Therefore, information about the prevalence and seasonal dynamics of the asymptomatic infections in the border regions and their contribution to transmission is required to guide the efforts of NMCPs to achieve malaria elimination.

4. The burden of *P. vivax* malaria and G6PD deficiency

Another characteristic of the rapidly evolving malaria epidemiology in the GMS is that the prevalence of *P. vivax* is increasing proportionally to *P. falciparum* [37] (**Table 1**). The resilience of vivax malaria to control efforts may be attributed to some intrinsic biological features of this parasite. First, *P. vivax* only invades reticulocytes, and thus the resulting parasitemia is normally far lower than that of *P. falciparum* malaria. This makes microscopy-based diagnosis and RDTs not sufficiently sensitive in detecting *P. vivax* infections [38–40]. Second, during blood-stage infections with *P. vivax*, gametocytes are formed before the manifestation of clinical symptoms, which allows transmission of the parasite before treatment. Third, *P. vivax* develops dormant hypnozoites in the liver of the human host, which awaken in the weeks and months following a primary attack and cause relapses. Finally, vivax malaria is often transmitted by outdoor biting mosquitoes, making the current insecticide-based control measures (LLIN and IRS) less effective. Because of these unique features, traditional malaria control efforts often fail to control *P. vivax* transmission. In addition, containment of *P. falciparum* has been prioritized in the GMS, partially because of the emerging artemisinin resistance. As a result, *P. falciparum* prevalence has decreased, while the proportion of *P. vivax* has increased.

In the GMS, the first-line therapy for vivax malaria remains chloroquine (CQ) and primaquine (PQ) (**Table 1**) [41]. Reports of clinical CQ resistance in many regions of the world and falling efficacy of PQ are of great concern for vivax malaria control [42–45]. Although some studies indicated that *P. vivax* in the GMS remained sensitive to CQ [46–51], others clearly documented CQ-resistant *P. vivax* [52–55]. In Myanmar, sporadic CQ-resistant *P. vivax* cases were first reported more than 20 years ago [52, 53]. A later report of 34% treatment failures in Dawei of Southern Myanmar suggests an increase of CQ resistance [55]. More recent studies identified both early and late treatment failures in Myawaddy of the Kayin State and Kawthaung of the Tanintharyi Region, Myanmar [56]. In northeastern Myanmar bordering China, a recent study showed 5.2% cumulative incidence of recurrent parasitemia during a 28-day follow-up of 587 *P. vivax* treated with CQ/PQ [57], suggesting sensitivity to CQ may also be deteriorating in this region. This reduced sensitivity of *P. vivax* to CQ requires close surveillance and potential implementation of more effective treatment measures such as ACTs [58].

Studies from Papua New Guinea suggest that 80% of the vivax infections may be attributed to relapses. A modeling approach predicts that as much as 96% of clinical attacks by *P. vivax* in Thailand are due to relapses [60]. For radical cure, WHO recommends a dose of 0.25–0.5 mg/kg of PQ daily for 14 days. However, the lower dose (total of 3.5 mg/kg) fails to prevent relapses in many different endemic sites [61]. Because of the potential risk of severe hemolysis that this drug could cause in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency, PQ is not widely prescribed [43, 62, 63]. In routine practice, G6PD status is not screened; the GMS

nations still use the lower total dose of PQ in fear of the possible harm to those with G6PD deficiency. Because evaluation of PQ efficacy in preventing relapses requires longer-term follow-up, the clinical efficacy of the current PQ regimen for radical cure of vivax malaria in the GMS is unknown. Even with longer follow-ups, it is still not possible to reliably determine whether a recurrent infection after day 28 is due to relapse or reinfection given that a relapse infection may be from reactivation of a different hypnozoite clone [64, 65]. For PQ efficacy, host factors also need to be considered. Recently, failures of the PQ radical cure have been linked to reduced activity of the hepatic cytochrome P450 (CYP) 2D6 [66], which mediates activation of PQ to its active metabolite(s) [67, 68]. Different CYP2D6 activities have differential effects on the pharmacokinetics of PQ [69]. CYP2D6 is involved in the metabolism of as many as 25% of drugs in clinical use and is also a member of the CYP450 family with the greatest prevalence and genetic polymorphism [70, 71]. About 70 CYP2D6 allelic variants have been found and grouped into 4 phenotypic classes of ultra-rapid, extensive, intermediate, and abolished protein activity [72]. The frequency of alleles with reduced function is as high as 50% in most Asian populations [73]. Thus, it is important to determine the extent by which reduced CYP2D6 activity is responsible for PQ failures in radical cure of vivax malaria [74].

The *G6PD* gene is extraordinarily polymorphic with more than 400 variants discovered based on biochemical diagnosis [75], among which 186 mutations are associated with G6PD deficiency [76]. The prevalence of G6PD deficiency and distribution of G6PD variants vary geographically [77]. In the GMS, G6PD deficiency is often highly prevalent among ethnic groups. Along the Thailand-Myanmar border, the prevalence of G6PD deficiency was above 10% [78–80], whereas in the Kachin ethnicity along the China-Myanmar border, it almost reached 30% [81]. In Thailand and Myanmar, the Mahidol variant (487G>A) is the most predominant and often accounts for ~90% of all mutations [79, 81–83]. According to the WHO classification, the Mahidol variant is a Class III mutation or mild-deficient variant with 60% enzyme activity [76]. However, this classification may not be accurate since patients with the Mahidol variant often had <1% of the normal G6PD activity [79, 84, 85]. Patients having the G6PD Mahidol variant (487G>A) rarely had acute hemolytic anemia after taking the normal dose of PQ [84, 86]. In contrast to the belief that PQ only induces mild hemolysis in patients with the Mahidol variant, there have been case reports showing that the normal dosage of 15 mg/kg/day for 3 days in vivax patients with this G6PD variant could lead to acute hemolytic anemia that required blood transfusion or even cause renal failure [87–89]. It is noteworthy that G6PD activity can vary substantially between individuals with the same variant and even within the same individual over time. Therefore, with the prevalence of vivax malaria in this region and the goal of malaria elimination, the deployment of point-of-care G6PD deficiency diagnostics is urgent [90]. In addition, there is a need to test whether weekly PQ of 0.75 mg/kg for 8 weeks, a dosage considered safe for the G6PD African variant [91], could be prescribed in the GMS without prior testing for G6PD deficiency.

5. Management of drug resistance in *P. falciparum*

ACTs have played an indispensable role in reducing global malaria-associated mortality and morbidity. However, these achievements are threatened by the recent emergence of artemisinin resistance in *P. falciparum* in the GMS [92–94]. Artemisinin resistance is associated with a parasite clearance half-life of >5 h as compared to a normal value of ~2 h [94–96].

Clinical artemisinin resistance was first detected in western Cambodia [92, 93, 96, 97] but is now detected in other GMS regions including Thailand, Laos, Vietnam, Southern Myanmar, and the China-Myanmar border area [94, 95, 98–103]. Out of fear of a catastrophic spread of artemisinin resistance to Africa, WHO deployed an artemisinin resistance containment plan in Cambodia [104]. Later, with the finding that artemisinin resistance has emerged independently in many areas of the GMS [105], the containment plan has been revised to a regional malaria elimination strategy [3, 4].

The principle of ACTs is that the fast-acting artemisinins rapidly reduce the parasite biomass, leaving the slow-eliminating partner drugs to clear the residual parasites. The emergence of artemisinin resistance means that a larger parasite mass is left for the partner drugs to clear after the usual 3-day ACT course, which increases the chance of resistance development to the partner drugs. Indeed, in the short period of time since the deployment of ACTs, clinical resistance to two ACTs, first artesunate/mefloquine [106] and more recently dihydroartemisinin/piperaquine (DHA/PPQ), has emerged in the GMS. These are the two most popular ACTs deployed in the GMS countries (**Table 1**). Since promising new antimalarials are still in the development pipeline, possible solutions to this problem include introduction of new ACTs, rotation of different ACTs, use of longer course of ACT treatment, and introduction of triple ACTs (artemisinin derivatives with two slow-eliminating partner drugs) [112]. To mitigate the threat of spread of artemisinin-resistant *P. falciparum* parasites, heightened surveillance is needed in sentinel sites of the GMS [113].

Tools for monitoring the epidemiology of antimalarial drug resistance include ex vivo or in vitro drug assays and molecular surveillance, which complement in vivo drug efficacy studies. It is noteworthy that the slow-clearance phenotype of clinical artemisinin resistance does not correspond to the 50% inhibitory concentrations of artemisinin drugs estimated from the conventional DNA replication-based in vitro assay but is better reflected in the newly developed ring-stage survival assay, which quantifies the number of early ring-stage parasites (0–3 h) that can survive the exposure to 700 nM of DHA for 6 h [114]. The discovery of mutations in the *kelch* domain protein K13 associated with artemisinin resistance provides a convenient molecular marker for a large-scale surveillance purpose [115]. To date, the correlations of K13 mutations with delayed parasite clearance have been established in several studies [95, 105, 115–117] but only a very limited number of K13 mutations were confirmed to confer in vitro artemisinin resistance through genetic manipulations [118, 119]. The K13 gene in the world *P. falciparum* populations harbors more than 108 nonsynonymous mutations, which showed marked geographic disparity in frequency and distribution [120]. Similarly, K13 mutations in the GMS also showed highly heterogeneous distribution [103, 121–125], possibly reflecting different drug histories and evolutionary origins of the parasite populations [126]. Clinical failures of DHA/PPQ have been associated with increased in vitro PPQ resistance and the molecular markers of PPQ resistance in western Cambodia include amplification of the aspartic protease genes *plasmepsin 2–3* and point mutation E415G in an exonuclease gene (PF3D7_12362500) [127, 128]. Molecular surveillance of artemisinin resistance in western Cambodia, Thailand, and Laos has detected the spread of a parasite clone with a long K13 haplotype carrying the C580Y mutation (the artemisinin-resistant mutation reaching near fixation in western Cambodia) to northeastern Thailand and southern Laos, which indicates a transnational selective sweep [129]. Importantly, this parasite lineage also harbors the *plasmepsin 2* amplification, which may preclude further use of DHA/PPQ in this region. In addition, this situation also necessitates implementation of

http://dx.doi.org/10.5772/intechopen.76337

stringent follow-ups of malaria cases after ACT treatment to ensure that recrudescent cases are treated with effective antimalarials. Thus, surveillance should be mandatory to delay the spread of the resistant parasites and to accelerate malaria elimination in the GMS.

6. Vectors

LLINs and IRS are the key vector-based malaria interventions that have been found to be highly effective in sub-Saharan Africa. However, these measures are much less efficient in the GMS [130]. The GMS has a complex vector system; most of the malaria vectors belong to species complexes or groups such as Dirus, Minimus, Maculatus, and Sundaicus, which vary significantly in terms of geographic distribution, ecology, behavior, and vectorial competence [131–133]. At least 19 species are known malaria vectors, some of which comprise cryptic species complexes [132]. In order to apply the appropriate control approaches in relation to the biology of the vector species, we first need to identify the mosquitoes to their species level and to differentiate the vector from nonvector species, which requires molecular assays [134]. These vector species display significant variations in geographical distribution and seasonal dynamics, and accordingly their roles in malaria transmission also vary in space and time [135]. In many endemic areas of the GMS, perennial malaria transmission is maintained by *Anopheles dirus* during the rainy season and *An. minimus* during the drier periods of the year [132, 136]. Environmental changes such as deforestation have caused changes in the vector species composition [137, 138] and benefited the survivorship of major vectors [40]. Since many of these vector species exhibit early evening and outdoor biting preferences, LLINs alone are not sufficient for interrupting malaria transmission [140]. In addition, the emergence and spread of insecticide resistance further compromise the effectiveness of the mosquito control measures [141–143].

7. Technological innovation for malaria elimination

The technical challenges discussed here suggest that the currently used malaria control tools (RDT, ACT, LLIN, and IRS) that were instrumental for the gains against malaria may not be sufficient for malaria elimination [144]. Additional tools are needed to achieve the final goal of malaria elimination in the GMS. First, residual transmission requires MDA to eliminate asymptomatic and submicroscopic parasite reservoirs. For the success of MDA, better knowledge of malaria epidemiology is needed so that targeted MDA can be implemented. Successful MDA programs also require strong community engagement. MDA has proved successful in eliminating malaria in Asia-Pacific regions such as Vanuatu and central China [145, 146]. In an earlier study conducted in Cambodian villages, MDA of artemisinin-PPQ at 10-day intervals for 6 months drastically reduced *P. falciparum* rates [147]. A recent pilot MDA study conducted in villages of Kayin State, Myanmar, showed that a 3-day supervised course of DHA/PPQ was well tolerated and highly effective in reducing asymptomatic *P. falciparum* carriage, whereas the effect on reducing *P. vivax* was transient presumably due to relapse [148]. Thus, drugs targeting the *P. vivax* hypnozoite reservoir are required for MDA in the GMS, where *P. vivax* is becoming the predominant parasite species [149]. The high prevalence of G6PD deficiency in the target populations demands prescreening using a point-of-care diagnostic for G6PD deficiency. From

a programmatic standpoint, such an operation requires substantial financial commitment. Second, effective management of malaria cases in the face of emergence and spread of drug resistance requires new therapies such as triple ACTs. Third, novel vector control approaches are desperately needed including larval control strategies [150], incorporation of ivermectin in the MDA program to reduce the life span of mosquitoes [151, 152], topical and spatial repellents against outdoor biting vectors [153, 154], genetically manipulated mosquitoes for population replacement [155], and next generation of LLINs and IRS [156]. It is imperative that new interventions are continuously developed and integrated into malaria elimination programs.

8. Conclusions

Malaria elimination in the GMS carries the urgency of eliminating artemisinin-resistant *P. falciparum* parasites before they become untreatable and spread to Africa. The changing malaria epidemiology with increasing proportion of *P. vivax* malaria requires an 8-aminoquinoline drug for radical cure, but it demands deployment of point-of-care diagnostics for G6PD deficiency due to its high prevalence in endemic human populations. In addition, the prevalent asymptomatic parasite reservoirs need to be targeted by a MDA approach. The diversity of *Anopheles* vectors in the GMS and decreasing effectiveness of indoor control measures, such as LLIN and IRS facing the outdoor malaria transmission, also require development and implementation of novel interventions for vector control. To meet the challenge of border malaria, coordinated efforts among the NMCPs targeting the mobile and migrant populations along international borders will prevent cross-border reintroduction of malaria. Altogether, a holistic attack on malaria using integrated approaches is necessary to achieve the goal of regional malaria elimination in the GMS.

Acknowledgements

We thank the National Institute of Allergy and Infectious Diseases, NIH, for financial support (U19AI089672).

Author details

Liwang Cui[1]*, Yaming Cao[2], Jaranit Kaewkungwal[3], Amnat Khamsiriwatchara[3], Saranath Lawpoolsri[3], Than Naing Soe[4], Myat Phone Kyaw[5] and Jetsumon Sattabongkot[3]

*Address all correspondence to: luc2@psu.edu

1 Department of Entomology, Pennsylvania State University, PA, USA

2 Department of Immunology, China Medical University, Shenyang, China

3 Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

4 Department of Public Health, Ministry of Health and Sports, Nay Pyi Taw, Myanmar

5 Myanmar Medical Association, Yangon, Myanmar

References

[1] Cui L, Yan G, Sattabongkot J, Cao Y, Chen B, Chen X, et al. Malaria in the greater Mekong subregion: Heterogeneity and complexity. Acta Tropica. 2012;**121**:227-239

[2] Hewitt S, Delacollette C, Chavez I. Malaria situation in the greater Mekong subregion. The Southeast Asian Journal of Tropical Medicine and Public Health. 2013;**44**(Suppl 1):46-72; discussion 306-307

[3] WHO. Strategy for malaria elimination in the greater Mekong subregion (2015-2030). http://iris.wpro.who.int/bitstream/handle/10665.1/10945/9789290617181_eng.pdf [Accessed: January 21, 2018]

[4] Smith Gueye C, Newby G, Hwang J, Phillips AA, Whittaker M, MacArthur JR, et al. The challenge of artemisinin resistance can only be met by eliminating *Plasmodium falciparum* malaria across the greater Mekong subregion. Malaria Journal. 2014;**13**:286

[5] Fidock DA. Microbiology. Eliminating malaria. Science. 2013;**340**:1531-1533

[6] Hewitt S, Delacollette C, Poirot E. Malaria control in the greater Mekong subregion: An overview of the current response and its limitations. The Southeast Asian Journal of Tropical Medicine and Public Health. 2013;**44**(Suppl 1):249-305; discussion 306-247

[7] WHO. World Malaria Report 2017. http://www.who.int/malaria/publications/world-malaria-report-2017/en/ [Accessed: January 21, 2018]

[8] Liu H, Nie R-H, Li C-F, Sun Y-H, Li G-S. Active detection of malaria cases in Myanmar Wa ethnical villages of China-Myanmar border. Parasitoses and Infectious Diseases. 2009;**7**:6-9

[9] Gueye CS, Teng A, Kinyua K, Wafula F, Gosling R, McCoy D. Parasites and vectors carry no passport: How to fund cross-border and regional efforts to achieve malaria elimination. Malaria Journal. 2012;**11**:344

[10] Parker DM, Carrara VI, Pukrittayakamee S, McGready R, Nosten FH. Malaria ecology along the Thailand-Myanmar border. Malaria Journal. 2015;**14**:388

[11] Xu J, Liu H. The challenges of malaria elimination in Yunnan Province, People's Republic of China. The Southeast Asian Journal of Tropical Medicine and Public Health. 2012;**43**:819-824

[12] Huguet JW, Chamratrithirong A. Thailand Migration Report. Bangkok, Thailand: International Organization for Migration, Thailand Office; 2011 http://publications.iom.int/system/files/pdf/tmr_2011.pdf [Accessed: January 21, 2018]

[13] Jitthai N. Migration and malaria. The Southeast Asian Journal of Tropical Medicine and Public Health. 2013;**44**(Suppl 1):166-200; discussion 306-167

[14] Guyant P, Canavati SE, Chea N, Ly P, Whittaker MA, Roca-Feltrer A, et al. Malaria and the mobile and migrant population in Cambodia: A population movement framework to inform strategies for malaria control and elimination. Malaria Journal. 2015;**14**:252

[15] Xu JW, Liu H. The relationship of malaria between Chinese side and Myanmar's five special regions along China-Myanmar border: A linear regression analysis. Malaria Journal. 2016;**15**:368

[16] Zhou G, Sun L, Xia R, Duan Y, Xu J, Yang H, et al. Clinical malaria along the China-Myanmar border, Yunnan Province, China, January 2011-August 2012. Emerging Infectious Diseases. 2014;**20**:675-678

[17] Lo E, Lam N, Hemming-Schroeder E, Nguyen J, Zhou G, Lee MC, et al. Frequent spread of *Plasmodium vivax* malaria maintains high genetic diversity at the Myanmar-China border without distance and landscape barriers. The Journal of Infectious Diseases. 2017;**216**(10):1254-1263

[18] Lo E, Zhou G, Oo W, Lee MC, Baum E, Felgner PL, et al. Molecular inference of sources and spreading patterns of *Plasmodium falciparum* malaria parasites in internally displaced persons settlements in Myanmar-China border area. Infection, Genetics and Evolution. 2015;**33**:189-196

[19] Parker DM, Matthews SA, Yan G, Zhou G, Lee MC, Sirichaisinthop J, et al. Microgeography and molecular epidemiology of malaria at the Thailand-Myanmar border in the malaria pre-elimination phase. Malaria Journal. 2015;**14**:198

[20] Wangroongsarb P, Satimai W, Khamsiriwatchara A, Thwing J, Eliades JM, Kaewkungwal J, et al. Respondent-driven sampling on the Thailand-Cambodia border. II. Knowledge, perception, practice and treatment-seeking behaviour of migrants in malaria endemic zones. Malaria Journal. 2011;**10**:117

[21] Wangroongsarb P, Sudathip P, Satimai W. Characteristics and malaria prevalence of migrant populations in malaria-endemic areas along the Thai-Cambodian border. The Southeast Asian Journal of Tropical Medicine and Public Health. 2012;**43**:261-269

[22] Khamsiriwatchara A, Sudathip P, Sawang S, Vijakadge S, Potithavoranan T, Sangvichean A, et al. Artemisinin resistance containment project in Thailand. (I): Implementation of electronic-based malaria information system for early case detection and individual case management in provinces along the Thai-Cambodian border. Malaria Journal. 2012;**11**:247

[23] Khamsiriwatchara A, Wangroongsarb P, Thwing J, Eliades J, Satimai W, Delacollette C, et al. Respondent-driven sampling on the Thailand-Cambodia border. I. Can malaria cases be contained in mobile migrant workers? Malaria Journal. 2011;**10**:120

[24] Sturrock HJ, Roberts KW, Wegbreit J, Ohrt C, Gosling RD. Tackling imported malaria: An elimination endgame. The American Journal of Tropical Medicine and Hygiene. 2015;**93**:139-144

[25] Ghani AC, Sutherland CJ, Riley EM, Drakeley CJ, Griffin JT, Gosling RD, et al. Loss of population levels of immunity to malaria as a result of exposure-reducing interventions: Consequences for interpretation of disease trends. PLoS One. 2009;**4**:e4383

[26] Lin JT, Saunders DL, Meshnick SR. The role of submicroscopic parasitemia in malaria transmission: What is the evidence? Trends in Parasitology. 2014;**30**:183-190

[27] Okell LC, Ghani AC, Lyons E, Drakeley CJ. Submicroscopic infection in *Plasmodium falciparum*-endemic populations: A systematic review and meta-analysis. The Journal of Infectious Diseases. 2009;**200**:1509-1517

[28] Moreira CM, Abo-Shehada M, Price RN, Drakeley CJ. A systematic review of sub-microscopic *Plasmodium vivax* infection. Malaria Journal. 2015;**14**:360

[29] Imwong M, Hanchana S, Malleret B, Renia L, Day NP, Dondorp A, et al. High-throughput ultrasensitive molecular techniques for quantifying low-density malaria parasitemias. Journal of Clinical Microbiology. 2014;**52**:3303-3309

[30] Zhao Y, Lv Y, Liu F, Wang Q, Li P, Zhao Z, et al. Comparison of methods for detecting asymptomatic malaria infections in the China-Myanmar border area. Malaria Journal. 2017;**16**:159

[31] Imwong M, Nguyen TN, Tripura R, Peto TJ, Lee SJ, Lwin KM, et al. The epidemiology of subclinical malaria infections in South-East Asia: Findings from cross-sectional surveys in Thailand-Myanmar border areas, Cambodia, and Vietnam. Malaria Journal. 2015;**14**:381

[32] Baum E, Sattabongkot J, Sirichaisinthop J, Kiattibutr K, Davies DH, Jain A, et al. Submicroscopic and asymptomatic *Plasmodium falciparum* and *Plasmodium vivax* infections are common in western Thailand—Molecular and serological evidence. Malaria Journal. 2015;**14**:95

[33] Pethleart A, Prajakwong S, Suwonkerd W, Corthong B, Webber R, Curtis C. Infectious reservoir of *Plasmodium* infection in Mae Hong Son Province, north-west Thailand. Malaria Journal. 2004;**3**:34

[34] Coleman RE, Kumpitak C, Ponlawat A, Maneechai N, Phunkitchar V, Rachapaew N, et al. Infectivity of asymptomatic *Plasmodium*-infected human populations to *Anopheles dirus* mosquitoes in western Thailand. Journal of Medical Entomology. 2004;**41**:201-208

[35] Kiattibutr K, Roobsoong W, Sriwichai P, Saeseu T, Rachaphaew N, Suansomjit C, et al. Infectivity of symptomatic and asymptomatic *Plasmodium vivax* infections to a southeast Asian vector, *Anopheles dirus*. International Journal for Parasitology. 2017;**47**:163-170

[36] Okell LC, Bousema T, Griffin JT, Ouedraogo AL, Ghani AC, Drakeley CJ. Factors determining the occurrence of submicroscopic malaria infections and their relevance for control. Nature Communications. 2012;**3**:1237

[37] Cotter C, Sturrock HJ, Hsiang MS, Liu J, Phillips AA, Hwang J, et al. The changing epidemiology of malaria elimination: New strategies for new challenges. Lancet. 2013; **382**:900-911

[38] Coleman RE, Maneechai N, Ponlawat A, Kumpitak C, Rachapaew N, Miller RS, et al. Short report: Failure of the OptiMAL rapid malaria test as a tool for the detection of asymptomatic malaria in an area of Thailand endemic for *Plasmodium falciparum* and P. *vivax*. The American Journal of Tropical Medicine and Hygiene. 2002;**67**:563-565

[39] Yan J, Li N, Wei X, Li P, Zhao Z, Wang L, et al. Performance of two rapid diagnostic tests for malaria diagnosis at the China-Myanmar border area. Malaria Journal. 2013;**12**:73

[40] Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A, Wernsdorfer WH. A review of malaria diagnostic tools: Microscopy and rapid diagnostic test (RDT). The American Journal of Tropical Medicine and Hygiene. 2007;**77**:119-127

[41] Baird KJ, Maguire JD, Price RN. Diagnosis and treatment of *Plasmodium vivax* malaria. Advances in Parasitology. 2012;**80**:203-270

[42] Baird JK. Resistance to therapies for infection by *Plasmodium vivax*. Clinical Microbiology Reviews. 2009;**22**:508-534

[43] Baird JK, Rieckmann KH. Can primaquine therapy for vivax malaria be improved? Trends in Parasitology. 2003;**19**:115-120

[44] Goller JL, Jolley D, Ringwald P, Biggs BA. Regional differences in the response of *Plasmodium vivax* malaria to primaquine as anti-relapse therapy. The American Journal of Tropical Medicine and Hygiene. 2007;**76**:203-207

[45] Price RN, von Seidlein L, Valecha N, Nosten F, Baird JK, White NJ. Global extent of chloroquine-resistant *Plasmodium vivax*: A systematic review and meta-analysis. The Lancet Infectious Diseases. 2014;**14**:982-991

[46] Luxemburger C, van Vugt M, Jonathan S, McGready R, Looareesuwan S, White NJ, et al. Treatment of vivax malaria on the western border of Thailand. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1999;**93**:433-438

[47] Pukrittayakamee S, Chantra A, Simpson JA, Vanijanonta S, Clemens R, Looareesuwan S, et al. Therapeutic responses to different antimalarial drugs in vivax malaria. Antimicrobial Agents and Chemotherapy. 2000;**44**:1680-1685

[48] Tasanor O, Ruengweerayut R, Sirichaisinthop J, Congpuong K, Wernsdorfer WH, Na-Bangchang K. Clinical-parasitological response and in-vitro sensitivity of *Plasmodium vivax* to chloroquine and quinine on the western border of Thailand. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2006;**100**:410-418

[49] Muhamad P, Ruengweerayut R, Chacharoenkul W, Rungsihirunrat K, Na-Bangchang K. Monitoring of clinical efficacy and in vitro sensitivity of *Plasmodium vivax* to chloroquine in area along Thai Myanmar border during 2009-2010. Malaria Journal. 2011;**10**:44

[50] Vijaykadga S, Rojanawatsirivej C, Congpoung K, Wilairatana P, Satimai W, Uaekowitchai C, et al. Assessment of therapeutic efficacy of chloroquine for vivax malaria in Thailand. The Southeast Asian Journal of Tropical Medicine and Public Health. 2004;**35**:566-569

[51] Congpoung K, Satimai W, Sujariyakul A, Intanakom S, Harnpitakpong W, Pranuth Y, et al. In vivo sensitivity monitoring of chloroquine for the treatment of uncomplicated vivax malaria in four bordered provinces of Thailand during 2009-2010. Journal of Vector Borne Diseases. 2011;**48**:190-196

[52] Myat-Phone-Kyaw, Myint-Oo, Myint-Lwin, Thaw-Zin, Kyin-Hla-Aye, Nwe-Nwe-Yin. Emergence of chloroquine-resistant *Plasmodium vivax* in Myanmar (Burma). Transactions of the Royal Society of Tropical Medicine and Hygiene. 1993;**87**:687

[53] Marlar T, Myat Phone K, Aye Yu S, Khaing Khaing G, Ma S, Myint O. Development of resistance to chloroquine by *Plasmodium vivax* in Myanmar. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1995;**89**:307-308

[54] Rijken MJ, Boel ME, Russell B, Imwong M, Leimanis ML, Phyo AP, et al. Chloroquine resistant *vivax* malaria in a pregnant woman on the western border of Thailand. Malaria Journal. 2011;**10**:113

[55] Guthmann JP, Pittet A, Lesage A, Imwong M, Lindegardh N, Min Lwin M, et al. *Plasmodium vivax* resistance to chloroquine in Dawei, southern Myanmar. Tropical Medicine & International Health. 2008;**13**:91-98

[56] Htun MW, Mon NCN, Aye KM, Hlaing CM, Kyaw MP, Handayuni I, et al. Chloroquine efficacy for *Plasmodium vivax* in Myanmar in populations with high genetic diversity and moderate parasite gene flow. Malaria Journal. 2017;**16**:281

[57] Yuan L, Wang Y, Parker DM, Gupta B, Yang Z, Liu H, et al. Therapeutic responses of *Plasmodium vivax* malaria to chloroquine and primaquine treatment in northeastern Myanmar. Antimicrobial Agents and Chemotherapy. 2015;**59**:1230-1235

[58] Baird JK. Resistance to chloroquine unhinges vivax malaria therapeutics. Antimicrobial Agents and Chemotherapy. 2011;**55**:1827-1830

[59] Robinson LJ, Wampfler R, Betuela I, Karl S, White MT, Li Wai Suen CS, et al. Strategies for understanding and reducing the *Plasmodium vivax* and *Plasmodium ovale* hypnozoite reservoir in Papua new Guinean children: A randomised placebo-controlled trial and mathematical model. PLoS Medicine. 2015;**12**:e1001891

[60] Adekunle AI, Pinkevych M, McGready R, Luxemburger C, White LJ, Nosten F, et al. Modeling the dynamics of *Plasmodium vivax* infection and hypnozoite reactivation in vivo. PLoS Neglected Tropical Diseases. 2015;**9**:e0003595

[61] White NJ. Determinants of relapse periodicity in *Plasmodium vivax* malaria. Malaria Journal. 2011;**10**:297

[62] Butcher GA. Antimalarial drugs and the mosquito transmission of *Plasmodium*. International Journal for Parasitology. 1997;**27**:975-987

[63] White NJ. The role of anti-malarial drugs in eliminating malaria. Malaria Journal. 2008; **7**(Suppl 1):S8

[64] Imwong M, Snounou G, Pukrittayakamee S, Tanomsing N, Kim JR, Nandy A, et al. Relapses of *Plasmodium vivax* infection usually result from activation of heterologous hypnozoites. The Journal of Infectious Diseases. 2007;**195**:927-933

[65] Chen N, Auliff A, Rieckmann K, Gatton M, Cheng Q. Relapses of *Plasmodium vivax* infection result from clonal hypnozoites activated at predetermined intervals. The Journal of Infectious Diseases. 2007;**195**:934-941

[66] Bennett JW, Pybus BS, Yadava A, Tosh D, Sousa JC, McCarthy WF, et al. Primaquine failure and cytochrome P-450 2D6 in *Plasmodium vivax* malaria. The New England Journal of Medicine. 2013;**369**:1381-1382

[67] Pybus BS, Marcsisin SR, Jin X, Deye G, Sousa JC, Li Q, et al. The metabolism of primaquine to its active metabolite is dependent on CYP 2D6. Malaria Journal. 2013;**12**:212

[68] Pybus BS, Sousa JC, Jin X, Ferguson JA, Christian RE, Barnhart R, et al. CYP450 phenotyping and accurate mass identification of metabolites of the 8-aminoquinoline, antimalarial drug primaquine. Malaria Journal. 2012;**11**:259

[69] Potter BM, Xie LH, Vuong C, Zhang J, Zhang P, Duan D, et al. Differential CYP 2D6 metabolism alters primaquine pharmacokinetics. Antimicrobial Agents and Chemotherapy. 2015;**59**:2380-2387

[70] Zhou SF, Liu JP, Lai XS. Substrate specificity, inhibitors and regulation of human cytochrome P450 2D6 and implications in drug development. Current Medicinal Chemistry. 2009;**16**:2661-2805

[71] Zhou SF. Polymorphism of human cytochrome P450 2D6 and its clinical significance: Part I. Clinical Pharmacokinetics. 2009;**48**:689-723

[72] Ingelman-Sundberg M, Sim SC, Gomez A, Rodriguez-Antona C. Influence of cytochrome P450 polymorphisms on drug therapies: Pharmacogenetic, pharmacoepigenetic and clinical aspects. Pharmacology & Therapeutics. 2007;**116**:496-526

[73] Bradford LD. CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. Pharmacogenomics. 2002;**3**:229-243

[74] Marcsisin SR, Reichard G, Pybus BS. Primaquine pharmacology in the context of CYP 2D6 pharmacogenomics: Current state of the art. Pharmacology & Therapeutics. 2016;**161**:1-10

[75] Nkhoma ET, Poole C, Vannappagari V, Hall SA, Beutler E. The global prevalence of glucose-6-phosphate dehydrogenase deficiency: A systematic review and meta-analysis. Blood Cells, Molecules & Diseases. 2009;**42**:267-278

[76] Minucci A, Moradkhani K, Hwang MJ, Zuppi C, Giardina B, Capoluongo E. Glucose-6-phosphate dehydrogenase (G6PD) mutations database: Review of the "old" and update of the new mutations. Blood Cells, Molecules & Diseases. 2012;**48**:154-165

[77] Howes RE, Dewi M, Piel FB, Monteiro WM, Battle KE, Messina JP, et al. Spatial distribution of G6PD deficiency variants across malaria-endemic regions. Malaria Journal. 2013;**12**:418

[78] Charoenkwan P, Tantiprabha W, Sirichotiyakul S, Phusua A, Sanguansermsri T. Prevalence and molecular characterization of glucose-6-phosphate dehydrogenase deficiency in northern Thailand. The Southeast Asian Journal of Tropical Medicine and Public Health. 2014;**45**:187-193

[79] Bancone G, Chu CS, Somsakchaicharoen R, Chowwiwat N, Parker DM, Charunwatthana P, et al. Characterization of G6PD genotypes and phenotypes on the northwestern Thailand-Myanmar border. PLoS One. 2014;**9**:e116063

[80] Kotepui M, Uthaisar K, PhunPhuech B, Phiwklam N. Prevalence and hematological indicators of G6PD deficiency in malaria-infected patients. Infectious Diseases of Poverty. 2016;**5**:36

[81] Li Q, Yang F, Liu R, Luo L, Yang Y, Zhang L, et al. Prevalence and molecular characterization of glucose-6-phosphate dehydrogenase deficiency at the China-Myanmar border. PLoS One. 2015;**10**:e0134593

[82] Matsuoka H, Wang J, Hirai M, Arai M, Yoshida S, Kobayashi T, et al. Glucose-6-phosphate dehydrogenase (G6PD) mutations in Myanmar: G6PD Mahidol (487G>A) is the most common variant in the Myanmar population. Journal of Human Genetics. 2004;**49**:544-547

[83] Nuchprayoon I, Sanpavat S, Nuchprayoon S. Glucose-6-phosphate dehydrogenase (G6PD) mutations in Thailand: G6PD Viangchan (871G>A) is the most common deficiency variant in the Thai population. Human Mutation. 2002;**19**:185

[84] Charoenlarp P, Areekul S, Pholpothi T, Harinasuta T. The course of primaquine-induced haemolysis in G-6-PD-deficient Thais. Journal of the Medical Association of Thailand. 1973;**56**:392-397

[85] Charoenlarp P, Areekul S, Harinasuta T, Sirivorasarn P. The haemolytic effect of a single dose of 45 mg of primaquine in G-6-PD deficient Thais. Journal of the Medical Association of Thailand. 1972;**55**:631-638

[86] Buchachart K, Krudsood S, Singhasivanon P, Treeprasertsuk S, Phophak N, Srivilairit S, et al. Effect of primaquine standard dose (15 mg/day for 14 days) in the treatment of vivax malaria patients in Thailand. The Southeast Asian Journal of Tropical Medicine and Public Health. 2001;**32**:720-726

[87] Karwacki JJ, Shanks GD, Kummalue T, Watanasook C. Primaquine induced hemolysis in a Thai soldier. The Southeast Asian Journal of Tropical Medicine and Public Health. 1989;**20**:555-556

[88] Kheng S, Muth S, Taylor WR, Tops N, Kosal K, Sothea K, et al. Tolerability and safety of weekly primaquine against relapse of *Plasmodium vivax* in Cambodians with glucose-6-phosphate dehydrogenase deficiency. BMC Medicine. 2015;**13**:203

[89] Chen X, He Y, Miao Y, Yang Z, Cui L. A young man with severe acute haemolytic anaemia. BMJ. 2017;**359**:j4263

[90] Domingo GJ, Satyagraha AW, Anvikar A, Baird K, Bancone G, Bansil P, et al. G6PD testing in support of treatment and elimination of malaria: Recommendations for evaluation of G6PD tests. Malaria Journal. 2013;**12**:391

[91] Alving AS, Johnson CF, Tarlov AR, Brewer GJ, Kellermeyer RW, Carson PE. Mitigation of the haemolytic effect of primaquine and enhancement of its action against exoerythrocytic forms of the Chesson strain of *Plasmodium vivax* by intermittent regimens of drug administration: A preliminary report. Bulletin of the World Health Organization. 1960;**22**:621-631

[92] Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM. Evidence of artemisinin-resistant malaria in western Cambodia. The New England Journal of Medicine. 2008;**359**:2619-2620

[93] Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. The New England Journal of Medicine. 2009;**361**:455-467

[94] Phyo AP, Nkhoma S, Stepniewska K, Ashley EA, Nair S, McGready R, et al. Emergence of artemisinin-resistant malaria on the western border of Thailand: A longitudinal study. Lancet. 2012;**379**:1960-1966

[95] Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, et al. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. The New England Journal of Medicine. 2014;**371**:411-423

[96] Amaratunga C, Sreng S, Suon S, Phelps ES, Stepniewska K, Lim P, et al. Artemisinin-resistant *Plasmodium falciparum* in Pursat province, western Cambodia: A parasite clearance rate study. The Lancet Infectious Diseases. 2012;**12**:851-858

[97] Noedl H, Se Y, Sriwichai S, Schaecher K, Teja-Isavadharm P, Smith B, et al. Artemisinin resistance in Cambodia: A clinical trial designed to address an emerging problem in Southeast Asia. Clinical Infectious Diseases. 2010;**51**:e82-e89

[98] Hien TT, Thuy-Nhien NT, Phu NH, Boni MF, Thanh NV, Nha-Ca NT, et al. In vivo susceptibility of *Plasmodium falciparum* to artesunate in Binh Phuoc Province, Vietnam. Malaria Journal. 2012;**11**:355

[99] Bustos MD, Wongsrichanalai C, Delacollette C, Burkholder B. Monitoring antimalarial drug efficacy in the greater Mekong subregion: An overview of in vivo results from 2008 to 2010. The Southeast Asian Journal of Tropical Medicine and Public Health. 2013;**44**(Suppl 1):201-230; discussion 306-207

[100] Kyaw MP, Nyunt MH, Chit K, Aye MM, Aye KH, Lindegardh N, et al. Reduced susceptibility of *Plasmodium falciparum* to artesunate in southern Myanmar. PLoS One. 2013;**8**:e57689

[101] Carrara VI, Lwin KM, Phyo AP, Ashley E, Wiladphaingern J, Sriprawat K, et al. Malaria burden and artemisinin resistance in the mobile and migrant population on the Thai-Myanmar border, 1999-2011: An observational study. PLoS Medicine. 2013;**10**:e1001398

[102] Thriemer K, Hong NV, Rosanas-Urgell A, Phuc BQ, Ha do M, Pockele E, et al. Delayed parasite clearance after treatment with dihydroartemisinin-piperaquine in *Plasmodium falciparum* malaria patients in Central Vietnam. Antimicrobial Agents and Chemotherapy. 2014;**58**:7049-7055

[103] Huang F, Takala-Harrison S, Jacob CG, Liu H, Sun X, Yang H, et al. A single mutation in K13 predominates in southern China and is associated with delayed clearance of *Plasmodium falciparum* following artemisinin treatment. The Journal of Infectious Diseases. 2015;**212**:1629-1635

[104] WHO. Global plan for artemisinin resistance containment (GPARC). 2011. p. 87

[105] Takala-Harrison S, Jacob CG, Arze C, Cummings MP, Silva JC, Dondorp AM, et al. Independent emergence of artemisinin resistance mutations among *Plasmodium falciparum* in Southeast Asia. The Journal of Infectious Diseases. 2015;**211**:670-679

[106] Wongsrichanalai C, Meshnick SR. Declining artesunate-mefloquine efficacy against *falciparum* malaria on the Cambodia-Thailand border. Emerging Infectious Diseases. 2008;**14**:716-719

[107] Amaratunga C, Lim P, Suon S, Sreng S, Mao S, Sopha C, et al. Dihydroartemisinin-piperaquine resistance in *Plasmodium falciparum* malaria in Cambodia: A multisite prospective cohort study. The Lancet Infectious Diseases. 2016;**16**:357-365

[108] Saunders DL, Vanachayangkul P, Lon C. Dihydroartemisinin-piperaquine failure in Cambodia. The New England Journal of Medicine. 2014;**371**:484-485

[109] Leang R, Taylor WR, Bouth DM, Song L, Tarning J, Char MC, et al. Evidence of *Plasmodium falciparum* malaria multidrug resistance to Artemisinin and Piperaquine in western Cambodia: Dihydroartemisinin-Piperaquine open-label multicenter clinical assessment. Antimicrobial Agents and Chemotherapy. 2015;**59**:4719-4726

[110] Spring MD, Lin JT, Manning JE, Vanachayangkul P, Somethy S, Bun R, et al. Dihydroartemisinin-piperaquine failure associated with a triple mutant including kelch13 C580Y in Cambodia: An observational cohort study. The Lancet Infectious Diseases. 2015;**15**:683-691

[111] Phuc BQ, Rasmussen C, Duong TT, Dong LT, Loi MA, Menard D, et al. Treatment failure of dihydroartemisinin/piperaquine for *Plasmodium falciparum* malaria, Vietnam. Emerging Infectious Diseases. 2017;**23**:715-717

[112] Menard D, Dondorp A. Antimalarial drug resistance: A threat to malaria elimination. Cold Spring Harbor Perspectives in Medicine. 2017;**7**:a025619

[113] Cui L, Mharakurwa S, Ndiaye D, Rathod PK, Rosenthal PJ. Antimalarial drug resistance: Literature review and activities and findings of the ICEMR network. The American Journal of Tropical Medicine and Hygiene. 2015;**93**:57-68

[114] Witkowski B, Amaratunga C, Khim N, Sreng S, Chim P, Kim S, et al. Novel phenotypic assays for the detection of artemisinin-resistant *Plasmodium falciparum* malaria in Cambodia: In-vitro and ex-vivo drug-response studies. The Lancet Infectious Diseases. 2013;**13**:1043-1049

[115] Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, Khim N, et al. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. Nature. 2014;**505**:50-55

[116] Nyunt MH, Hlaing T, Oo HW, Tin-Oo LL, Phway HP, Wang B, et al. Molecular assessment of artemisinin resistance markers, polymorphisms in the k13 propeller, and a multidrug-resistance gene in the eastern and western border areas of Myanmar. Clinical Infectious Diseases. 2015;**60**:1208-1215

[117] Miotto O, Amato R, Ashley EA, MacInnis B, Almagro-Garcia J, Amaratunga C, et al. Genetic architecture of artemisinin-resistant *Plasmodium falciparum*. Nature Genetics. 2015;**47**:226-234

[118] Ghorbal M, Gorman M, Macpherson CR, Martins RM, Scherf A, Lopez-Rubio JJ. Genome editing in the human malaria parasite *Plasmodium falciparum* using the CRISPR-Cas9 system. Nature Biotechnology. 2014;**32**:819-821

[119] Straimer J, Gnadig NF, Witkowski B, Amaratunga C, Duru V, Ramadani AP, et al. Drug resistance. K13-propeller mutations confer artemisinin resistance in *Plasmodium falciparum* clinical isolates. Science. 2015;**347**:428-431

[120] Menard D, Khim N, Beghain J, Adegnika AA, Shafiul-Alam M, Amodu O, et al. A worldwide map of *Plasmodium falciparum* K13-propeller polymorphisms. The New England Journal of Medicine. 2016;**374**:2453-2464

[121] Wang Z, Shrestha S, Li X, Miao J, Yuan L, Cabrera M, et al. Prevalence of K13-propeller polymorphisms in *Plasmodium falciparum* from China-Myanmar border in 2007-2012. Malaria Journal. 2015;**14**:168

[122] Tun KM, Imwong M, Lwin KM, Win AA, Hlaing TM, Hlaing T, et al. Spread of artemisinin-resistant *Plasmodium falciparum* in Myanmar: A cross-sectional survey of the K13 molecular marker. The Lancet Infectious Diseases. 2015;**15**:415-421

[123] Wang Z, Wang Y, Cabrera M, Zhang Y, Gupta B, Wu Y, et al. Artemisinin resistance at the China-Myanmar border and association with mutations in the K13 propeller gene. Antimicrobial Agents and Chemotherapy. 2015;**59**:6952-6959

[124] Ye R, Hu D, Zhang Y, Huang Y, Sun X, Wang J, et al. Distinctive origin of artemisinin-resistant *Plasmodium falciparum* on the China-Myanmar border. Scientific Reports. 2016;**6**:20100

[125] Putaporntip C, Kuamsab N, Kosuwin R, Tantiwattanasub W, Vejakama P, Sueblinvong T, et al. Natural selection of K13 mutants of *Plasmodium falciparum* in response to artemisinin combination therapies in Thailand. Clinical Microbiology and Infection. 2016;**22**:285 e281-285 e288

[126] Talundzic E, Okoth SA, Congpuong K, Plucinski MM, Morton L, Goldman IF, et al. Selection and spread of artemisinin-resistant alleles in Thailand prior to the global artemisinin resistance containment campaign. PLoS Pathogens. 2015;**11**:e1004789

[127] Witkowski B, Duru V, Khim N, Ross LS, Saintpierre B, Beghain J, et al. A surrogate marker of piperaquine-resistant *Plasmodium falciparum* malaria: A phenotype-genotype association study. The Lancet Infectious Diseases. 2017;**17**:174-183

[128] Amato R, Lim P, Miotto O, Amaratunga C, Dek D, Pearson RD, et al. Genetic markers associated with dihydroartemisinin-piperaquine failure in *Plasmodium falciparum* malaria in Cambodia: A genotype-phenotype association study. The Lancet Infectious Diseases. 2017;**17**:164-173

[129] Imwong M, Suwannasin K, Kunasol C, Sutawong K, Mayxay M, Rekol H, et al. The spread of artemisinin-resistant *Plasmodium falciparum* in the greater Mekong subregion: A molecular epidemiology observational study. The Lancet Infectious Diseases. 2017;**17**:491-497

[130] Smithuis FM, Kyaw MK, Phe UO, van der Broek I, Katterman N, Rogers C, et al. Entomological determinants of insecticide-treated bed net effectiveness in western Myanmar. Malaria Journal. 2013;**12**:364

[131] Suwonkerd W, Ritthison W, Ngo CT, Tainchum K, Bangs MJ, Chareonviriphap T. Vector biology and malaria transmission in Southeast Asia. In: Manguin S, editor. *Anopheles* Mosquitoes—New Insights into Malaria Vectors. Rijeka: Intech; 2013. pp. 274-325

[132] Sinka ME, Bangs MJ, Manguin S, Chareonviriyaphap T, Patil AP, Temperley WH, et al. The dominant *Anopheles* vectors of human malaria in the Asia-Pacific region: Occurrence data, distribution maps and bionomic precis. Parasites & Vectors. 2011;**4**:89

[133] Hii J, Rueda LM. Malaria vectors in the greater Mekong subregion: Overview of malaria vectors and remaining challenges. The Southeast Asian Journal of Tropical Medicine and Public Health. 2013;**44**(Suppl 1):73-165; discussion 306-167

[134] Manguin S, Garros C, Dusfour I, Harbach RE, Coosemans M. Bionomics, taxonomy, and distribution of the major malaria vector taxa of *Anopheles* subgenus *Cellia* in Southeast Asia: An updated review. Infection, Genetics and Evolution. 2008;**8**:489-503

[135] Morgan K, Somboon P, Walton C. Understanding *Anopheles* diversity in Southeast Asia and its applications for malaria control. In: Manguin S, editor. *Anopheles* Mosquitoes—New Insights into Malaria Vectors. Rijeka: Intech; 2013. pp. 327-355

[136] Tainchum K, Ritthison W, Chuaycharoensuk T, Bangs MJ, Manguin S, Chareonviriyaphap T. Diversity of *Anopheles* species and trophic behavior of putative malaria vectors in two malaria endemic areas of northwestern Thailand. Journal of Vector Ecology. 2014;**39**:424-436

[137] Sriwichai P, Karl S, Samung Y, Sumruayphol S, Kiattibutr K, Payakkapol A, et al. Evaluation of CDC light traps for mosquito surveillance in a malaria endemic area on the Thai-Myanmar border. Parasites & Vectors. 2015;**8**:636

[138] Wang Y, Zhong D, Cui L, Lee MC, Yang Z, Yan G, et al. Population dynamics and community structure of *Anopheles* mosquitoes along the China-Myanmar border. Parasites & Vectors. 2015;**8**:445

[139] Zhong D, Wang X, Xu T, Zhou G, Wang Y, Lee MC, et al. Effects of microclimate condition changes due to land use and land cover changes on the survivorship of malaria vectors in China-Myanmar border region. PLoS One. 2016;**11**:e0155301

[140] Smithuis FM, Kyaw MK, Phe UO, van der Broek I, Katterman N, Rogers C, et al. The effect of insecticide-treated bed nets on the incidence and prevalence of malaria in children in an area of unstable seasonal transmission in western Myanmar. Malaria Journal. 2013;**12**:363

[141] Chareonviriyaphap T, Bangs MJ, Suwonkerd W, Kongmee M, Corbel V, Ngoen-Klan R. Review of insecticide resistance and behavioral avoidance of vectors of human diseases in Thailand. Parasites & Vectors. 2013;**6**:280

[142] Van Bortel W, Trung HD, Thuan le K, Sochantha T, Socheat D, Sumrandee C, et al. The insecticide resistance status of malaria vectors in the Mekong region. Malaria Journal. 2008;**7**:102

[143] Verhaeghen K, Van Bortel W, Trung HD, Sochantha T, Coosemans M. Absence of knockdown resistance suggests metabolic resistance in the main malaria vectors of the Mekong region. Malaria Journal. 2009;**8**:84

[144] Baird JK. Asia-Pacific malaria is singular, pervasive, diverse and invisible. International Journal for Parasitology. 2017;**47**:371-377

[145] Kaneko A, Taleo G, Kalkoa M, Yamar S, Kobayakawa T, Bjorkman A. Malaria eradication on islands. Lancet. 2000;**356**:1560-1564

[146] Hsiang MS, Hwang J, Tao AR, Liu Y, Bennett A, Shanks GD, et al. Mass drug administration for the control and elimination of *Plasmodium vivax* malaria: An ecological study from Jiangsu province, China. Malaria Journal. 2013;**12**:383

[147] Song J, Socheat D, Tan B, Dara P, Deng C, Sokunthea S, et al. Rapid and effective malaria control in Cambodia through mass administration of artemisinin-piperaquine. Malaria Journal. 2010;**9**:57

[148] Landier J, Kajeechiwa L, Thwin MM, Parker DM, Chaumeau V, Wiladphaingern J, et al. Safety and effectiveness of mass drug administration to accelerate elimination of artemisinin-resistant *falciparum* malaria: A pilot trial in four villages of eastern Myanmar. Wellcome Open Research. 2017;**2**:81

[149] Newby G, Hwang J, Koita K, Chen I, Greenwood B, von Seidlein L, et al. Review of mass drug administration for malaria and its operational challenges. The American Journal of Tropical Medicine and Hygiene. 2015;**93**:125-134

[150] Geissbuhler Y, Kannady K, Chaki PP, Emidi B, Govella NJ, Mayagaya V, et al. Microbial larvicide application by a large-scale, community-based program reduces malaria infection prevalence in urban Dar es Salaam, Tanzania. PLoS One. 2009;**4**:e5107

[151] Chaccour CJ, Kobylinski KC, Bassat Q, Bousema T, Drakeley C, Alonso P, et al. Ivermectin to reduce malaria transmission: A research agenda for a promising new tool for elimination. Malaria Journal. 2013;**12**:153

[152] Kobylinski KC, Ubalee R, Ponlawat A, Nitatsukprasert C, Phasomkulsolsil S, Wattanakul T, et al. Ivermectin susceptibility and sporontocidal effect in greater Mekong subregion *Anopheles*. Malaria Journal. 2017;**16**:280

[153] Sluydts V, Durnez L, Heng S, Gryseels C, Canier L, Kim S, et al. Efficacy of topical mosquito repellent (picaridin) plus long-lasting insecticidal nets versus long-lasting insecticidal nets alone for control of malaria: A cluster randomised controlled trial. The Lancet Infectious Diseases. 2016;**16**:1169-1177

[154] Gryseels C, Uk S, Sluydts V, Durnez L, Phoeuk P, Suon S, et al. Factors influencing the use of topical repellents: Implications for the effectiveness of malaria elimination strategies. Scientific Reports. 2015;**5**:16847

[155] Wang S, Jacobs-Lorena M. Genetic approaches to interfere with malaria transmission by vector mosquitoes. Trends in Biotechnology. 2013;**31**:185-193

[156] Barreaux P, Barreaux AMG, Sternberg ED, Suh E, Waite JL, Whitehead SA, et al. Priorities for broadening the malaria vector control tool kit. Trends in Parasitology. 2017;**33**:763-774

Chapter 9

Ending Malaria Transmission in the Asia Pacific Malaria Elimination Network (APMEN) Countries: Challenges and the Way Forward

Kinley Wangdi and Archie CA Clements

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75405

Abstract

Member countries in the Asia Pacific Malaria Elimination Network (APMEN) are pursuing the global goal of malaria elimination by 2030. Different countries are in various phases of malaria elimination and this review aims to present a compilation of available evidence on the challenges and way forward for malaria elimination in APMEN countries. Malaria transmission in these States is complex. APMEN member countries include the largest populations living in areas of malaria transmission risk outside Africa. They are a global source for spread of artemisinin-based combination therapy (ACT) resistance, include the biggest burden of *Plasmodium vivax* and zoonotic malaria, and face many geopolitical and socio-economic factors that will challenge malaria elimination efforts. These challenges can be addressed in part through operational research to identify country-specific solutions, making better use of operational data such as through spatial decision support system (SDSS) approaches, strengthening surveillance, and cross-border initiative for coordinated action.

Keywords: *Plasmodium falciparum*, *P. vivax*, drug-resistance, malaria elimination, APMEN, challenges

1. Background

Malaria imposes great health and socio-economic burden on humanity, with an estimated 3.2 billion people at risk of being infected with malaria [1]. In 2016, there were approximately 216 million cases with 445,000 deaths, most of which were in children aged under 5 years in Africa [2, 3]. However, substantial progress has been made in fighting malaria, with global

Figure 1. Member countries of the Asia Pacific Malaria Elimination Network countries (APMEN).

incidence reducing by 41% and mortality rates by 62% between 2000 and 2015 [1]. In 2016, malaria remained endemic in 91 countries and territories as compared to 108 in 2000 [2]. It is estimated that most (90%) of total malaria cases were in the World Health Organisation (WHO) African Region, followed by the South-East Asian Region (SEAR) (7%) and the Eastern Mediterranean Region (2%) [4]. A number of factors have been attributed for this reduction, including wide-scale deployment of malaria control interventions, economic development in endemic countries, urbanisation, and unprecedented financial support for malaria control interventions [5–8]. In 2016, an estimated US$ 2.7 billion was invested in malaria control and elimination efforts globally by governments of malaria endemic countries and international partners [1, 9].

Recognising the need to hasten progress in reducing the burden of malaria, WHO developed the *Global Technical Strategy for Malaria 2016–2030* (GTS) [5], which sets out a vision for accelerating progress towards malaria elimination. The WHO strategy is complemented by the Roll Back Malaria advocacy plan, *Action and Investment to Defeat Malaria 2016–2030* (AIM) [10]. GTS and AIM set an ambitious global target of eliminating malaria in at least 21 countries by 2020, identified as E-2020 countries by WHO and 35 countries by 2030 [1, 2, 10].

In line with the global efforts to eliminate malaria, the Asia Pacific Malaria Elimination Network (APMEN) was established in 2009, initially including 10 countries (Bhutan, China,

No. of cases/year Country	2010	2011	2012	2013	2014	2015	2016
Bangladesh	79,300	69,700	13,750	5000	12,990	8000	6000
Bhutan	400	190	≤100	≤100	≤100	≤100	≤100
Cambodia	175,000	203,600	146,000	76,500	89,700	120,300	83,300
China	5000	3000	240	≤100	≤100	≤100	≤10
DPR Korea	13,520	16,760	21,850	14,410	10,540	800	2700
India	21,090,000	17,930,000	14,640,000	11,540,000	11,850,000	12,670,000	13,170,000
Indonesia	2,715,000	2,469,000	2,453,000	2,017000	1,479,000	1,274,000	1,281,000
Lao PDR	51,000	42,800	112,700	93,500	117,300	87,900	27,390
Malaysia	5000	4000	4000	2900	3100	240	270
Nepal	43,400	32,700	20,520	16,230	8000	7000	4000
Philippines	53,200	25,970	18,630	16,290	12,210	20,580	16,630
PNG*	1,342,000	1,130,000	1,452,000	1,617,000	1,260,000	1,014000	1,407,000
Republic of Korea	1300	500	400	400	600	600	600
Solomon Islands	95,900	66,200	55,000	56,400	30,780	39,400	86,000
Sri Lanka	—	—	—	—	—	—	—
Thailand	32,500	24,900	32,600	33,300	37,900	8000	11,520
Vanuatu	13,780	10,000	7000	5000	1900	600	4000
Vietnam	25,460	22,630	26,610	23,140	21,200	12,560	6000

Source: World malaria report 2017 (WHO [1, 16])*PNG, Papua New Guinea.

Table 1. Malaria transmission trends in the Asia Pacific Malaria Elimination Network (APMEN) countries based on the estimated malaria cases during 2010–2016.

Democratic People's Republic of Korea (DPR Korea), Indonesia, Malaysia, the Philippines, Republic of Korea, Solomon Islands, Sri Lanka, and Vanuatu) that now have expanded to 18 countries (adding Bangladesh, Cambodia, Lao People's Democratic Republic (Lao PDR), India, Nepal, Papua New Guinea, Thailand, and Vietnam) [11] (**Figure 1**). APMEN countries encompass the largest malaria reporting area outside the African region. APMEN serves the country partners and together with regional partners from the academic, development, non-governmental and private sectors, and global agencies including the WHO, collaboratively address the unique challenges of malaria elimination in the region through leadership, advocacy, capacity building, knowledge exchange and building evidence to support more effective, sustained malaria elimination programmes across the region [12].

Each member State has defined elimination goals based on malaria transmission trends (**Table 1**). Countries with low incidence of malaria are targeting elimination at the national level, while countries with higher incidence are planning to eliminate malaria at the sub-national level before pursuing elimination at the national level. However, all countries are committed to eliminating malaria in the Asia Pacific region by 2030 [13]. Sri Lanka eliminated malaria in 2012 and WHO certified Sri Lanka malaria free nation in 2016 [14]. Bhutan and the Republic of Korea have targeted to eliminate malaria in 2018 and 2019 respectively [15, 16]. Bangladesh, China, Malaysia, Philippines, and Vanuatu plan to eliminate malaria by 2020; DPR Korea, Cambodia, Lao People's Demographic Republic (Lao PDR), and Papua New Guinea (PNG) are planning to eliminate by 2025, and Nepal by 2026; finally India, Indonesia, Thailand, and Vietnam plan to eliminate malaria by 2030 [15]. The success of malaria elimination in APMEN States will greatly enhance the global drive towards malaria elimination. Therefore, the aim of this review is to present a compilation of available evidence on the challenges and way forward for malaria elimination in APMEN countries.

2. Epidemiological drivers of malaria in APMEN countries

Malaria elimination in APMEN countries faces many challenges. The challenges include large numbers of people living in malaria risk areas; presence of all forms of human malaria: *Plasmodium falciparum*, *P. vivax*, *P. ovale, P. malariae*, and *P. knowlesi*; the high incidence of *P. vivax* malaria, which is particularly difficult to control due to the dormant stages of its life cycle within the human host, and zoonotic malaria caused by *P. knowlesi*, which has animal reservoirs; anti-malarial drug resistance in *P. falciparum* and *P. vivax* parasites; diverse vectors with different feeding behaviour and insecticide resistance; forest malaria; human migration across porous international borders and cross-border malaria; and inadequacies in health systems in the region.

2.1. *Plasmodium vivax* Malaria

Plasmodium vivax is an important but relatively neglected malaria parasite globally [17]. This form of malaria is more widespread than *P. falciparum* malaria with 2.9 billion people at risk of infection, of which 90% live in the Asia Pacific region [18–22]. *P. vivax* is more difficult to treat than *P. falciparum* due to dormant liver stages (hypnozoites) [23–25], and the development of transmissible blood stages (gametocytes) before clinical symptoms [26]. These characteristics enable the parasite to adapt to environmental challenges and evade control interventions in place and time.

In many countries embarking on malaria elimination, *P. falciparum* incidence declines more rapidly than *P. vivax* incidence, due to the greater effectiveness of interventions for the former. Treating all stages of the parasite (radical cure) is a critical strategy for the successful control and ultimate elimination of *P. vivax*. In order to achieve radical cure of *P. vivax*, blood stage parasites, as well as the hypnozoites, need to be cleared. The only current widely available drug against hypnozoites is the 8-aminoquinoline compound, primaquine [27]. Unfortunately, individuals who have a genetic deficiency for glucose-6-phosphate dehydrogenase (G6PD) enzyme are at risk of severe haemolysis when treated with the drug [28–30]. In addition, primaquine requires prolonged daily administration over seven to 14 days. The complexities of prescribing reliable, safe and effective radical cure of *P. vivax* highlights the urgent need for innovative new approaches to assure schizonticidal and hypnozoiticidal treatment; without which, *P. vivax* elimination is unlikely in most settings.

2.2. Zoonotic malaria

Plasmodium knowlesi infections have been reported in a number of Asian Pacific countries [31–34]. This zoonotic species of malaria, which also infects macaque monkeys that form the main animal reservoir, was probably present in humans but was undiagnosed until molecular detection methods were developed that could distinguish *P. knowlesi* from the morphologically similar human malaria parasite *Plasmodium malariae* [35, 36]. Recently, the first case of human infection with *Plasmodium cynomolgi* was reported in Peninsular Malaysia that resembles *P. vivax* morphologically [37]. The role of animal reservoirs of malaria transmittable to humans is an almost wholly neglected question in the elimination agenda in the Asia-Pacific region [38].

2.3. Characteristics of populations at risk

Nearly 2.1 billion people in the Asia-Pacific region live in areas where there is risk of malaria transmission of which 16.8% live in high-risk areas [2, 39] (**Figure 2**). These high-risk areas include settlements located in remote parts of endemic countries including border areas. Many of these high-risk areas are characterised by forest and forest fringe environment with high malaria transmission, poor geographical accessibility, high population mobility, and low human density. In addition, most of these areas are inhabited by ethnic minorities, refugees and displaced people who are difficult to access and often experience high degree of poverty [40, 41]. Furthermore, these areas are frequented by people engaged in activities with increased risk of malaria exposure, such as tourism and pilgrimages, forest-related work such as logging, gem-mining, latex harvesting, fishing, road construction and other industrial occupations [41–45].

2.4. Antimalarial drug-resistance

Historically, countries in the Mekong Region including Cambodia and Thailand are global epicentres of emerging antimalarial drug resistance [46]. Chloroquine resistance was first reported in this area in the 1970s, followed by resistance to other anti-malarial drugs [47]. Over the past decade, artemisinin-based combination therapy (ACT) became the first-line protocol for the management of *P. falciparum* infections world over. However, parasites that

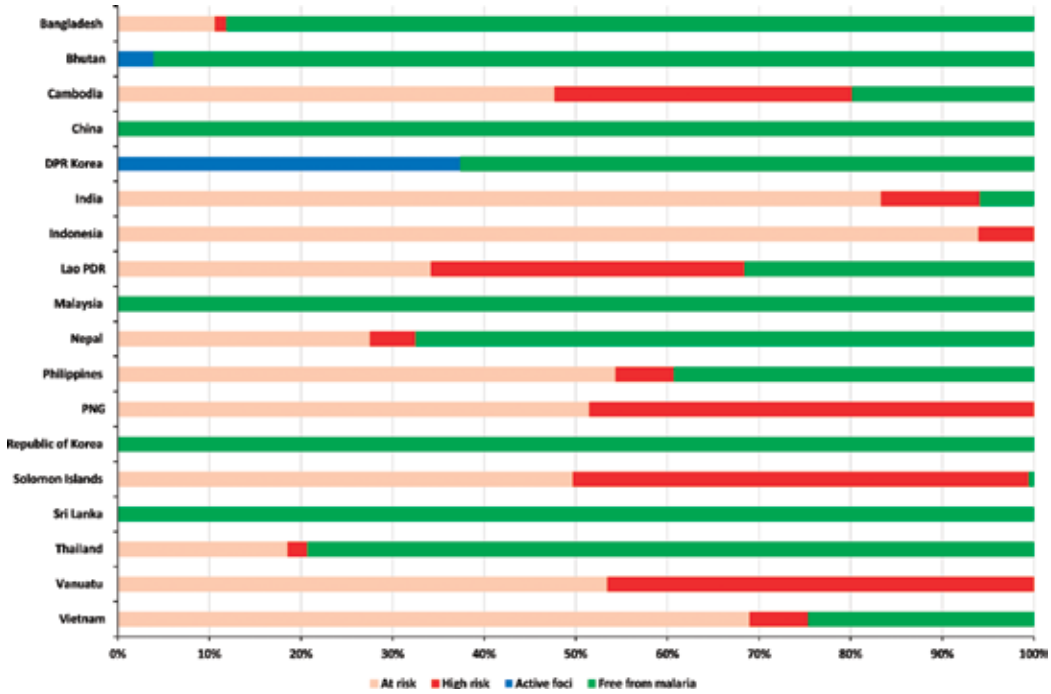


Figure 2. Population at risk of malaria in Asia Pacific Malaria Elimination Network (APMEN) countries for data based on 2016. (at risk- low risk + high risk). Source: World malaria report 2017 [1].

are drug-resistant to artemisinin and its derivatives have recently emerged in various parts of Southeast Asia challenging all control strategies for treatment and elimination efforts [48–51]. Presently, resistance to mefloquine continues to be a concern in Thailand and Cambodia, where artesunate-mefloquine is used as first line treatment [47]. Artemether-lumefantrine remains highly effective in most parts of the world, with the exception of Cambodia [52, 53]. There are evidences of resistance to ACT in Vietnam [2, 54]. In India, ACT is used universally across the country yet declining efficacy to artesunate plus sulphadoxine-pyrimethamine has already been reported in its northeastern region [55–57] however, there have been no reports of ACT resistance in other APMEN member States (**Figure 3**).

Chloroquine has remained the main choice of treatment for *P. vivax* blood stage infections, however, this policy is under threat from emerging drug resistant *P. vivax* strains [58]. A number of APMEN countries have reported *P. vivax* resistance to chloroquine. There are reports of resistance in some States of India [59–62], central Vietnam [63], and Thai-Myanmar border [64]. However, *P. vivax* is still sensitive to chloroquine in Cambodia [65], border area of Yunnan Province of China and Myanmar [66], central China [67], and Nepal [68, 69].

2.5. Vector control

Vector control remains one of the main preventive strategies of containing malaria transmission in APMEN countries. However, a lack of technical capacity in entomology and vector control represents a key gap in elimination programmes. In addition, the diversity of malaria vectors in the

http://dx.doi.org/10.5772/intechopen.75405

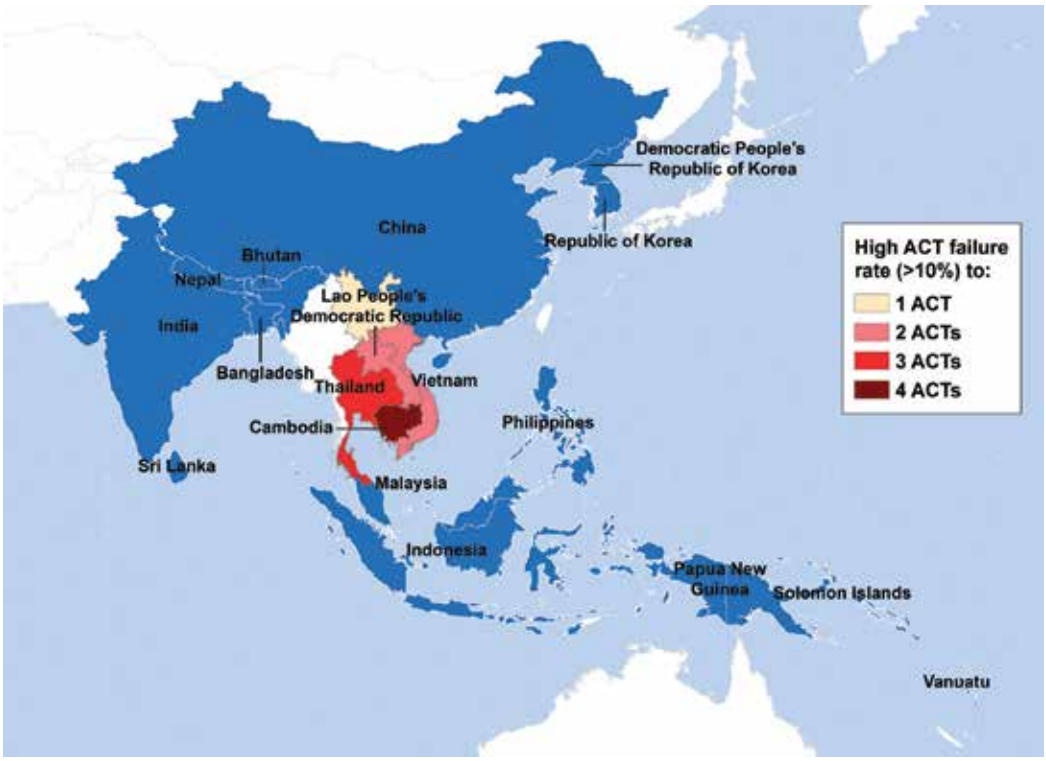


Figure 3. Distribution of malarial multidrug resistance for data based on 2016. ACT- artemisinin-based combination therapy; 1 ACT- resistance to one ACT; 2 ACT- resistance to two ACTs; 3 ACTs- resistance to three ACTs; 4 ACTs- resistance to four ACTs. Source: World malaria report 2017 [1].

Asia-Pacific region (19 different species) poses unique challenges for elimination [70, 71] (**Table 2**). There is considerable variation in bionomical characteristics of mosquito vectors making control efforts difficult. The commonest malaria vector species in the region, including *Anopheles dirus, An. baimaii,* and *An. minimus* [72, 73], are able to avoid indoor sprayed surfaces because of their exophilic and exophagic characteristics [70, 74, 75] rendering most domicile-based interventions, like long-lasting insecticidal nets (LLIN) and indoor residual spraying (IRS), less effective [74, 76]. Other challenges include insecticide resistance [77] and absence of local vector surveillance [78]. To address these challenges, APMEN instituted the APMEN Vector Control Working Group (VcWG) in 2010 [79]. The working group fosters information exchange between vector control experts and national programme managers of APMEN countries to formulate strategies to counter the challenges faced in the region. The Working Group has supported a range of activities to build vector control capacity in the region, including providing training fellowships to vector control officers in priority areas, supporting community efficacy studies of interventions, and consolidating information on vector management practices in the region [78].

2.6. Forest malaria

Forest malaria constitutes bulk of transmission in APMEN countries [42, 43, 80–83]. Many species of *Anopheles* mosquitoes that transmit malaria agents are abundant in natural forests

Country°	Main vectors*
Bangladesh [210]	*An. dirus, An. minimus, An. aconitus, An. philippinensis, An. sundaicus, An. barbirostris, An. subpictus, An. culicifacies, An. fluviatilis, An. maculatus*
Bhutan [210]	*An. minimus*
Cambodia [211]	*An. dirus, An. minimus, An. maculatus, An. epiroticus*
China [73, 212]	*An. sinensis, An. lesteri, An. dirus, An. minimus, An. maculatus*
DPR Korea [210]	*An. lesteri, An. sinensis, An. sineroides, An. kleini, An. yatsus hiroensis, An. lindesayi japonicas, An. koreicus*
India [213]	*An. culicifacies, An. baimaii, An. fluviatilis, An. minimus, An. stephensi, An. maculatus, An. sundaicus*
Indonesia [214]	*An. aconitus, An. balabacensis, An. bancrofti, An. barbirostris, An. barbumbrosus, An. farauti, An. flavirostris, An. karwari, An. kochi, An. koliensis, An. leucosphyrus, An. maculatus, An. nigerrimus, An. parangensis, An. punctulatus, An. sinensis, An. subpictus, An. sundaicus, An. tessellatus, An. vagus*
Lao PDR [211]	*An. dirus, An. minimus, An. maculatus, An. jeyporiensis*
Malaysia [211]	*An. balabacensis, An. campestris, An. cracens, An. donaldi, An. flavirostris, An. latens, An. letifer, An. maculatus, An. sundaicus*
Nepal [210]	*An. fluviatilis, An. annularis, An. maculatus*
Philippines [215]	*An. flavirostris, An. balabacensis, An. maculatus, An. litoralis, An. mangyanus*
PNG [216, 217]	*An. farauti, An. koliensis, An. punctulatus, An. bancroftii, An. karwari*
Republic of Korea [218]	*An. kleini, An. pullus, An. belenrae, An. sineroides, An. sinensis, An. lesteri*
Solomon Islands [217]	*An. punctulatus, An. koliensis, An. farauti*
Sri Lanka [210, 219]	*An. culicifacies, An. annularis, An. subpictus, An. tessellatus, An. stephensi*
Thailand [210]	*An. dirus, An. minimus, An. maculatus, An. aconitus, An. epiroticus*
Vanuatu [217]	*An. farauti*
Vietnam [211]	*An. dirus, An. minimus, An. maculatus, An. aconitus, An. jeyporiensis, An. subpictus, An. sinensis, An. pampanai, An. epiroticus*

*An., *Anopheles* names refer either to the group, complex or species when specific identifications have been done.
°Corresponding references are in brackets.

Table 2. List of the main malaria vectors in the Asia Pacific Malaria Elimination Network (APMEN) countries.

and forested plantations. Both the forests and occurrence of deforestation impact increasing malaria risk and transmission, particularly in border areas. Forested areas provide conducive environment for vector proliferation and survival [84, 85]. Forest vectors usually prefer tree canopy coverage and are known to take shelter in tree holes [86–88]. Forest flora and sugar availability have also been shown to be crucial determinants of vectorial capacity [89]. In addition, leaves falling into larval habitats assure sustainable micro-climatic conditions for larvae of vectors like *An. dirus*, which is a dominant vector in Southeast Asia [90]. Further, there are usually abundant bodies of water including ponds, streams, and rivers in forested areas supporting vector multiplication and survival thereby sustaining malaria transmission

in the region [80, 90–93]. Deforestation increases the risk of malaria through a number of favourable conditions for the *Anopheles* mosquito by creating mosquito-breeding sites in the stumps of trees, ditches and puddles on the ground. The direct sunlight on the pools of water increases temperatures promoting mosquito breeding. Increased human activities in deforested areas such as logging, increased large-scale agricultural activities, mining, building of hydropower projects, and the collection of wood for fuel, all enhance contact with mosquitoes and thereby increased malaria transmission [94–96].

Populations in border areas are at greater risk of malaria infections because they frequently visit forests, forest fringe areas, or forested plantations at or near the border [42, 75, 97, 98]. Occupational exposures affect malarial receptivity by age group–for example, in forest fringe villages, adult infections are more prevalent due to forest-related activities such as logging, rubber tapping, bamboo cutting, charcoaling, foraging, and overnight stays in the forests [99]. Migration of the population working in the forest and forest fringe results in spread via carriers to new areas previously free from malaria transmission [100]. Despite high coverage of preventive measures such as LLIN or insecticide-treated nets (ITNs) and IRS in the member States of APMEN, populations working and staying overnight in the forest are not protected [43, 82, 101]. A lack of infrastructure such as roads and healthcare facilities hinder malaria control activities and delayed treatment.

2.7. Migration and cross-border malaria

One of the main challenges that continues thwart malaria elimination is cross-border malaria [94, 102]. People migrate across international borders for a number of reasons including work opportunities, visiting friends and relatives, and displacement as a result of natural and manmade calamities (such as ethnic conflicts) and major development projects. Malaria control in border areas is often difficult for being heavily forested, mountainous and inaccessible terrain, and unregulated population movements across the borders [103, 104]. Open porous international borders allow unchecked movement of people [105–111]. Such cross-border migration is likely to derail the malaria control activities of the neighbouring countries and risk introduction of drug-resistant parasites [112]. Mobile populations along the border areas often live in poverty and have poor access to healthcare services. Movement of people across international borders has contributed to maintaining high transmission hotspots adjacent to border points [73, 105, 113].

2.8. Misalignment of programmatic approaches

There are differences in programmatic approaches among neighbouring countries in the APMEN region making the coordination of control and preventive measures challenging [114, 115]. For example, there are differences in malaria control activities across Laos-Vietnam border. In Laos, the mainstay of malaria control is distribution of LLINs but on the Vietnamese side there is a stronger focus on IRS [114, 115]. Even where the approaches are similar, the specific antimalarial drugs or insecticides used can influence effectiveness due to parasite or vector resistance. Deltamethrin (synthetic pyrethroid) is used for IRS in Bhutan, however, DDT is used in the neighbouring State of Assam in India [116–118]. Effective control or elimination requires coordinated efforts for control interventions.

3. Way forward

In light of the aforementioned challenges in the APMEN member States, some of the possible solutions for way forward include carrying out operational research (OR) to understand the micro-epidemiology of malaria in each country, the use of technologically-assisted solutions for managing operational data (including spatial decision support systems (SDSS)), strengthening surveillance and initiating cross-border initiative.

3.1. Operational research (OR)

As countries move forward with malaria elimination, this effort requires adjustments on the way national malaria programmes operate. For example, the strategies for case detection and surveillance are radically different in control and elimination programmes. Countries may face constraints or bottlenecks as they make the transition from control to elimination for which OR can help to remove these bottlenecks, thereby enabling countries to make the transition from control to elimination phases more rapidly [119, 120]. OR in health is defined as search for knowledge on interventions, strategies, or tools that can enhance the quality, effectiveness, or coverage of programmes [121], and results in improved policy-making, better design and implementation of health systems, and more efficient methods of service delivery [122–125]. The goal is to strengthen health services and improve healthcare delivery in disease-endemic countries and it has an additional critical role to play in helping solve major implementation problems [121, 126–128]. The key elements of OR are that the research questions are generated by identifying the constraints and challenges encountered during the implementation of programme activities, thus can be imbedded into routine programmatic activities [129]. The WHO and Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM) have been encouraging programmes to conduct OR as part of their donor-funded activities [119, 130].

A significant limitation of national programmes has been the poor ability, even inability, to manage operational data collected through surveillance and other health information systems [131]. OR can be used to address these knowledge gaps and provide solutions to this limitation. OR has been under-utilised in APMEN member States [132, 133]. However, some countries including China [134], Bhutan [108], India [135, 136], Nepal [137], Solomon Islands [138], and countries in the Greater Mekong Sub-region (GMS) [120] are starting to address the challenges in malaria elimination efforts through OR in areas such as artemisinin resistance.

A key challenge is a lack of operational research capacity of member States [133]. One of the ways to overcome this shortcoming is to develop research capacity through the Structured Operational Research and Training Initiative (SORT IT), a global partnership-based initiative led by the Special Programme for Research and Training in Tropical Diseases (TDR) of WHO [131, 139, 140].

3.2. Role of geospatial data analysis

Malaria has a focal spatial distribution in pre-elimination and elimination phases, with hotspots of transmission in which the risk of malaria (including asymptomatic parasitaemias) and number of cases are higher than in surrounding areas [141, 142]. The scale at which spatial

heterogeneity occurs ranges from micro-geographical setting beginning with household or village level [143–149] to municipalities [150], sub-districts [111], district [151–153], subnational [105, 154–156], national [40], regional [157], and global scales [70]. These spatial clusters of malaria have the potential to be sources of spread into neighbouring regions and countries if there is no focused intervention in the hotspot areas. Given the spatial heterogeneity of the disease, focused interventions in areas with higher incidence of disease are likely to have greater impact than uniform resource allocation [158]. Therefore, the spatial distribution of malaria and its interventions should be taken into account in national malaria elimination plans.

Risk mapping and temporal forecasting of malaria using environmental and climatic factors as spatial and/or temporal risk predictors has been routinely undertaken [107, 159, 160]. Environmental data for geospatial and temporal analysis can be collected through satellite sensors or meteorological stations [159–162]. Image analysis techniques can be applied to satellite data to derive useful variables for the investigation of environmental drivers of malaria, such as land surface temperature, cold cloud duration (an indirect measure of rainfall), land use or land cover class, and normalised difference vegetation index (NDVI) [85, 161]. The NDVI can be used as proxy for rainfall through the measure of the greenness of the earth's surface and hence vegetation cover [163]. Meteorological data can be interpolated with statistical techniques to estimate values of climatic variables, such as rainfall, temperature, and humidity, for locations where meteorological data are not available [164]. Currently these approaches have mainly been used in research context, and more research including OR needs to be conducted to establish how these approaches can be of practical benefit to malaria control and elimination programmes.

3.3. Spatial decision support systems

In recent years, spatial decision support systems (SDSSs) have been increasingly used in malaria elimination programmes in some countries of Asia-Pacific region to support planning, monitoring and evaluation, including Vanuatu, Solomon Islands and Bhutan [110, 165]. SDSSs have also been employed for other vector-borne disease control programmes such as dengue in Thailand and Singapore [166–168].

SDSSs are technology-driven systems for the collection, mapping, displaying and dissemination of disease data. They provide computerised support for decision making that helps spatially-explicit resource allocation decisions [107, 169]. Key elements of SDSS include: (i) data inputs from a variety of sources (including geospatial data layers), (ii) automated outputs to guide informed and strategic decision making for designated applications, (iii) enabling application/intervention outcomes re-entered back into the SDSS as a cyclical input, and (iv) expert knowledge integrated throughout all stages of the spatial decision support process [170] (**Figure 4**). In most recent examples, data are fed into the SDSS in the field using personal digital assistants (PDAs). The SDSS contains modules for planning, monitoring and evaluating coverage of target populations with IRS and LLINs, and for mapping malaria surveillance data. A mechanism is provided to link routinely collected data with associated spatial information. Spatial queries and analyses can be conducted and cartographic maps and reports of the areas of interest can be produced. Summary statistics of key indicators and maps are fed back to field teams to enhance implementation of interventions.

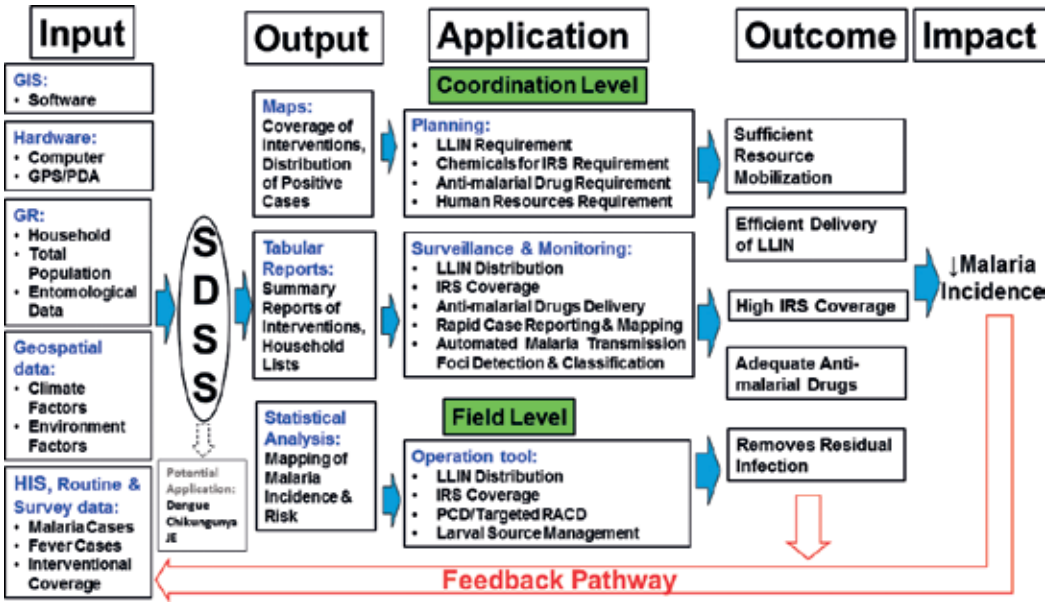


Figure 4. Framework of spatial decision support system for malaria control and prevention with potential use in other vector borne diseases. (GIS geographical information system, PDA personnel digital assistant, GPS global positioning system, SDSS spatial decision support system, GR geographic reconnaissance, LLIN long-lasting insecticidal net, IRS indoor residual spraying, PCD passive case detection, RACD active case detection, JE Japanese encephalitis) (Wangdi et al. [110]).

Limited evaluation to date suggests that these systems support health programmes with a powerful and user-friendly operational tool for evidence-based decision making. Maps are an important SDSS output that provide a visual aid for decision making [170]. An example of map used to monitor LLIN coverage during a mass LLIN distribution in Bhutan is shown in **Figure 5**. This map can inform programme officials of the progress of the campaign and more importantly identifies areas that require catch up activities to achieve target coverage. Malaria incidence maps provide important inputs to policy makers to implement targeted interventions aimed at disease prevention and management. Spatial targeting of malaria interventions, supported by SDSS, will result in more efficient and effective allocation of intervention resources in transmission hotspots helping achieve substantial transmission reduction [135, 156, 158, 171].

3.4. Strengthening surveillance-response and cross-border initiatives

For countries embarking on malaria elimination, malaria surveillance systems need revamping. The main objectives of surveillance in malaria elimination are to detect infections (both symptomatic and asymptomatic), and ensure radical cure. This is in contrast to the malaria control phase in which the main objectives of surveillance is to quantify the level of malaria transmission and to support preventive action at the population level [172, 173]. In most countries, malaria surveillance is based on passive case detection. Passive surveillance involves reporting malaria cases by a health facility, which can be limited by incomplete reporting, healthcare seeking in the private sector (not captured by government systems), and poor diagnostic capacity, particularly in low transmission settings [174]. Prompt detection and radical treatment of

http://dx.doi.org/10.5772/intechopen.75405

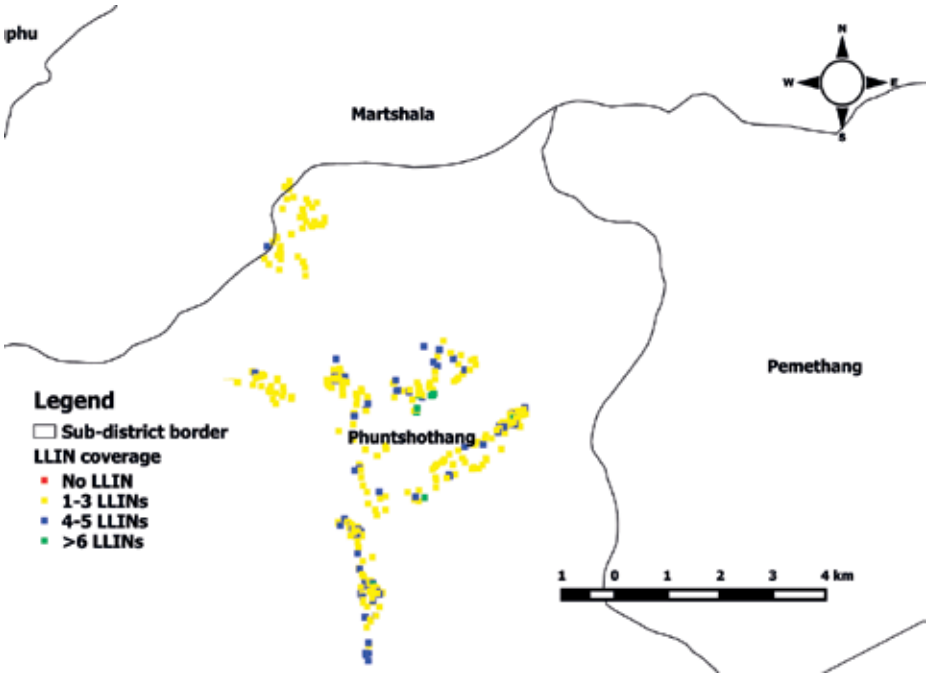


Figure 5. Sample output map for monitoring the coverage of long-lasting insecticidal net in Bhutan (Samdrup Jongkhar) (in this map there was no households without LLIN) (Wangdi et al. [110]).

imported malaria cases is critical for malaria elimination for sustaining the malaria elimination efforts. However, importation of malaria is inevitable, even in countries that have eliminated malaria. Passive case detection (PCD) could capture imported cases and allow interventions that would prevent resurgence in the presence of robust health system [175]. However, in areas with high transmission intensities in APMEN countries [70, 176], and unchecked migration across borders [103–111], there is likely to be significant transmission even in low transmission settings. Therefore, imported infections must be prevented through border screening, regional and cross-border initiatives and dialogue, proactive case detection, and treatment in high-risk population groups and travellers preventing resurgence of the disease [177].

Active surveillance addresses some of the limitations of PCD and generally involves cross-sectional surveys of defined sample populations, where the primary malaria indicator is the proportion of persons infected with malaria parasites (parasite prevalence) [178]. These surveys enable detection of asymptomatic infections that perpetuate transmission [179], and provide an opportunity to concurrently assess coverage of malaria interventions [180], but they are expensive and difficult to implement, and are not efficient in low-transmission settings.

One of the most efficient ways to enhance passive surveillance is through reactive case detection (RACD). When an index case of clinical malaria is detected in a community, RACD is carried out in all the households located within a certain distance of the index case. During the RACD, follow-up activities differ widely and can include testing of fever using RDTs or microscopy for any residual malaria infection and treating those who test positive. In addition, vector control activities including IRS and LLINs are intensified. RACD has been implemented in Africa and

Asia with mixed results [110, 181–186]. Nevertheless, RACD provides an opportunity for public health workers to concurrently assess coverage of malaria interventions including LLINs, and should be advocated and practised. Another efficient way to evaluate the efficacy of vector control methods, also applied in Africa and Asia, is to estimate the human antibody response to *Anopheles* saliva in human populations [187–189].

Diagnostic techniques used for testing blood during RACD will significantly impact the programme effectiveness. Estimating parasite prevalence using microscopy is time and labour intensive, and often inaccurate in operational settings [190]. Newly available rapid diagnostic tests (RDTs) offer on-the-spot results, but have limitations in specificity, sensitivity, quality, and cost [190–193]. Both methods (microscopy and RDTs) may fail to detect a substantial proportion of low-density parasitaemias [186, 194, 195]. Polymerase chain reaction (PCR) provides enhanced sensitivity but results are not available immediately [196], instead Real-time PCR may present a consistent, accurate, and efficient tool for surveillance to assist malaria elimination in the future [196].

Cross-border movement of populations impacts the maintenance of 'hotspots' of high transmission along international borders [77, 94, 97, 108, 137, 197–200], and spread of drug-resistance seen along the international border of Thailand and Cambodia [201]. Then, cross-border initiatives should be initiated through sharing of programme data including insecticide resistance, blood testing at the border areas, and treatment of symptomatic cases [177, 202–208]. Such successful cross-border case studies in the region have led to significant reduction in malaria burden in the study areas [209].

4. Conclusions

Successful malaria elimination in the APMEN member States will greatly enhance the global drive to eliminate malaria. Malaria transmission in these States is complex. APMEN member States include the largest populations living in areas of malaria transmission risk outside Africa. They are a global source of ACT resistance, highest burden of *P. vivax* and zoonotic malaria, and face many geopolitical and socioeconomic factors that will challenge malaria elimination efforts. These challenges can be addressed in part through operational research to identify country specific solutions, making better use of operational data such as through implementing SDSS approaches, and strengthening surveillance and cross-border collaborations.

Abbreviations

ACT	artemisinin-based combination therapy
AIM	action and investment to defeat malaria 2016–2030
APMEN	Asia Pacific Malaria Elimination Network
DDT	dichlorodiphenyltrichloroethane

DPR Korea	Democratic People's Republic of Korea
G6PD	glucose-6-phosphate dehydrogenase
GFATM	Global Fund to Fight AIDS, Tuberculosis and Malaria
GIS	geographic information systems
GMS	Greater Mekong Sub-region
GST	Global Technical Strategy for Malaria
IRS	indoor residual spraying
ITN	insecticide-treated nets
Lao PDR	Lao People's Demographic Republic
LLIN	long-lasting insecticidal nets
MIS	malaria indicator survey
NDVI	normalised difference vegetation index
OR	operational research
PCD	passive case detection
PCR	polymerase chain reaction
RACD	reactive case detection
RDT	rapid diagnostic test
PNG	Papua New Guinea
SDSS	spatial decision support systems
SEAR	South-East Asian Region
SORT IT	Structured Operational Research and Training Initiative
TDR	Research and Training in Tropical Diseases
VcWG	Vector Control Working Group
WHO	World Health Organisation

Author details

Kinley Wangdi* and Archie CA Clements

*Address all correspondence to: kinley.wangdi@anu.edu.au

Department of Global Health, Research School of Population Health, The Australian National University, Canberra, ACT, Australia

References

[1] WHO. World Malaria Report 2017. Geneva, Switzerland: WHO Library Cataloguing-in-Publication Data; 2017

[2] WHO. World Malaria Report 2016. Switzerland: WHO Library Cataloguing-in-Publication Data Geneva; 2016

[3] Malaria; 2016. [http://www.who.int/malaria/media/world-malaria-report-2016/en/]

[4] WHO. World Malaria Report 2014. Switzerland: World Health Organization, Geneva, Switzerland, Geneva; 2014

[5] WHO. Global Technical Strategy for Malaria 2016-2030. WHO Library Cataloguing-in-Publication Data; 2015

[6] Wang SQ, Li YC, Zhang ZM, Wang GZ, Hu XM, Qualls WA, Xue RD. Prevention measures and socio-economic development result in a decrease in malaria in Hainan, China. Malaria Journal. 2014;**13**:362

[7] Tusting LS, Willey B, Lucas H, Thompson J, Kafy HT, Smith R, Lindsay SW. Socioeconomic development as an intervention against malaria: A systematic review and meta-analysis. Lancet. 2013;**382**:963-972

[8] Datta SC, Reimer JJ. Malaria and economic development. Review of Development Economics. 2013;**17**:1-15

[9] Malaria; 2017. [http://www.who.int/malaria/media/world-malaria-report-2017/en/]

[10] Action and Investiment to Defeat Malaria 2016-2030; 2015. [https://rollbackmalaria.com/about-rbm/aim-2016-2030/]

[11] Gosling RD, Whittaker M, Gueye CS, Fullman N, Baquilod M, Kusriastuti R, Feachem RG. Malaria elimination gaining ground in the Asia Pacific. Malaria Journal. 2012;**11**:346

[12] APMEN: Elimination 2030: Working Together for a Malria-free Aisa Pacific. 2014;**2017**. [http://apmen.org/web/1/elimination/]

[13] Chairman's Statement of 9th East Asia Summit; 2014. [http://dfat.gov.au/international-relations/regional-architecture/eas/Pages/chairmans-statement-of-the-9th-east-asia-summit.aspx]

[14] WHO certifies Sri Lanka malaria-free; 2016. [http://www.searo.who.int/mediacentre/releases/2016/1631/en/]

[15] Malaria elimination goals; 2015. [http://apmen.org/web/1/elimination/]

[16] WHO: Malaria Control and Elimination in the Western Pacific (2016-2020). (Pacific WHOROftW ed. Manila, Philippines. 2017

[17] Feachem RG, Phillips AA, Hwang J, Cotter C, Wielgosz B, Greenwood BM, Sabot O, Rodriguez MH, Abeyasinghe RR, Ghebreyesus TA, Snow RW. Shrinking the malaria map: Progress and prospects. Lancet. 2010;**376**:1566-1578

[18] Baird JK. Neglect of *Plasmodium vivax* malaria. Trends in Parasitology. 2007;**23**:533-539

[19] Ballut PC, Siqueira AM, Orlando AC, Alexandre MA, Alecrim MG, Lacerda MV. Prevalence and risk factors associated to pruritus in *Plasmodium vivax* patients using chloroquine in the Brazilian Amazon. Acta Tropica. 2013;**128**:504-508

[20] Mendis K, Sina BJ, Marchesini P, Carter R. The neglected burden of *Plasmodium vivax* malaria. The American Journal of Tropical Medicine and Hygiene. 2001;**64**:97-106

[21] Sharma VP, Dev V, Phookan S. Neglected *Plasmodium vivax* malaria in northeastern states of India. The Indian Journal of Medical Research. 2015;**141**:546-555

[22] Waheed AA, Ghanchi NK, Rehman KA, Raza A, Mahmood SF, Beg MA. Vivax malaria and chloroquine resistance: A neglected disease as an emerging threat. Malaria Journal. 2015;**14**:146

[23] Gray KA, Dowd S, Bain L, Bobogare A, Wini L, Shanks GD, Cheng Q. Population genetics of *Plasmodium falciparum* and *Plasmodium vivax* and asymptomatic malaria in Temotu Province, Solomon Islands. Malaria Journal. 2013;**12**:429

[24] Noviyanti R, Coutrier F, Utami RA, Trimarsanto H, Tirta YK, Trianty L, Kusuma A, Sutanto I, Kosasih A, Kusriastuti R, et al. Contrasting transmission dynamics of co-endemic *Plasmodium vivax* and *P. falciparum*: Implications for malaria control and elimination. PLoS Neglected Tropical Diseases. 2015;**9**:e0003739

[25] Jennison C, Arnott A, Tessier N, Tavul L, Koepfli C, Felger I, Siba PM, Reeder JC, Bahlo M, Mueller I, Barry AE. *Plasmodium vivax* populations are more genetically diverse and less structured than sympatric *Plasmodium falciparum* populations. PLoS Neglected Tropical Diseases. 2015;**9**:e0003634

[26] Bousema T, Drakeley C. Epidemiology and infectivity of *Plasmodium falciparum* and *Plasmodium vivax* gametocytes in relation to malaria control and elimination. Clinical Microbiology Reviews. 2011;**24**:377-410

[27] Domingo GJ, Satyagraha AW, Anvikar A, Baird K, Bancone G, Bansil P, Carter N, Cheng Q, Culpepper J, Eziefula C, et al. G6PD testing in support of treatment and elimination of malaria: Recommendations for evaluation of G6PD tests. Malaria Journal. 2013;**12**:391

[28] von Seidlein L, Auburn S, Espino F, Shanks D, Cheng Q, McCarthy J, Baird K, Moyes C, Howes R, Menard D, et al. Review of key knowledge gaps in glucose-6-phosphate dehydrogenase deficiency detection with regard to the safe clinical deployment of 8-aminoquinoline treatment regimens: A workshop report. Malaria Journal. 2013;**12**:112

[29] Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. Lancet. 2008; **371**:64-74

[30] Howes RE, Battle KE, Satyagraha AW, Baird JK, Hay SI. G6PD deficiency: Global distribution, genetic variants and primaquine therapy. Advances in Parasitology. 2013;**81**: 133-201

[31] Moyes CL, Henry AJ, Golding N, Huang Z, Singh B, Baird JK, Newton PN, Huffman M, Duda KA, Drakeley CJ, et al. Defining the geographical range of the *Plasmodium knowlesi* reservoir. PLoS Neglected Tropical Diseases. 2014;**8**:e2780

[32] Khim N, Siv S, Kim S, Mueller T, Fleischmann E, Singh B, Divis PC, Steenkeste N, Duval L, Bouchier C, et al. *Plasmodium knowlesi* infection in humans, Cambodia, 2007-2010. Emerging Infectious Diseases. 2011;**17**:1900-1902

[33] Ta TT, Salas A, Ali-Tammam M, Martinez Mdel C, Lanza M, Arroyo E, Rubio JM. First case of detection of *Plasmodium knowlesi* in Spain by real time PCR in a traveller from Southeast Asia. Malaria Journal. 2010;**9**:219

[34] Lubis IND, Wijaya H, Lubis M, Lubis CP, Divis PCS, Beshir KB, Sutherland CJ. Contribution of *Plasmodium knowlesi* to multispecies human malaria infections in north Sumatera, Indonesia. The Journal of Infectious Diseases. 2017;**215**:1148-1155

[35] Singh B, Kim Sung L, Matusop A, Radhakrishnan A, Shamsul SS, Cox-Singh J, Thomas A, Conway DJ. A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings. Lancet. 2004;**363**:1017-1024

[36] Singh B, Daneshvar C. Human infections and detection of *Plasmodium knowlesi*. Clinical Microbiology Reviews. 2013;**26**:165-184

[37] Ta TH, Hisam S, Lanza M, Jiram AI, Ismail N, Rubio JM. First case of a naturally acquired human infection with *Plasmodium cynomolgi*. Malaria Journal. 2014;**13**:68

[38] Baird JK. Asia-Pacific malaria is singular, pervasive, diverse and invisible. International Journal for Parasitology. 2017;**47**:371-377

[39] Overview: Malaria in the Asia-Pacific; 2014. [un.org.au/files/2014/04/Malaria-in-the-Asia-Pacific-WMD-2014.pdf]

[40] Bui HM, Clements AC, Nguyen QT, Nguyen MH, Le XH, Hay SI, Tran TH, Wertheim HF, Snow RW, Horby P. Social and environmental determinants of malaria in space and time in Viet Nam. International Journal for Parasitology. 2011;**41**:109-116

[41] Erhart A, Ngo DT, Phan VK, Ta TT, Van Overmeir C, Speybroeck N, Obsomer V, Le XH, Le KT, Coosemans M, D'Alessandro U. Epidemiology of forest malaria in Central Vietnam: A large scale cross-sectional survey. Malaria Journal. 2005;**4**:58

[42] Erhart A, Thang ND, Hung NQ, Toi le V, Hung le X, Tuy TQ, Cong le D, Speybroeck N, Coosemans M, D'Alessandro U. Forest malaria in Vietnam: A challenge for control. American Journal of Tropical Medicine and Hygiene. 2004;**70**:110-118

[43] Grietens KP, Xuan XN, Ribera J, Duc TN, Bortel W, Ba NT, Van KP, Xuan HL, D'Alessandro U, Erhart A. Social determinants of long lasting insecticidal hammock use among the Ra-glai ethnic minority in Vietnam: Implications for forest malaria control. PLoS One. 2012;**7**:e29991

[44] Liu Y, Hsiang MS, Zhou H, Wang W, Cao Y, Gosling RD, Cao J, Gao Q. Malaria in overseas labourers returning to China: An analysis of imported malaria in Jiangsu Province, 2001-2011. Malaria Journal. 2014;**13**:29

[45] Pichainarong N, Chaveepojnkamjorn W. Malaria infection and life-style factors among hilltribes along the Thai-Myanmar border area, northern Thailand. The Southeast Asian Journal of Tropical Medicine and Public Health. 2004;**35**:834-839

[46] WHO: Containment of Malaria Multi-Drug Resistance on the Cambodia-Thailand Border. WHO-Mekong Malaria Programme; 2007

[47] Satimai W, Sudathip P, Vijaykadga S, Khamsiriwatchara A, Sawang S, Potithavoranan T, Sangvichean A, Delacollette C, Singhasivanon P, Kaewkungwal J, Lawpoolsri S. Artemisinin resistance containment project in Thailand. II: Responses to mefloquine-artesunate combination therapy among falciparum malaria patients in provinces bordering Cambodia. Malaria Journal. 2012;**11**:300

[48] Dondorp AM, Fairhurst RM, Slutsker L, Macarthur JR, Breman JG, Guerin PJ, Wellems TE, Ringwald P, Newman RD, Plowe CV. The threat of artemisinin-resistant malaria. The New England Journal of Medicine. 2011;**365**:1073-1075

[49] Enserink M. Malaria's drug miracle in danger. Science. 2010;**328**:844-846

[50] Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, Lwin KM, Ariey F, Hanpithakpong W, Lee SJ, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. The New England Journal of Medicine. 2009;**361**:455-467

[51] Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, Sreng S, Anderson JM, Mao S, Sam B, et al. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. The New England Journal of Medicine. 2014;**371**:411-423

[52] WHO. Global report on antimalarial drug efficacy and drug resistance: 2000-2010. WHO Library Cataloguing-in-Publication Data; 2010

[53] Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM. Evidence of artemisinin-resistant malaria in western Cambodia. The New England Journal of Medicine. 2008;**359**:2619-2620

[54] Hien TT, Thuy-Nhien NT, Phu NH, Boni MF, Thanh NV, Nha-Ca NT, Thai le H, Thai CQ, Toi PV, Thuan PD, et al. In vivo susceptibility of *Plasmodium falciparum* to artesunate in Binh Phuoc Province, Vietnam. Malaria Journal. 2012;**11**:355

[55] Mishra N, Prajapati SK, Kaitholia K, Bharti RS, Srivastava B, Phookan S, Anvikar AR, Dev V, Sonal GS, Dhariwal AC, et al. Surveillance for artemisinin resistance in *Plasmodium falciparum* in India using the kelch13 molecular marker. Antimicrobial Agents and Chemotherapy. 2015;**59**:2548-2553

[56] Saha P, Guha SK, Das S, Mullick S, Ganguly S, Biswas A, Bera DK, Chattopadhyay G, Das M, Kundu PK, et al. Comparative efficacies of artemisinin combination therapies in *Plasmodium falciparum* malaria and polymorphism of pfATPase6, pfcrt, pfdhfr, and pfdhps genes in tea gardens of Jalpaiguri District, India. Antimicrobial Agents and Chemotherapy. 2012;**56**:2511-2517

[57] Patel P, Bharti PK, Bansal D, Ali NA, Raman RK, Mohapatra PK, Sehgal R, Mahanta J, Sultan AA, Singh N. Prevalence of mutations linked to antimalarial resistance in *Plasmodium falciparum* from Chhattisgarh, Central India: A malaria elimination point of view. Scientific Reports. 2017;**7**:16690

[58] Price RN, von Seidlein L, Valecha N, Nosten F, Baird JK, White NJ. Global extent of chloroquine-resistant *Plasmodium vivax*: A systematic review and meta-analysis. The Lancet Infectious Diseases. 2014;**14**:982-991

[59] Srivastava HC, Yadav RS, Joshi H, Valecha N, Mallick PK, Prajapati SK, Dash AP. Therapeutic responses of *Plasmodium vivax* and *P. falciparum* to chloroquine, in an area of western India where *P. vivax* predominates. Annals of Tropical Medicine and Parasitology. 2008;**102**:471-480

[60] Adak T, Sharma VP, Orlov VS. Studies on the *Plasmodium vivax* relapse pattern in Delhi, India. The American Journal of Tropical Medicine and Hygiene. 1998;**59**:175-179

[61] Yadav RS, Ghosh SK. Radical curative efficacy of five-day regimen of primaquine for treatment of *Plasmodium vivax* malaria in India. The Journal of Parasitology. 2002; **88**:1042-1044

[62] Singh RK. Emergence of chloroquine-resistant vivax malaria in South Bihar (India). Transactions of the Royal Society of Tropical Medicine and Hygiene. 2000;**94**:327

[63] Thanh PV, Hong NV, Van NV, Louisa M, Baird K, Xa NX, Peeters Grietens K, Hung le X, Duong TT, Rosanas-Urgell A, et al. Confirmed *Plasmodium vivax* resistance to chloroquine in Central Vietnam. Antimicrobial Agents and Chemotherapy. 2015;**59**:7411-7419

[64] Rungsihirunrat K, Muhamad P, Chaijaroenkul W, Kuesap J, Na-Bangchang K. *Plasmodium vivax* drug resistance genes; Pvmdr1 and Pvcrt-o polymorphisms in relation to chloroquine sensitivity from a malaria endemic area of Thailand. The Korean Journal of Parasitology. 2015;**53**:43-49

[65] Amaratunga C, Sreng S, Mao S, Tullo GS, Anderson JM, Chuor CM, Suon S, Fairhurst RM. Chloroquine remains effective for treating *Plasmodium vivax* malaria in Pursat province, Western Cambodia. Antimicrobial Agents and Chemotherapy. 2014;**58**:6270-6272

[66] Liu H, Yang HL, Tang LH, Li XL, Huang F, Wang JZ, Li CF, Wang HY, Nie RH, Guo XR, et al. Monitoring *Plasmodium vivax* chloroquine sensitivity along China-Myanmar border of Yunnan Province, China during 2008-2013. Malaria Journal. 2014;**13**:364

[67] Lu F, Wang B, Cao J, Sattabongkot J, Zhou H, Zhu G, Kim K, Gao Q, Han ET. Prevalence of drug resistance-associated gene mutations in *Plasmodium vivax* in Central China. The Korean Journal of Parasitology. 2012;**50**:379-384

[68] Ranjitkar S, Schousboe ML, Thomsen TT, Adhikari M, Kapel CM, Bygbjerg IC, Alifrangis M. Prevalence of molecular markers of anti-malarial drug resistance in *Plasmodium vivax* and *Plasmodium falciparum* in two districts of Nepal. Malaria Journal. 2011;**10**:75

[69] Pant DK, Joshi AB, Lekhak B. Assessment of therapeutic efficacy of anti malarial drug (Chloroquine) against *Plasmodium vivax* malaria in Kanchanpur District. Journal of Nepal Health Research Council. 2006;**4**:41-45

[70] Sinka ME, Bangs MJ, Manguin S, Rubio-Palis Y, Chareonviriyaphap T, Coetzee M, Mbogo CM, Hemingway J, Patil AP, Temperley WH, et al. A global map of dominant malaria vectors. Parasites & Vectors. 2012;**5**:69

[71] Morgan K, Somboon P, Walton C. Understanding *Anopheles* diversity in Southeast Asia and its applications for malaria control. In: Manguin S, editor. *Anopheles* Mosquitoes - New Insights into Malaria Vectors. Rijeka: InTech; 2013. Ch. 11

[72] Dev V, Manguin S. Biology, distribution and control of *Anopheles (Cellia) minimus* in the context of malaria transmission in northeastern India. Parasites & Vectors. 2016;**9**:585

[73] Zhang S, Guo S, Feng X, Afelt A, Frutos R, Zhou S, Manguin S. *Anopheles* vectors in mainland China while approaching malaria elimination. Trends in Parasitology. 2017;**33**:889-900

[74] Trung HD, Bortel WV, Sochantha T, Keokenchanh K, Briet OJ, Coosemans M. Behavioural heterogeneity of *Anopheles* species in ecologically different localities in Southeast Asia: A challenge for vector control. Tropical Medicine & International Health. 2005;**10**:251-262

[75] Manh CD, Beebe NW, Van VN, Quang TL, Lein CT, Nguyen DV, Xuan TN, Ngoc AL, Cooper RD. Vectors and malaria transmission in deforested, rural communities in north-Central Vietnam. Malaria Journal. 2010;**9**:259

[76] Somboon P, Lines J, Aramrattana A, Chitprarop U, Prajakwong S, Khamboonruang C. Entomological evaluation of community-wide use of lambdacyhalothrin-impregnated bed nets against malaria in a border area of north-West Thailand. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1995;**89**:248-254

[77] Dhiman S, Goswami D, Rabha B, Gopalakrishnan R, Baruah I, Singh L. Malaria epidemiology along indo-Bangladesh border in Tripura state, India. The Southeast Asian Journal of Tropical Medicine and Public Health. 2010;**41**:1279-1289

[78] Vector control; 2010. [http://apmen.org/focus-areas/vector-control/]

[79] APMEN: APMEN Vector Working Group (VcWG): Vector Control Working Group Objectives and Governance. APMEN; 2010. [http://apmen.org/focus-areas/vector-control/]

[80] Bharti PK, Chand SK, Singh MP, Mishra S, Shukla MM, Singh R, Singh N. Emergence of a new focus of *Plasmodium malariae* in forest villages of district Balaghat, Central India: Implications for the diagnosis of malaria and its control. Tropical Medicine & International Health. 2013;**18**:12-17

[81] Sharma SK, Tyagi PK, Padhan K, Upadhyay AK, Haque MA, Nanda N, Joshi H, Biswas S, Adak T, Das BS, et al. Epidemiology of malaria transmission in forest and plain ecotype villages in Sundargarh District, Orissa, India. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2006;**100**:917-925

[82] Van Bortel W, Trung HD, Hoi le X, Van Ham N, Van Chut N, Luu ND, Roelants P, Denis L, Speybroeck N, D'Alessandro U, Coosemans M. Malaria transmission and vector behaviour in a forested malaria focus in Central Vietnam and the implications for vector control. Malaria Journal. 2010;**9**:373

[83] Durnez L, Mao S, Denis L, Roelants P, Sochantha T, Coosemans M. Outdoor malaria transmission in forested villages of Cambodia. Malaria Journal. 2013;**12**:329

[84] O'Loughlin SM, Okabayashi T, Honda M, Kitazoe Y, Kishino H, Somboon P, Sochantha T, Nambanya S, Saikia PK, Dev V, Walton C. Complex population history of two *Anopheles dirus* mosquito species in Southeast Asia suggests the influence of Pleistocene climate change rather than human-mediated effects. Journal of Evolutionary Biology. 2008;**21**:1555-1569

[85] Haque U, Hashizume M, Glass GE, Dewan AM, Overgaard HJ, Yamamoto T. The role of climate variability in the spread of malaria in Bangladeshi highlands. PLoS One. 2010;**5**:e14341

[86] Zhou G, Munga S, Minakawa N, Githeko AK, Yan G. Spatial relationship between adult malaria vector abundance and environmental factors in western Kenya highlands. The American Journal of Tropical Medicine and Hygiene. 2007;**77**:29-35

[87] Yadav RS, Sharma VP, Chand SK. Mosquito breeding and resting in treeholes in a forest ecosystem in Orissa. Indian Journal of Malariology. 1997;**34**:8-16

[88] Gunasekaran K, Sahu SS, Parida SK, Sadanandane C, Jambulingam P, Das PK. Anopheline fauna of Koraput district, Orissa state, with particular reference to transmission of malaria. The Indian Journal of Medical Research. 1989;**89**:340-343

[89] Manda H, Gouagna LC, Foster WA, Jackson RR, Beier JC, Githure JI, Hassanali A. Effect of discriminative plant-sugar feeding on the survival and fecundity of *Anopheles gambiae*. Malaria Journal. 2007;**6**:113

[90] Obsomer V, Defourny P, Coosemans M. The *Anopheles dirus* complex: Spatial distribution and environmental drivers. Malaria Journal. 2007;**6**:26

[91] Sahu SS, Parida SK, Sadanandane C, Gunasekaran K, Jambulingam P, Das PK. Breeding habitats of malaria vectors: *A. fluviatilis*, *A. annularis* and *A. culicifacies*, in Koraput district, Orissa. Indian Journal of Malariology. 1990;**27**:209-216

[92] Kumar DS, Andimuthu R, Rajan R, Venkatesan MS. Spatial trend, environmental and socioeconomic factors associated with malaria prevalence in Chennai. Malaria Journal. 2014;**13**:14

[93] Sharma VP, Dev V. Biology & control of *Anopheles culicifacies* Giles 1901. The Indian Journal of Medical Research. 2015;**141**:525-536

[94] Wangdi K, Gatton ML, Kelly GC, Clements AC. Cross-border malaria: A major obstacle for malaria elimination. Advances in Parasitology. 2015;**89**:79-107

[95] Hahn MB, Gangnon RE, Barcellos C, Asner GP, Patz JA. Influence of deforestation, logging, and fire on malaria in the Brazilian Amazon. PLoS One. 2014;**9**:e85725

[96] Guerra CA, Snow RW, Hay SI. A global assessment of closed forests, deforestation and malaria risk. Annals of Tropical Medicine and Parasitology. 2006;**100**:189-204

[97] Chaveepojnkamjorn W, Pichainarong N. Malaria infection among the migrant population along the Thai-Myanmar border area. The Southeast Asian Journal of Tropical Medicine and Public Health. 2004;**35**:48-52

[98] Tangena JA, Thammavong P, Wilson AL, Brey PT, Lindsay SW. Risk and control of mosquito-borne diseases in southeast Asian rubber plantations. Trends in Parasitology. 2016;**32**:402-415

[99] Dysoley L, Kaneko A, Eto H, Mita T, Socheat D, Borkman A, Kobayakawa T. Changing patterns of forest malaria among the mobile adult male population in Chumkiri District, Cambodia. Acta Tropica. 2008;**106**:207-212

[100] Wisit Chaveepojnkamjorn D. Behavioral factors and malaria infection among the migrant population, Chiang Rai province. Journal of the Medical Association of Thailand. 2005;**88**:1293-1301

[101] Singh N, Singh OP, Sharma VP. Dynamics of malaria transmission in forested and deforested regions of Mandla District, Central India (Madhya Pradesh). Journal of the American Mosquito Control Association. 1996;**12**:225-234

[102] Hlongwana KW, Tsoka-Gwegweni J. Towards the implementation of malaria elimination policy in South Africa: The stakeholders' perspectives. Global Health Action. 2017;**10**:1288954

[103] Stern A. International population movements and public health in the Mekong region: An overview of some issues concerning mapping. The Southeast Asian Journal of Tropical Medicine and Public Health. 1998;**29**:201-212

[104] Xu J, Liu H. The challenges of malaria elimination in Yunnan Province, People's Republic of China. The Southeast Asian Journal of Tropical Medicine and Public Health. 2012;**43**:819-824

[105] Clements A, Barnett AG, Cheng ZW, Snow RW, Zhou HN. Space-time variation of malaria incidence in Yunnan province, China. Malaria Journal. 2009;**8**:18

[106] Hu H, Singhasivanon P, Salazar NP, Thimasarn K, Li X, Wu Y, Yang H, Zhu D, Supavej S, Looarecsuwan S. Factors influencing malaria endemicity in Yunnan Province, PR China (analysis of spatial pattern by GIS). Geographical information system. The Southeast Asian Journal of Tropical Medicine and Public Health. 1998;**29**:191-200

[107] Reid H, Haque U, Clements AC, Tatem AJ, Vallely A, Ahmed SM, Islam A, Haque R. Mapping malaria risk in Bangladesh using Bayesian geostatistical models. The American Journal of Tropical Medicine and Hygiene. 2010;**83**:861-867

[108] Wangdi K, Banwell C, Gatton ML, Kelly GC, Namgay R, Clements AC. Malaria burden and costs of intensified control in Bhutan, 2006-14: An observational study and situation analysis. The Lancet Global Health. 2016;**4**:e336-e343

[109] Mohapatra PK, Narain K, Prakash A, Bhattacharyya DR, Mahanta J. Risk factors of malaria in the fringes of an evergreen monsoon forest of Arunachal Pradesh. National Medical Journal of India. 2001;**14**:139-142

[110] Wangdi K, Banwell C, Gatton ML, Kelly GC, Namgay R, Clements AC. Development and evaluation of a spatial decision support system for malaria elimination in Bhutan. Malaria Journal. 2016;**15**:180

[111] Wangdi K, Kaewkungwal J, Singhasivanon P, Silawan T, Lawpoolsri S, White NJ. Spatio-temporal patterns of malaria infection in Bhutan: A country embarking on malaria elimination. Malaria Journal. 2011;**10**:89

[112] Childs DZ, Cattadori IM, Suwonkerd W, Prajakwong S, Boots M. Spatiotemporal patterns of malaria incidence in northern Thailand. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2006;**100**:623-631

[113] Carme B. Substantial increase of malaria in inland areas of eastern French Guiana. Tropical Medicine & International Health. 2005;**10**:154-159

[114] Anh NQ, Hung le X, Thuy HN, Tuy TQ, Caruana SR, Biggs BA, Morrow M. KAP surveys and malaria control in Vietnam: Findings and cautions about community research. The Southeast Asian Journal of Tropical Medicine and Public Health 2005;**36:**572-577

[115] Hung le Q, Vries PJ, Giao PT, Nam NV, Binh TQ, Chong MT, Quoc NT, Thanh TN, Hung LN, Kager PA. Control of malaria: A successful experience from Viet Nam. Bulletin of the World Health Organization. 2002;**80**:660-666

[116] Dev V, Phookan S, Sharma VP, Dash AP, Anand SP. Malaria parasite burden and treatment seeking behavior in ethnic communities of Assam, northeastern India. The Journal of Infection. 2006;**52**:131-139

[117] Mittal PK, Wijeyaratne P, Pandey S. Status of insecticide resistance of malaria, Kala-azar and Japanese encephalitis vectors in Bangladesh, Bhutan, India and Nepal (BBIN). Environmental Health Project Activity Report. 2004;**129**:44-48

[118] Wangdi K, Singhasivanon P, Silawan T, Lawpoolsri S, White NJ, Kaewkungwal J. Development of temporal modelling for forecasting and prediction of malaria infections using time-series and ARIMAX analyses: A case study in endemic districts of Bhutan. Malaria Journal. 2010;**9**:251

[119] WHO. Planning Meeting for Operational Research on Malaria Elimination. Geneva, Switzerland; 2014

[120] Guyant P, Corbel V, Guerin PJ, Lautissier A, Nosten F, Boyer S, Coosemans M, Dondorp AM, Sinou V, Yeung S, White N. Past and new challenges for malaria control and elimination: The role of operational research for innovation in designing interventions. Malaria Journal. 2015;**14**:279

[121] Zachariah R, Harries AD, Ishikawa N, Rieder HL, Bissell K, Laserson K, Massaquoi M, Van Herp M, Reid T. Operational research in low-income countries: What, why, and how? The Lancet Infectious Diseases. 2009;**9**:711-717

[122] Nunn P, Harries A, Godfrey-Faussett P, Gupta R, Maher D, Raviglione M. The research agenda for improving health policy, systems performance, and service delivery for tuberculosis control: A WHO perspective. Bulletin of the World Health Organization. 2002;**80**:471-476

[123] Reynolds J. Introduction. Socio-Economic Planning Sciences. 1987;**21**:73-77

[124] Datta S. Applications of O.R. in health in developing countries: A review. Social Science & Medicine. 1993;**37**:1441-1450

[125] Harries AD. Integration of operational research into National Tuberculosis Control Programmes. Tuberculosis (Edinburgh, Scotland). 2003;**83**:143-147

[126] Operational and Implementation research; 2016. [http://www.who.int/tdr/diseases-topics/operational-implementation-research/en/]

[127] Malhotra S, Zodpey SP. Operations research in public health. Indian Journal of Public Health. 2010;**54**:145-150

[128] Monks T. Operational research as implementation science: Definitions, challenges and research priorities. Implementation Sci. 2016;**11**:1-10

[129] Mahendradhata Y, Probandari A, Widjanarko B, Riono P, Mustikawati D, Tiemersma EW, Alisjahbana B. Embedding operational research into national disease control programme: Lessons from 10 years of experience in Indonesia. Global Health Action. 2014;**7**:25412

[130] WHO. Guide to Operational Research in programs supported by the global fund; 2016. [http://www.who.int/hiv/pub/operational/globalfund/en/]

[131] Zachariah R, Ford N, Maher D, Bissell K, Van den Bergh R, van den Boogaard W, Reid T, Castro KG, Draguez B, von Schreeb J, et al. Is operational research delivering the goods? The journey to success in low-income countries. The Lancet Infectious Diseases. 2012;**12**:415-421

[132] Zhou SS, Rietveld AE, Velarde-Rodriguez M, Ramsay AR, Zhang SS, Zhou XN, Cibulskis RE. Operational research on malaria control and elimination: A review of projects published between 2008 and 2013. Malaria Journal. 2014;**13**:473

[133] Ramsay A, Olliaro P, Reeder JC. The need for operational research and capacity-building in support of the global technical strategy for malaria 2016-2030. Malaria Journal. 2016;**15**:235

[134] Chen SB, Ju C, Chen JH, Zheng B, Huang F, Xiao N, Zhou X, Ernest T, Zhou XN. Operational research needs toward malaria elimination in China. Advances in Parasitology. 2014;**86**:109-133

[135] Srivastava A, Nagpal B, Joshi P, Paliwal J, Dash A. Identification of malaria hot spots for focused intervention in tribal state of India: A GIS based approach. International Journal of Health Geographics. 2009;**8**:30

[136] Dev V, Sangma BM, Dash AP. Persistent transmission of malaria in Garo hills of Meghalaya bordering Bangladesh, north-East India. Malaria Journal. 2010;**9**:263

[137] Dhimal M, O'Hara RB, Karki R, Thakur GD, Kuch U, Ahrens B. Spatio-temporal distribution of malaria and its association with climatic factors and vector-control interventions in two high-risk districts of Nepal. Malaria Journal. 2014;**13**:457

[138] Atkinson JA, Johnson ML, Wijesinghe R, Bobogare A, Losi L, O'Sullivan M, Yamaguchi Y, Kenilorea G, Vallely A, Cheng Q, et al. Operational research to inform a sub-national surveillance intervention for malaria elimination in Solomon Islands. Malaria Journal. 2012;**11**:101

[139] Ramsay A, Harries AD, Zachariah R, Bissell K, Hinderaker SG, Edginton M, Enarson DA, Satyanarayana S, Kumar AM, Hoa NB, et al. The structured operational research and training initiative for public health programmes. Public Health Action. 2014;**4**:79-84

[140] Zachariah R, Rust S, Berger SD, Guillerm N, Bissell K, Delaunois P, Reid AJ, Kumar AM, Olliaro PL, Reeder JC, et al. Building global capacity for conducting operational research using the SORT IT model: Where and who? PLoS One. 2016;**11**:e0160837

[141] Bousema T, Griffin JT, Sauerwein RW, Smith DL, Churcher TS, Takken W, Ghani A, Drakeley C, Gosling R. Hitting hotspots: Spatial targeting of malaria for control and elimination. PLoS Medicine. 2012;**9**:e1001165

[142] Ernst KC, Adoka SO, Kowuor DO, Wilson ML, John CC. Malaria hotspot areas in a highland Kenya site are consistent in epidemic and non-epidemic years and are associated with ecological factors. Malaria Journal. 2006;**5**:78

[143] Alemu K, Worku A, Berhane Y, Kumie A. Spatiotemporal clusters of malaria cases at village level, Northwest Ethiopia. Malaria Journal. 2014;**13**:223

[144] 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. Malaria Journal. 2015;**14**:176

[145] Pinchoff J, Henostroza G, Carter BS, Roberts ST, Hatwiinda S, Hamainza B, Hawela M, Curriero FC. Spatial patterns of incident malaria cases and their household contacts in a single clinic catchment area of Chongwe District, Zambia. Malaria Journal. 2015;**14**:305

[146] Ayele DG, Zewotir TT, Mwambi HG. Spatial distribution of malaria problem in three regions of Ethiopia. Malaria Journal. 2013;**12**:207

[147] Rosas-Aguirre A, Speybroeck N, Llanos-Cuentas A, Rosanas-Urgell A, Carrasco-Escobar G, Rodriguez H, Gamboa D, Contreras-Mancilla J, Alava F, Soares IS, et al. Hotspots of malaria transmission in the Peruvian Amazon: Rapid assessment through a parasitological and serological survey. PLoS One. 2015;**10**:e0137458

[148] Rulisa S, Kateera F, Bizimana JP, Agaba S, Dukuzumuremyi J, Baas L, de Dieu Harelimana J, Mens PF, Boer KR, de Vries PJ. Malaria prevalence, spatial clustering and risk factors in a low endemic area of eastern Rwanda: A cross sectional study. PLoS One. 2013;**8**:e69443

[149] Wen L, Li C, Lin M, Yuan Z, Huo D, Li S, Wang Y, Chu C, Jia R, Song H. Spatio-temporal analysis of malaria incidence at the village level in a malaria-endemic area in Hainan, China. Malaria Journal. 2011;**10**:88

[150] Braz RM, Guimaraes RF, Carvalho Junior OA, Tauil PL. Spatial dependence of malaria epidemics in municipalities of the Amazonian ecosystem. Revista Brasileira de Epidemiologia. 2014;**17**:615-628

[151] Brooker S, Clarke S, Njagi JK, Polack S, Mugo B, Estambale B, Muchiri E, Magnussen P, Cox J. Spatial clustering of malaria and associated risk factors during an epidemic

in a highland area of western Kenya. Tropical Medicine & International Health. 2004;**9**: 757-766

[152] Haque U, Huda M, Hossain A, Ahmed SM, Moniruzzaman M, Haque R. Spatial malaria epidemiology in Bangladeshi highlands. Malaria Journal. 2009;**8**:185

[153] Ahmed S, Galagan S, Scobie H, Khyang J, Prue CS, Khan WA, Ram M, Alam MS, Haq MZ, Akter J, et al. Malaria hotspots drive hypoendemic transmission in the Chittagong Hill districts of Bangladesh. PLoS One. 2013;**8**:e69713

[154] Kohara Melchior LA, Chiaravalloti Neto F. Spatial and spatio-temporal analysis of malaria in the state of acre, western Amazon, Brazil. Geospatial Health. 2016;**11**:443

[155] Sluydts V, Heng S, Coosemans M, Van Roey K, Gryseels C, Canier L, Kim S, Khim N, Siv S, Mean V, et al. Spatial clustering and risk factors of malaria infections in Ratanakiri Province, Cambodia. Malaria Journal. 2014;**13**:387

[156] Zhang W, Wang L, Fang L, Ma J, Xu Y, Jiang J, Hui F, Wang J, Liang S, Yang H, Cao W. Spatial analysis of malaria in Anhui province, China. Malaria Journal. 2008;**7**:206

[157] Gething PW, Casey DC, Weiss DJ, Bisanzio D, Bhatt S, Cameron E, Battle KE, Dalrymple U, Rozier J, Rao PC, et al. Mapping *Plasmodium falciparum* mortality in Africa between 1990 and 2015. The New England Journal of Medicine. 2016;**375**:2435-2445

[158] Clements ACA, Reid HL, Kelly GC, Hay SI. Further shrinking the malaria map: How can geospatial science help to achieve malaria elimination? The Lancet Infectious Diseases. 2013;**13**:709-718

[159] Hay SI, Tatem AJ, Graham AJ, Goetz SJ, Rogers DJ. Global environmental data for mapping infectious disease distribution. Advances in Parasitology. 2006;**62**:37-77

[160] Yang GJ, Gao Q, Zhou SS, Malone JB, McCarroll JC, Tanner M, Vounatsou P, Bergquist R, Utzinger J, Zhou XN. Mapping and predicting malaria transmission in the People's Republic of China, using integrated biology-driven and statistical models. Geospatial Health. 2010;**5**:11-22

[161] Dlamini SN, Franke J, Vounatsou P. Assessing the relationship between environmental factors and malaria vector breeding sites in Swaziland using multi-scale remotely sensed data. Geospatial Health. 2015;**10**:302

[162] Kamanga A, Renn S, Pollard D, Bridges DJ, Chirwa B, Pinchoff J, Larsen DA, Winters AM. Open-source satellite enumeration to map households: Planning and targeting indoor residual spraying for malaria. Malaria Journal. 2015;**14**:345

[163] Sudre B, Rossi M, Van Bortel W, Danis K, Baka A, Vakalis N, Semenza JC. Mapping environmental suitability for malaria transmission, Greece. Emerging Infectious Diseases. 2013;**19**:784-786

[164] Hay SI, Lennon JJ. Deriving meteorological variables across Africa for the study and control of vector-borne disease: A comparison of remote sensing and spatial interpolation of climate. Tropical Medicine & International Health. 1999;**4**:58-71

[165] Kelly GC, Seng CM, Donald W, Taleo G, Nausien J, Batarii W, Iata H, Tanner M, Vestergaard LS, Clements AC. A spatial decision support system for guiding focal indoor residual interventions in a malaria elimination zone. Geospatial Health. 2011;**6**:21-31

[166] Sithiprasasna R, Patpoparn S, Attatippaholkun W, Suvannadabba S, Srisuphanunt M. The geographic information system as an epidemiological tool in the surveillance of dengue virus-infected *Aedes* mosquitos. The Southeast Asian Journal of Tropical Medicine and Public Health. 2004;**35**:918-926

[167] Teng TB. New initiatives in dengue control in Singapore. Dengue Bulletin. 2001;**25**:1-6

[168] Ai-Leen GT, Song RJ. The use of GIS in ovitrap monitoring for dengue control in Singapore. Dengue Bulletin. 2000;**24**:110-116

[169] Keenan PB. Spatial Decision Support Systems: Decision Making Support Systems: Achievements and Challenges for the New Decade. Idea Group Publishing; 2003

[170] Kelly GC, Tanner M, Vallely A, Clements A. Malaria elimination: Moving forward with spatial decision support systems. Trends in Parasitology. 2012;**28**:297-304

[171] Mendis K. Spatial technology & malaria control. The Indian Journal of Medical Research. 2009;**130**:498-500

[172] WHO. Disease Surveillance for Malaria Elimination: An Operational Manual. World Health Organization. Switzerland: Geneva; 2012

[173] WHO. Disease Surveillance for Malaria Control. Geneva, Switzerland: World Health Organization; 2012

[174] Singh N, Bharti PK, Kumre NS. Active v. Passive surveillance for malaria in remote tribal belt of Central India: Implications for malaria elimination. Pathogens and Global Health. 2016;**110**:178-184

[175] Moonen B, Cohen JM, Snow RW, Slutsker L, Drakeley C, Smith DL, Abeyasinghe RR, Rodriguez MH, Maharaj R, Tanner M, Targett G. Operational strategies to achieve and maintain malaria elimination. Lancet. 2010;**376**:1592-1603

[176] Walton C, Handley JM, Tun-Lin W, Collins FH, Harbach RE, Baimai V, Butlin RK. Population structure and population history of *Anopheles dirus* mosquitoes in Southeast Asia. Molecular Biology and Evolution. 2000;**17**:962-974

[177] Sturrock HJ, Roberts KW, Wegbreit J, Ohrt C, Gosling RD. Tackling imported malaria: An elimination endgame. The American Journal of Tropical Medicine and Hygiene. 2015;**93**:139-144

[178] Hay SI, Smith DL, Snow RW. Measuring malaria endemicity from intense to interrupted transmission. The Lancet Infectious Diseases. 2008;**8**:369-378

[179] Alves FP, Gil LH, Marrelli MT, Ribolla PE, Camargo EP, Da Silva LH. Asymptomatic carriers of *Plasmodium* spp. as infection source for malaria vector mosquitoes in the Brazilian Amazon. Journal of Medical Entomology. 2005;**42**:777-779

[180] WHO. Malaria indicator survey: basic documentation for survey design and implementation/Roll Back Malaria Monitoring and Evaluation Reference Group. 1211 Geneva 27, Switzerland; 2005

[181] Cotter C, Sudathip P, Herdiana H, Cao Y, Liu Y, Luo A, Ranasinghe N, Bennett A, Cao J, Gosling RD. Piloting a programme tool to evaluate malaria case investigation and reactive case detection activities: Results from 3 settings in the Asia Pacific. Malaria Journal. 2017;**16**:347

[182] Hustedt J, Canavati SE, Rang C, Ashton RA, Khim N, Berne L, Kim S, Sovannaroth S, Ly P, Menard D, et al. Reactive case-detection of malaria in Pailin Province, western Cambodia: Lessons from a year-long evaluation in a pre-elimination setting. Malaria Journal. 2016;**15**:132

[183] Larsen DA, Chisha Z, Winters B, Mwanza M, Kamuliwo M, Mbwili C, Hawela M, Hamainza B, Chirwa J, Craig AS, et al. Malaria surveillance in low-transmission areas of Zambia using reactive case detection. Malaria Journal. 2015;**14**:465

[184] Parker DM, Landier J, von Seidlein L, Dondorp A, White L, Hanboonkunupakarn B, Maude RJ, Nosten FH. Limitations of malaria reactive case detection in an area of low and unstable transmission on the Myanmar-Thailand border. Malaria Journal. 2016;**15**:571

[185] Rossi G, Van den Bergh R, Nguon C, Debackere M, Vernaeve L, Khim N, Kim S, Menard D, De Smet M, Kindermans JM. Adapting reactive case detection strategies for falciparum malaria in a low-transmission area in Cambodia. Clinical Infectious Diseases. 2017

[186] Sturrock HJ, Novotny JM, Kunene S, Dlamini S, Zulu Z, Cohen JM, Hsiang MS, Greenhouse B, Gosling RD. Reactive case detection for malaria elimination: Real-life experience from an ongoing program in Swaziland. PLoS One. 2013;**8**:e63830

[187] Brosseau L, Drame PM, Besnard P, Toto JC, Foumane V, Le Mire J, Mouchet F, Remoue F, Allan R, Fortes F, et al. Human antibody response to *Anopheles* saliva for comparing the efficacy of three malaria vector control methods in Balombo, Angola. PLoS One. 2012;**7**:e44189

[188] Drame PM, Poinsignon A, Marie A, Noukpo H, Doucoure S, Cornelie S, Remoue F. New salivary biomarkers of human exposure to malaria vector bites. In: Manguin S, editor. *Anopheles* Mosquitoes - New Insights into Malaria Vectors. Rijeka: InTech; 2013 Ch. 23

[189] Ya-Umphan P, Cerqueira D, Parker DM, Cottrell G, Poinsignon A, Remoue F, Brengues C, Chareonviriyaphap T, Nosten F, Corbel V. Use of an *Anopheles* salivary biomarker to assess malaria transmission risk along the Thailand-Myanmar border. The Journal of Infectious Diseases. 2017;**215**:396-404

[190] Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A, Wernsdorfer WH. A review of malaria diagnostic tools: Microscopy and rapid diagnostic test (RDT). The American Journal of Tropical Medicine and Hygiene. 2007;**77**:119-127

[191] Chiodini PL, Bowers K, Jorgensen P, Barnwell JW, Grady KK, Luchavez J, Moody AH, Cenizal A, Bell D. The heat stability of *Plasmodium* lactate dehydrogenase-based and

histidine-rich protein 2-based malaria rapid diagnostic tests. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2007;**101**:331-337

[192] Maltha J, Gillet P, Jacobs J. Malaria rapid diagnostic tests in endemic settings. Clinical Microbiology and Infection. 2013;**19**:399-407

[193] Murray CK, Gasser RA Jr, Magill AJ, Miller RS. Update on rapid diagnostic testing for malaria. Clinical Microbiology Reviews. 2008;**21**:97-110

[194] Littrell M, Sow GD, Ngom A, Ba M, Mboup BM, Dieye Y, Mutombo B, Earle D, Steketee RW. Case investigation and reactive case detection for malaria elimination in northern Senegal. Malaria Journal. 2013;**12**:331

[195] Lin JT, Saunders DL, Meshnick SR. The role of submicroscopic parasitemia in malaria transmission: What is the evidence? Trends in Parasitology. 2014;**30**:183-190

[196] Rogawski ET, Congpuong K, Sudathip P, Satimai W, Sug-aram R, Aruncharus S, Darakapong A, Kitchakarn S, Meshnick SR. Active case detection with pooled real-time PCR to eliminate malaria in Trat province, Thailand. The American Journal of Tropical Medicine and Hygiene. 2012;**86**:789-791

[197] Delacollette C, D'Souza C, Christophel E, Thimasarn K, Abdur R, Bell D, Dai TC, Gopinath D, Lu S, Mendoza R, et al. Malaria trends and challenges in the Greater Mekong subregion. The Southeast Asian Journal of Tropical Medicine and Public Health. 2009;**40**:674-691

[198] Haque U, Overgaard HJ, Clements AC, Norris DE, Islam N, Karim J, Roy S, Haque W, Kabir M, Smith DL, Glass GE. Malaria burden and control in Bangladesh and prospects for elimination: An epidemiological and economic assessment. The Lancet Global Health. 2014;**2**:e98-105

[199] Dev V, Sharma VP, Hojai D. Malaria transmission and disease burden in Assam: Challenges and opportunities. Journal of Parasitic Diseases. 2009;**33**:13-22

[200] Jitthai N. Migration and malaria. The Southeast Asian Journal of Tropical Medicine and Public Health. 2013;**44**(Suppl 1):166-200 discussion 306-167

[201] Bhumiratana A, Intarapuk A, Sorosjinda-Nunthawarasilp P, Maneekan P, Koyadun S. Border malaria associated with multidrug resistance on Thailand-Myanmar and Thailand-Cambodia borders: Transmission dynamic, vulnerability, and surveillance. BioMed Research International. 2013;**2013**:363417

[202] Edwards HM, Canavati SE, Rang C, Ly P, Sovannaroth S, Canier L, Khim N, Menard D, Ashton RA, Meek SR, Roca-Feltrer A. Novel cross-border approaches to optimise identification of asymptomatic and Artemisinin-resistant *Plasmodium* infection in mobile populations crossing Cambodian Borders. PLoS One. 2015;**10**:e0124300

[203] Yangzom T, Gueye CS, Namgay R, Galappaththy GN, Thimasarn K, Gosling R, Murugasampillay S, Dev V. Malaria control in Bhutan: Case study of a country embarking on elimination. Malaria Journal. 2012;**11**:9

[204] Blumberg L, Frean J, Moonasar D. Successfully controlling malaria in South Africa. South African Medical Journal. 2014;**104**:224-227

[205] Gueye CS, Teng A, Kinyua K, Wafula F, Gosling R, McCoy D. Parasites and vectors carry no passport: How to fund cross-border and regional efforts to achieve malaria elimination. Malaria Journal. 2012;**11**:344

[206] Wen S, Harvard KE, Gueye CS, Canavati SE, Chancellor A, Ahmed BN, Leaburi J, Lek D, Namgay R, Surya A, et al. Targeting populations at higher risk for malaria: A survey of national malaria elimination programmes in the Asia Pacific. Malaria Journal. 2016;**15**:271

[207] Wondji CS, Coleman M, Kleinschmidt I, Mzilahowa T, Irving H, Ndula M, Rehman A, Morgan J, Barnes KG, Hemingway J. Impact of pyrethroid resistance on operational malaria control in Malawi. Proceedings of the National Academy of Sciences of the United States of America. 2012;**109**:19063-19070

[208] Sonkong K, Chaiklieng S, Neave P, Suggaravetsiri P. Factors affecting delay in seeking treatment among malaria patients along Thailand-Myanmar border in Tak Province, Thailand. Malaria Journal. 2015;**14**:3

[209] Xu JW, Li Y, Yang HL, Zhang J, Zhang ZX, Yang YM, Zhou HN, Havumaki J, Li HX, Liu H, et al. Malaria control along China-Myanmar border during 2007-2013: An integrated impact evaluation. Infectious Diseases of Poverty. 2016;**5**:75

[210] SEARO: Malaria vectors of South-East Asia. SEARO W ed. 2017

[211] Suwonkerd W, Ritthison W, Ngo CT, Tainchum K, Bangs MJ, Chareonviriyaphap T. Vector biology and malaria transmission in Southeast Asia. In: Manguin S, editor. *Anopheles* Mosquitoes - New Insights into Malaria Vectors. Rijeka: InTech; 2013 Ch. 10

[212] Huang JX, Xia ZG, Zhou SS, Pu XJ, Hu MG, Huang DC, Ren ZP, Zhang SS, Yang MN, Wang DQ, Wang JF. Spatio-temporal analysis of malaria vectors in national malaria surveillance sites in China. Parasites & Vectors. 2015;**8**:146

[213] Wangdi K, Gatton ML, Kelly GC, Banwell C, Dev V, Clements AC. Malaria elimination in India and regional implications. The Lancet Infectious Diseases. 2016;**16**:e214-e224

[214] Elyazar IR, Sinka ME, Gething PW, Tarmidzi SN, Surya A, Kusriastuti R, Winarno BJK, Hay SI, Bangs MJ. The distribution and bionomics of *Anopheles* malaria vector mosquitoes in Indonesia. Advances in Parasitology. 2013;**83**:173-266

[215] WHO. Case Study on Malaria Elimination in the Philippines Launched. Geneva 27, Switzerland: WHO; 2015

[216] Cooper RD, Waterson DG, Frances SP, Beebe NW, Pluess B, Sweeney AW. Malaria vectors of Papua New Guinea. International Journal for Parasitology. 2009;**39**:1495-1501

[217] Beebe NW, Russell TL, Burkot TR, Lobo NF, Cooper RD. The systematics and bionomics of malaria vectors in the Southwest Pacific. In: Manguin S, editor. *Anopheles* Mosquitoes - New Insights into Malaria Vectors. Rijeka: InTech; 2013. Ch. 12

[218] Chang KS, Yoo DH, Ju YR, Lee WG, Roh JY, Kim HC, Klein TA, Shin EH. Distribution of malaria vectors and incidence of vivax malaria at Korean army installations near the demilitarized zone, Republic of Korea. Malaria Journal. 2016;**15**:259

[219] Gayan Dharmasiri AG, Perera AY, Harishchandra J, Herath H, Aravindan K, Jayasooriya HTR, Ranawaka GR, Hewavitharane M. First record of *Anopheles stephensi* in Sri Lanka: A potential challenge for prevention of malaria reintroduction. Malaria Journal. 2017;**16**:326

Chapter 10

Malaria Elimination in the People's Republic of China: Current Progress, Challenges, and Prospects

Shaosen Zhang, Li Zhang, Jun Feng, Jianhai Yin, Xinyu Feng, Zhigui Xia, Roger Frutos, Sylvie Manguin and Shuisen Zhou

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.77282

Abstract

In China, the malaria elimination program was launched in 2010 with the objective to eliminate this disease by 2020. Large-scale malaria control and elimination actions have been conducted with significant success since inception of the nationwide program. The incidence of locally acquired malaria has declined sharply along with the concomitant decrease of malaria-endemic areas from 762 counties reporting malaria in 2010 to just two counties adjacent to border areas (Yunnan, China-Myanmar and Tibet, China-India) in 2016. In total, 1723 counties (79%) and 134 prefectures (52%) had completed the malaria elimination internal assessment by the end of 2016. The year 2017 was the first year without report of indigenous malaria cases throughout the country. Hence, this chapter is meant to share the lessons learned from malaria elimination in China benefiting countries on the way to malaria elimination.

Keywords: malaria elimination, China, surveillance and responses, 1-3-7 model

1. Introduction

Although significant progress on malaria control and elimination has been made worldwide, malaria remains a major public health threat to human beings. According to the World Malaria Report published by the World Health Organization (WHO), a total of 216 million malaria cases were reported worldwide with 445,000 deaths in 2016 [1]. These malaria cases were mainly reported from Africa (90%) and Southeast Asia (7%). With the available intervention tools, several countries have been certified to be malaria-free, and others are in the

Anopheles species/ taxa	Sibling species prevalent in the People's Republic of China	Species identification tools	Breeding habitats	Feeding behavior (peak biting activity)	Resting behavior	Insecticide susceptibility status	Distribution range
Anopheles sinensis	—	Morphological characters	Rice field, canal, ditch, pond	Zoophily (first option) Anthropophily (second option)	Exophily	Resistance to organochlorine, dichloro-diphenyl-trichloroethane (DDT), and deltamethrin in some provinces	Latitude below 33°N
Anopheles minimus s.l.	*An. minimus*, *An. harrisoni*	Morphological characters; PCR	Heliophobic stream, canal, ditch, rice field	Zoophily/ anthropophily (reported only in Hainan Province)	Endophily	Sensitive to all insecticides used currently	Latitude below 32.5°N
Anopheles lesteri	—	Morphological characters; PCR	Heliophobic, canal, ditch, rice field, filter well	Anthropophily	Endophily	Sensitive to all insecticides used currently	Latitude 22°N~33°N
Anopheles dirus s.l.	*An. dirus*, *An. baimaii*	Morphological characters; PCR	Heliophobic, stream in forest, pit with water, footprint of cattle	Anthropophily	Exophily	Sensitive to all insecticides used currently	Latitude below 23°N

Table 1. Bionomical characteristics of malaria vectors in the People's Republic of China (reference [14]).

process of elimination in the foreseeable future [1]. Among others, within the broad objective of worldwide malaria elimination [2], 21 countries have the potential to eliminate malaria by 2020; these are marked as E-2020 countries by WHO [1]. Although some of E-2020 countries were moving forward to achieve elimination goals, 11[1] have reported an increase of indigenous malaria cases since 2015, and 5 countries[2] reported >100 cases in 2016 compared to 2015. World malaria elimination is currently at crossroads [3].

Among the E-2020 countries, China has made a significant progress on malaria elimination. Both the malaria-endemic territories and indigenous cases have decreased dramatically [4–6]. Furthermore, no indigenous cases were reported in China in 2017. Along with the decrease of indigenous malaria cases, the distribution of *Plasmodium* species associated to the reported malaria cases had changed as well. Only 2 *Plasmodium* species, i.e., *Plasmodium falciparum* and *P. vivax*, were present prior to the elimination program, but now all 4 human malaria parasites are encountered in China (i.e., *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*), as well as the simian species *P. knowlesi* [1, 7–12]. The predominant *Anopheles* vectors had also changed over the same period due to environmental changes and anti-malaria interventions. Prior to malaria elimination, 4 main species/complexes of vectors were recorded, i.e., *Anopheles lesteri*, *An. dirus s.l.*, *An. minimus s.l.*, and *An. sinensis* (**Table 1**). Currently, after 7 years of malaria elimination efforts, only 2 species/complexes are recorded to be prevalent, i.e., *An. minimus s.l.* (mainly *An. minimus* and *An. harrisoni*) and *An. sinensis.* Moreover, the geographic distribution range of *An. sinensis* has expanded and the proportion increased too [13–16]. Considering the progress of malaria elimination in China and the challenges still to be met, useful information has been generated which could be shared with the communities working on malaria elimination. This chapter is thus aiming at detailing the strategy of the Chinese national malaria elimination program, current progress, and lessons learned in defeating malaria.

2. Malaria elimination strategy in China

The national malaria elimination strategy was developed based on the malaria control situation and response to the Global Eradication of Malaria Initiative proposed by the United Nation Millennium Development Goals (MDGs) in September 2000 [5, 17, 18]. The overall strategy comprised specific objectives and key measures taking into account the different epidemiological contexts and diversity of malaria transmission models all over the country. The overall goals of malaria elimination in China were set to achieve zero indigenous cases in the country by 2015, leaving apart the border areas of Yunnan Province with Myanmar and Tibet Autonomous Region with India to achieve complete elimination in the country by 2020.

2.1. Intermediate objectives

The strategy and key measures for malaria elimination in China were developed in line with the WHO guidelines [19]. However, counties had variable endemicity based on which a classification

[1] *Botswana, Cabo Verde, South Africa, Swaziland, Costa Rica, Ecuador, El Salvador, Mexico, Saudi Arabia, Timor-Leste, Malaysia.*
[2] *Botswana, South Africa, Swaziland, Ecuador, Saudi Arabia.*

was established according to the different types of area and intensity of malaria transmission. According to the magnitude of transmission and incidence, all counties were classified into 4 types, including Type I, local transmission and incidence ≥1/10,000 over the past 3 years; Type II, local transmission and incidence <1/10,000 over the past 3 years; Type III, no indigenous cases reported over the past 3 years but still with risk of transmission; and Type IV, malaria-free [20]. The classification of malaria-endemic areas is presented in **Figure 1** [17, 20].

	Classification	No of counties	Target population
Type I	Local infections are detected in the past three consecutive years and the incidence rate equals or higher than 1/10,000	75	30,965,000
Type II	Local infections are detected in the past three consecutive years, and the incidence rate is lower than 1/10,000 at least in one of those 3 years	684	440,792,000
Type III	No local infections reported in the past three consecutive years but risk of transmission exists	1,430	620,281,000
Type IV	Malaria-free area	669	180,882,000

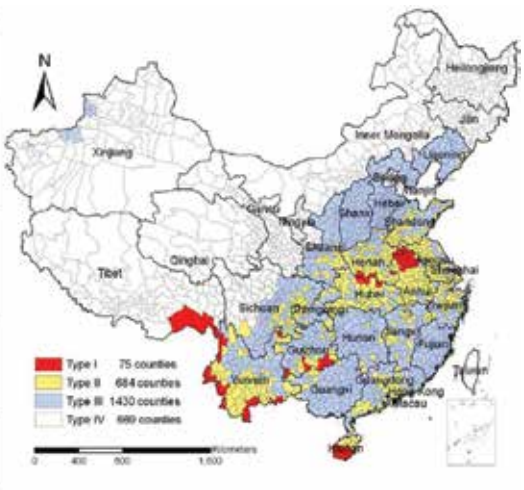


Figure 1. Stratification of malaria-endemic areas for data based on 2010 (references [5, 17]).

Several intermediate progress objectives were also set in a graduated way depending on the type of area. By 2015, (i) all Type III counties should achieve the objective of malaria elimination; (ii) at the same time, Types I and II counties, except Yunnan border counties, are expected to report zero indigenous malaria cases; (iii) the incidence of indigenous malaria cases in Type I counties located in the Yunnan border areas should be reduced to <1/10,000; (iv) it is expected that by 2017, no indigenous cases should be reported in the whole country; (v) by the year 2018, all Types I and II counties, except Yunnan border counties, must have fully achieved malaria elimination; and (vi) malaria elimination should be achieved all over the country by the year 2020.

2.2. Implementation requirements for malaria elimination

In order to achieve malaria elimination, specific requirements were assigned depending upon the type of county. The Type I counties should strengthen the management of infectious source and implement vector control measures to reduce the incidence of malaria. The Type II counties should eliminate the infectious source of malaria to interrupt local malaria transmission. The Type III counties should enhance the monitoring and disposition of the imported cases to prevent the secondary transmission. The Type IV counties should deal appropriately with imported cases. These requirements were also made flexible enough to be adjusted according to the control process and changes in the dynamics of the disease. These requirements were based on specific and standardized key measures [17].

2.2.1. Strengthening control and management of infectious sources

- **Timely malaria case detection**. Both public and private clinics and health facilities at all levels should take blood samples from patients including clinical diagnosed malaria cases, suspected malaria cases and febrile patients without confirmed origin of infection. Blood smear for microscopic tests or auxiliary detection using Rapid Diagnosis Test (RDT) kits should be carried out with blood samples. For RDT-positive samples, blood smears must be collected and kept for verification.
- **Timely surveillance and response to all malaria cases** [20, 21]
  - **Strengthening malaria case reporting.** Public or private clinics and health facilities at all levels have the responsibility to report malaria cases within 24 hours (**1 day**) after diagnosis. This is a requirement from the law on the Prevention and Control of Infectious Diseases in the People's Republic of China (http://www.gov.cn/banshi/2005-08/01/content_19107.htm).
  - **Case verification.** Centers for Disease Control and Prevention (CDCs) at the county level are made responsible for the verification of individual cases reported through the information system and for laboratory test assessment (RDT and microscope). Case investigation and verification are required to be accomplished within **3 days** after reporting.
  - **Management of malaria foci.** CDCs at the county level are responsible for investigation on identified malaria foci and further disposal within **7 days** after index case reporting. A reactive case detection must be carried out by collecting blood samples from inhabitants around the index case (fever displayed over the last 2 weeks). Collected blood samples must be analyzed by microscope or RDT. Meanwhile, vector control measures (i.e., indoor residual spraying (IRS)) must be implemented, and information materials for public awareness and health education must be provided to all families in different foci.
- **Treatment**
  - Full dose and **whole medication**[3] should be given to treat malaria patients according to the national guidelines. Public or private clinics and health facilities at all levels should treat all the detected malaria patients according to the national guidelines for anti-malarial drug use, issued by the Ministry of Health. All patients must be followed up across the whole course of treatment.
  - **Anti-relapse treatment.** In non-transmission malaria season, patients diagnosed with *vivax* malaria during the previous year must be given anti-relapse treatment.

2.2.2. Strengthening vector control

- **Anti-mosquito interventions.** During the malaria transmission season, it is encouraged to transform and improve the environment so as to reduce the number of breeding sites and

[3]Whole medication refers to the 8-day treatment for vivax malaria with primaquine (total dose 180 mg over 8 days) and chloroquine (total dose 1200 mg over 3 days) as first-line drugs. Artemisinin combination therapy (ACT) was used as first-line treatment to treat uncomplicated falciparum malaria.

decrease the density of mosquitoes in combination with the patriotic health movement[4] and new rural village development [22]. Measures of IRS and insecticide-treated net (ITN) are required to be applied in malaria foci.

- **Strengthening personal protection**. To prevent or minimize mosquito bites during the malaria transmission season, personal protective measures must be implemented such as repellents, mosquito-repellent incense, mosquito nets, wire mesh screening of doors and windows, *etc*.

2.2.3. Strengthening health education

- **Strengthening people awareness.** Mass media such as newspapers, radio, TV, and internet posts should cover "World Malaria Day and National Malaria Day" activities and implement various ways to widely broadcast knowledge on malaria and the malaria elimination policy to improve public awareness and motivate community participation in malaria control and elimination.
- **Strengthening health education for primary and junior high school students.** Education department should deploy and arrange the primary and junior high school health education. CDCs should strengthen the technical support to health education on malaria in these schools. Primary and junior high schools in Types I and II counties should keep malaria and malaria control awareness as a recurrent topic in regular health education courses or theme activities. They should encourage pupils to pass such awareness to their family members by the way of "child educate adult."
- **Strengthening health education at the community level.** In Types I and II counties, the local CDCs should organize and support malaria public awareness by the way of advertisements and regularly update posted news in hospital waiting rooms, community health service centers, rural hospitals, village clinics, and large construction sites. They should also develop awareness material in minority nations' language.

2.2.4. Strengthening malaria control in mobile/migrant populations

- **Strengthening the management of malaria among travelers**. The Department of Health and Exit & Entry Administration should regularly release public information about the world malaria situation and related information inside the country. Tourism departments should release such information to tourists too. The Department of Tourism and Commerce has the obligation to aid the Department of Health in providing information to people traveling to malaria-endemic areas, as well as track information on malaria patients. Mechanism for shared information should be established among different sectors. This cross sector mechanism is responsible for increasing the anti-malaria public awareness, for providing consultation services to cross-border populations, for screening individuals with fever who have been traveling to malaria-endemic areas, and for reporting the detected malaria cases.

[4]The patriotic health movement is referring to a community-based health movement focusing on cleaning, pest control, environment reforming, and other health-related activities, with the aim of preventing infectious diseases.

- **Strengthening malaria control and prevention among expatriates**. China is involved in many large construction projects in malaria epidemic areas in different countries, e.g., Ethiopia and Zambia in Africa, Myanmar and Indonesia in Southeast Asia. Some of the projects like road, railway, or dam constructions involve high malaria risk exposure. CDCs provide appropriate information and training to the personnel employed in these projects before expatriates' deployment. The management and registration of malaria cases among expatriates are conducted locally in coordination with health agencies and CDCs. Public security departments provide assistance to investigate malaria cases among expatriates as requested by the Department of Health.

2.2.5. Improving malaria surveillance

- **Strengthening capacity building for malaria diagnosis.** The National Institute of Parasitic Diseases, China CDC is responsible for managing national malaria diagnosis reference laboratory. Its mandate is to provide a final laboratory confirmation and identify the source of any malaria case. Reference laboratories at all levels should regularly assess the skills of test and conduct quality control to ensure operation of the laboratory network.
- **Malaria surveillance in the post-elimination stage.** The status of malaria-free area must be maintained in counties having achieved malaria elimination, as well as in previously non-endemic provinces. This must be done through regular training of the medical personnel in malaria diagnosis and treatment. Prevention of re-establishment of malaria transmission is also required and must be conducted by intensifying the monitoring of imported cases.

3. Progress on malaria elimination in China

3.1. Status after seven years of implementation of malaria elimination program

A total of 28,886 malaria cases were reported in Mainland China (excluding Hong Kong, Macau, and Taiwan) from 2010 to 2016 (**Table 2**) [9, 10, 23–26]. During this period, indigenous cases, as well as the number of areas with local transmission, were substantially reduced. There were 40 indigenous cases reported from 10 counties in 2015, a decrease of 99.06% from 2010. By 2015, all Type III counties had achieved malaria elimination goals (no occurrence of indigenous cases for at least 3 consecutive years). Except for border counties in Yunnan, 96.43% (54/56) of the Type I counties reported no indigenous case over the same period. The malaria incidence in 19 Type I counties in Yunnan border area was lower than 10/100,000. All positive cases were reported through the China Information System for Disease Control and Prevention (CISDCP). The increase in incidence and number of detected cases after 6 years of implementation of the malaria elimination program was linked to the large number of imported cases, while the number of indigenous cases was reduced by 99.93% between 2010 and 2016 (**Table 2**) [8, 27–30]. The number of provinces with imported cases increased from 22 in 2010 to 30 (all the provinces in Mainland China except Tibet) in 2015. A total of 3318 imported cases were reported in 2016 (**Table 2**). The top 5 countries of origin of the imported cases were Myanmar (15.9%), Angola (12.5%), Nigeria (7.7%), Equatorial Guinea (7.5%),

Year	Total reported cases	Local cases					Imported cases							No. of death cases
		Clinical diagnosis	*P. vivax*	*P. falciparum*	Mix	Subtotal	Clinical diagnosis	*P. vivax*	*P. falciparum*	*P. malariae*	*P. ovale*	Mix	Subtotal	
2010	7855	0	4165	97	0	4262	NA*	NA	1161	NA	NA	NA	3593	19
2011	4498	364	885	56	3	1308	372	1253	1468	62**	0	35	3190	33
2012	2718	32	228	16	5	281	35	900	1403	60**	0	39	2437	15
2013	4128	6	77	9	0	92	29	859	2899	51	133	65	4036	23
2014	3078	5	45	6	0	56	20	798	1876	52	232	44	3022	25
2015	3288	1	38	1	0	40	22	840	1991	76	272	47	3248	20
2016	3321	0	3	0	0	3	15	709	2158	64	311	61	3318	15

°The number of malaria cases reported in 2017 will be published by the end of 2018.

*NA indicates that data were not available in the annual reporting system.

**Before 2013, the data recorded in the annual reporting system did not separate *P. malariae* and *P. ovale.*

Table 2. Malaria-attributable morbidity in the People's Republic of China during 2010–2016°.

and Cameroon (7.1%). In response to the increasing risk from imported cases, joint coordination and transfer of information were established among different agencies, in particular between China CDC and port quarantines. The latter are responsible for frontline screening and detection providing timely reports of positive cases. Clinics and hospitals are in charge of case treatment, while CDCs must follow up all the reported cases and carry out the individual case investigation. A successful example of such organization is given by the Shanglin County, Guangxi [31], for reporting 1,052 imported malaria cases in 2013, all of which were successfully treated and no death cases occurred. Furthermore, although *Anopheles* mosquitoes were present [14], no secondary transmission occurred.

Along the border between China and countries of the Greater Mekong Subregion (GMS) (Myanmar, Lao PDR, and Vietnam), 3 frontline barriers were established jointly by CDCs and port quarantines [32]. These 3 barriers consisted of (i) a strengthened health system in all 25 border counties with a capacity to immediate and comprehensive response to each malaria case (first line), (ii) establishment of 68 malaria service points at the border to provide consultation and screening to the migrant/mobile population (MMP) (second line), and (iii) a coordination process for response to malaria along the international borders between China, Myanmar, Lao PDR, and Vietnam covering 42 border counties (20 in Yunnan and 22 in the 3 other countries).

The main risks clearly identified after implementation of the national malaria elimination program were re-introduction through imported cases and the associated secondary transmission by local malaria vectors. Sustainable vector control is therefore essential. With the support from the national malaria surveillance system and national malaria diagnosis reference laboratory network, all the confirmed malaria cases were examined, including a total number of 2,215 foci investigated within 7 days after case detection and verification (**Table 3**). Long-lasting insecticidal net (LLIN) or ITN was delivered to the communities with high malaria incidence and presence of highly efficient malaria vectors, such as *Anopheles dirus* s.l. or *Anopheles minimus* s.l. [14]. IRS was carried out in active malaria foci (the definition of active foci is given in Ref. [19, 33]). In 2015, a total number of 29,611 LLIN/ITN were delivered, and 1,697,188 persons were protected by IRS in response to malaria foci (**Table 4**). Another key element in the protection of people against imported malaria was the training and education of the personnel. Annual joint health training workshops were carried out by the Departments of Health, Education, and Inspection and Quarantine on the National Malaria Day (April 26th) since 2008. Altogether, 74.9 million educational documents were delivered during workshops from 2010 to 2015 (**Table 5**). To these, one must add all posters and flyers delivered through port quarantines. Capacity building for health professional personnel corresponded to 464,500 working days in CDCs; 848,764 working days for clinical doctors; and 186,368 working days for microscopists during 2010–2015 (**Table 5**). With respect to port quarantines, 16,141 working days of training were accomplished with a malaria awareness rate of 100%.

A key issue in successful implementation of a program relies on the governmental commitment and support for sustained allocation of resources. The government at all levels has adopted malaria elimination as a component of the socioeconomic strategy. A national action plan for malaria elimination was issued jointly by 13 ministries in 2010 with clear goals and strategy, followed by a sustainable budget plan to ensure the financial support for malaria elimination. As a

Province	Cases reported within 24 h			Case investigation within 3 days		Number of foci investigated and disposed within 7 days
	Total reported cases	Reported cases within 24 h	Proportion of reported cases	Investigated cases within 3 days	Proportion of investigated cases	
Beijing	89	89	100%	89	100%	0
Tianjin	17	17	100%	17	100%	0
Hebei	44	44	100%	44	100%	21
Shanxi	12	12	100%	12	100%	15
Inner Mongolia	6	6	100%	1	16.67%	0
Liaoning	65	65	100%	65	100%	62
Jilin	21	21	100%	21	100%	0
Heilongjiang	8	8	100%	8	100%	0
Shanghai	42	42	100%	42	100%	29
Jiangsu	408	408	100%	408	100%	408
Zhejiang	195	195	100%	195	100%	160
Anhui	129	129	100%	129	100%	117
Fujian	94	94	100%	94	100%	12
Jiangxi	52	52	100%	52	100%	43
Shandong	219	219	100%	217	99.09%	199
Henan	185	185	100%	184	99.46%	180
Hubei	125	125	100%	122	97.60%	12
Hunan	129	129	100%	128	99.22%	46
Guangdong	155	155	100%	144	92.90%	1
Guangxi	236	236	100%	236	100%	33
Hainan	14	14	100%	14	100%	12
Chongqing	33	33	100%	31	93.94%	26
Sichuan	294	294	100%	292	99.32%	272
Guizhou	17	17	100%	17	100%	0
Yunnan	622	622	100%	618	99.36%	481
Tibet	8	8	100%	8	100%	0
Shaanxi	81	81	100%	81	100%	80
Gansu	22	22	100%	21	95.45%	6
Qinghai	1	1	100%	0	0	0
Ningxia	6	6	100%	6	100%	0
Xinjiang	4	4	100%	4	100%	0
Total	**3333**	**3333**	**100%**	**3300**	**99.01%**	**2215**

*1-3-7 model is referring to case reported within **1 day** (24 hours), case verification and investigation within **3 days**, and foci investigation and disposal within **7 days**. This is summarized as work model for malaria surveillance and response for malaria elimination program [20, 21].

Table 3. Progress indicators of 1-3-7* model in 2015.

Province	Number of delivered LLIN/ITN	Number of people protected by IRS/house*
Beijing	0	0
Tianjin	0	0
Hebei	6	353
Shanxi	0	188
Inner Mongolia	0	0
Liaoning	0	1120
Jilin	0	0
Heilongjiang	0	0
Shanghai	0	301
Jiangsu	0	7299
Zhejiang	0	1850
Anhui	207	1094
Fujian	180	535
Jiangxi	0	11,142
Shandong	0	336
Henan	2096	17,814
Hubei	79	918
Hunan	4	408
Guangdong	1552	1,327,650
Guangxi	10	1961
Hainan	6910	20,106
Chongqing	2	45,280
Sichuan	7	11,248
Guizhou	7418	12,771
Yunnan	628	229,535
Tibet	10,000	1537
Shaanxi	512	3546
Gansu	0	196
Qinghai	0	0
Ningxia	0	0
Xinjiang	0	0
Total	**29,611**	**1,697,188**

*Vector control measures mainly implemented in malaria foci for targeting population at risk.

Table 4. Progress indicators of vector control measures in 2015.

key player in malaria elimination in the central government, the National Health Commission (NHC, previously known as the Ministry of Health before 2011 and National Health and Family Planning Commission during 2011–2018) has established a multidisciplinary technical committee

Province	Number of trained people (person/time)			Number of delivered health education materials
	Malaria health workers	Clinical doctors	Microscopists	
Beijing	208	0	176	185,000
Tianjin	240	300	300	25,700
Hebei	41,499	95,926	10,652	3,064,121
Shanxi	596	0	871	57,000
Inner Mongolia	204	408	204	3200
Liaoning	7554	88,609	5835	143,600
Jilin	226	0	226	4000
Heilongjiang	0	0	0	0
Shanghai	21,516	48,584	9377	1,246,268
Jiangsu	26,415	23,963	16,468	7,365,562
Zhejiang	11,447	19,304	8749	1,676,164
Anhui	59,229	42,417	18,671	14,323,973
Fujian	7653	8777	3095	556,489
Jiangxi	12,696	24,829	9272	1,621,293
Shandong	34,624	56,382	15,494	7,040,504
Henan	62,005	183,085	19,486	7,968,270
Hubei	27,199	38,291	10,062	4,078,954
Hunan	22,018	46,761	11,666	4,722,609
Guangdong	737	375	1008	429,401
Guangxi	23,682	57,461	10,914	4,505,439
Hainan	10,838	5288	2607	2,058,430
Chongqing	1027	0	235	0
Sichuan	1900	0	825	372,200
Guizhou	21,643	28,120	8827	3,342,021
Yunnan	28,962	35,103	7479	4,200,909
Tibet	—	—	—	—
Shaanxi	21,951	34,313	10,970	3,615,648
Gansu	12,851	9136	2178	1,631,944
Qinghai	—	—	—	—
Ningxia	469	0	144	0
Xinjiang	5111	1332	577	670,755
Total	464,500	848,764	186,368	74,909,454

Note: "-" denotes data not available.

Table 5. Progress indicators of health education and capacity building during 2010–2015.

comprising malaria experts, i.e., epidemiologists, entomologists, clinical doctors, parasitologists, program managers, *etc*. With the support of this committee, NHC has issued a series of guidelines and standards adapting and updating the WHO guidelines [2, 19, 33, 34], such as technical guidelines for malaria elimination, malaria treatment and anti-malarial drug use, standards for malaria control and elimination, and malaria diagnosis, to cite a few [18].

3.2. Successful implementation

A working model, named 1-3-7, for malaria surveillance and response was implemented as a national malaria program. The definition of "1-3-7" is as follow:

- "1," within 1 day (24 hours): all malaria cases must be reported to the Chinese Information System for Disease Control and Prevention (CISDCP), an internet-based reporting system. The case information will be notified through a four-level system "county→ prefecture→ province→ national." The response at different levels is implemented according to national guidelines. Malaria is classified as a category B notifiable infectious disease, and case reporting through CISDCP has been implemented since 2004 [35]. All private and public registered clinics and hospitals must report malaria cases through CISDCP after diagnosis. CDCs are the key operators of CISDCP (**Figure 2**). This ensures that malaria case information is timely transmitted from bottom to top.

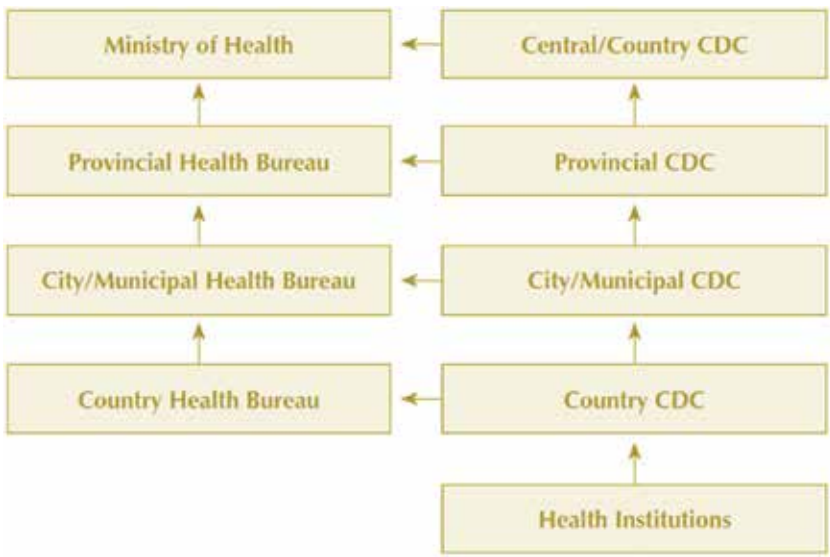


Figure 2. Vertical reporting structure of the China information system for disease control and prevention (CISDCP) (CDC, Centers for Disease Control and Prevention).

- "3," within 3 days: all the reported malaria cases should be confirmed and visited by CDCs' staffs at the county level to verify the infectious origin of the cases (whether imported or locally acquired). Meanwhile, blood samples of patients are taken and sent to the reference laboratory for further verification.

- "7," within 7 days: the outbreak focus should be investigated, and vector control and health promotion measures must be implemented. Vector control measures need to be conducted in active foci only, which are considered to have potential risk of onward transmission. The scope of investigation is the household of the reported patient and neighboring households. However, it can be expanded, if necessary.

Following the implementation of this 1-3-7 model, local malaria transmission was interrupted effectively in most parts of China, which accelerated the malaria elimination process [20, 21, 36]. Based on this success, the 1-3-7 model was recommended by WHO as an example for malaria surveillance model at elimination stage, in "Strategy for malaria elimination in the Greater Mekong Subregion: 2015-2030" and "Malaria surveillance, monitoring & evaluation: a reference manual" [37, 38].

However, the 1-3-7 strategy is not sufficient to successfully achieve malaria elimination. Other aspects must be considered. **The first aspect** to consider is the establishment of a network of malaria surveillance and diagnosis reference laboratories. After the launch of the malaria elimination program in 2010, and following the suggestions from the WHO guidelines [2, 19, 33, 34], a network for malaria diagnosis reference labs was gradually established [39]. By 2015, 23 provinces were enrolled into the National Reference Laboratory Network (NRLN). Laboratories at all levels worked together to ensure the quality of malaria diagnosis all over the country in a bottom-up approach (**Figure 3**) [39]. **Another key aspect** to consider is the involvement of communities. The community level is essential for a successful implementation. In addition to cross sector coordination, information was shared with different subnational divisions and in particular the community level. This is especially important when managing malaria cases among

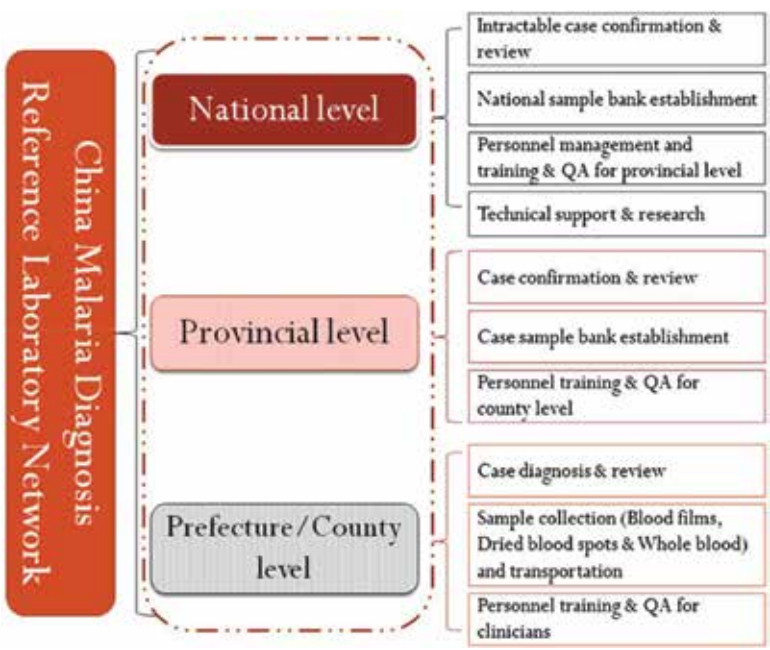


Figure 3. Structure of the National Reference Laboratory Network (QA, quality assurance).

http://dx.doi.org/10.5772/intechopen.77282

mobile/migrant populations. Five provinces in Central China, i.e., Jiangsu, Shandong, Henan, Anhui, and Hubei, and 3 provinces in Southern China, i.e., Guangdong, Guangxi, and Hainan, coordinated their actions at all levels (**Figure 4**). This joint coordination efficiently contributed to control malaria outbreaks and reduce malaria incidence [40, 41]. **The last aspect** to be considered is international collaboration. Malaria elimination cannot be achieved through the efforts of few countries only. It must be a general and coordinated international effort. In this respect, China has received support from international agencies such as WHO and Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM) to conduct this international collaboration [42]. At the same time, China developed international collaborations with African and GMS countries to implement a coordinated strategy for controlling and eliminating malaria [43, 44], and Chinese

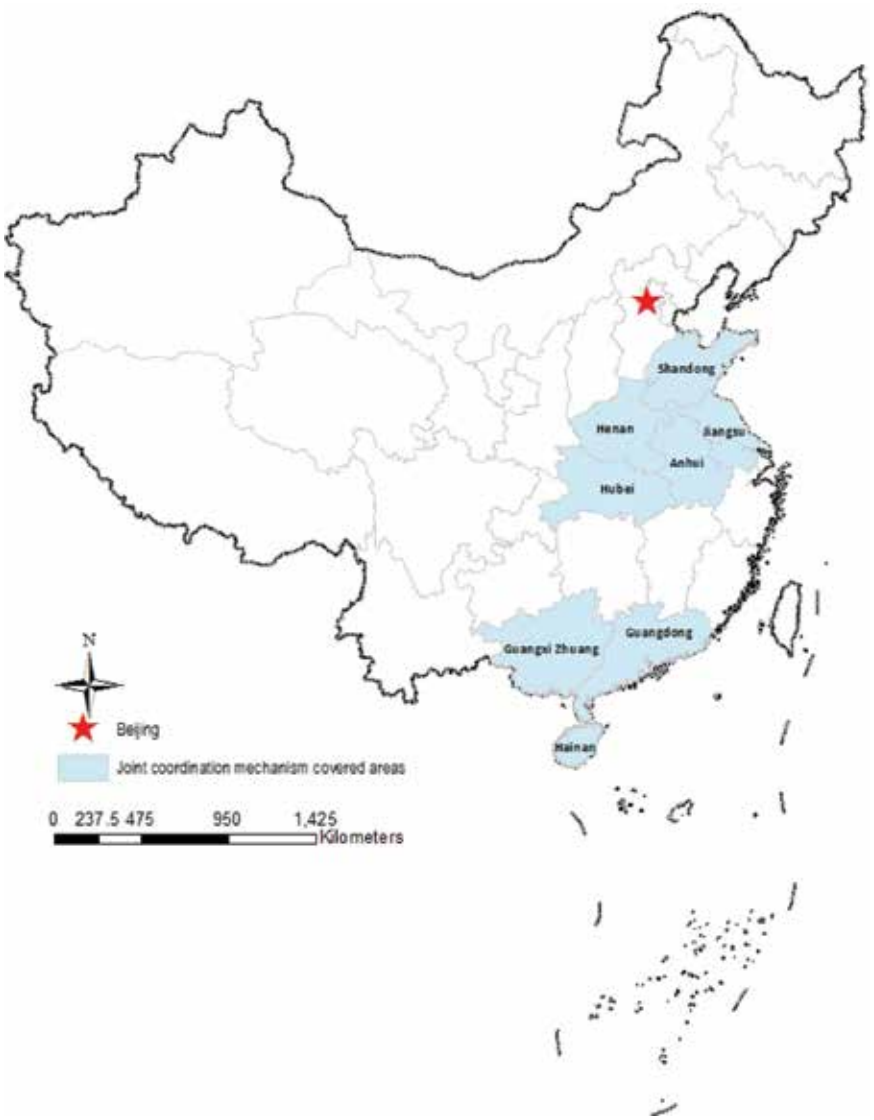


Figure 4. Cross province coordination mechanism for malaria control based on national mechanisms established since the 1950s–1970s. At that time, few population movements were occurring in the Yunnan Province.

students are being trained in Europe on molecular approaches applied on *Anopheles* mosquitoes [13–15]. These international collaborations on malaria and vector control toward elimination provided strong support to reduce malaria incidence in China and will be the basis for sustaining malaria elimination efforts.

4. Gaps and challenges

Although the malaria elimination program has made significant progress in China, there are still challenges. The main challenge is to accomplish malaria elimination along border areas. China is sharing 4060 km long international borders with GMS countries. These borders are devoid of natural barriers and porous, permitting population movement and facilitating cross border malaria transmission. The China-Myanmar border is particularly difficult to access for healthcare services due to ongoing armed conflict on the Myanmar side of the border resulting in proliferation of disease vectors and uninterrupted disease transmission [13, 15, 32, 45–48]. A related challenge is the prevention of re-introduction and re-establishment of malaria considered as a real threat to malaria elimination [2, 33, 34, 37]. Surveillance is highly recommended as a key intervention in the post-malaria elimination stage. However, decisive and rapid response to imported malaria is vital to prevent re-introduction and sustain malaria elimination [11, 27, 29, 49–53]. A shift from community to hospitals at the county and higher level for primary diagnosis was also recorded [8, 27], indicative of a more active role of the main city airports as ports of entry. In addition, owing to the sharp decrease of indigenous cases, it becomes difficult to maintain the capacity of intervention and proper training at the local level. Novel and innovative capacity building and training modules must thus be developed for both clinicians and CDC staffs.

5. Perspectives

The main objective in China with respect to malaria will be focused on how to sustain malaria elimination and prevent transmission re-establishment in accordance with the WHO's newly updated guidelines [33]. This requires a specific and sustainable investment even at post-elimination stage. Malaria is on the list of the "Health China 2030" blueprint issued by the State Council of China in 2016. This will ensure the required sustainable investment. Following this blueprint, a series of technical guidelines for malaria surveillance and response at post-elimination stage, as well as protocols for preventing malaria transmission re-establishment, are under development for short release. Meanwhile, mechanisms for maintaining anti-malaria capacity in health system are implemented. A National Technical Competition for Parasitic Disease Diagnosis and Test is organized annually for health workers from clinical agencies and CDCs [54]. This competition is an efficient way to maintain awareness and efficiency in malaria detection within the health system and prevent erosion of capacity along with malaria elimination. However, malaria elimination is primarily an international endeavor. Broad engagement and sustained investments are needed with support from multiple international partners [55–57]. In 2013, Chinese President Xi Jinping proposed the "One Belt and One Road Initiatives" to the world for international cooperation and development. Cooperation in health is one of the key components as it relates to the mutual benefits. China has already been actively involved

in global health governance [58], but a higher level of involvement is now expected, and the Chinese experience in malaria elimination will definitely be put at use within the "One Belt and One Road Initiative" [59]. Several platforms are currently under development, such as the Malaria Elimination Network in Lancang-Mekong Region (MENLMR) and the China-Africa Cooperation Program. Both GMS and sub-Saharan Africa are strongly affected by malaria, including drug resistance [60–62], high disease burden [1], and low level of resources [1, 3, 34]. They are thus primary targets for focused interventions enabling malaria elimination. Furthermore, these countries are experiencing innumerable challenges to achieve their planned malaria elimination program and in dire need of international support to bridge the funding gap [1–3, 37]. Although China has applied a successful model and did significant progress on malaria elimination, the Chinese model and experience cannot directly be implemented in these countries. Evaluation and field tests are needed as preliminary steps for operational feasibility. Pilot areas have been identified, and demonstrative projects have been therefore launched jointly by China and the targeted countries to assess the level of feasibility. These preliminary projects will provide evidence-based suggestions to develop a suitable strategy and model for each country to realize the ultimate goal of malaria elimination.

6. Conclusions

China has made substantial progress on malaria elimination and is on the way to achieve the elimination goal on time by 2020. The lessons drawn based on experiences in China will make a good reference for the countries aiming at malaria elimination. Challenges identified in the malaria elimination process in China might help other countries formulating appropriate strategies in time and place. International collaboration is strongly advocated to achieve the global issue to eliminate the most important infectious disease of the current times.

Acknowledgements

All the staffs in 31 provincial CDCs who have provided support to the data collection are acknowledged. This study was supported by TDR training grant (B40084).

Conflict of interest

The authors declare jointly that there is no conflict of interest.

Acronyms

ACT	Artemisinin combination therapy
CDC	Centers for Disease Control and Prevention

CISDCP	China Information System for Disease Control and Prevention
DDT	Dichlorodiphenyltrichloroethane
E-2020	In 2016, the WHO identified 21 countries with the potential to eliminate malaria by the year 2020. These countries were known as "E-2020 countries
GFATM	Global Fund to Fight AIDS, Tuberculosis and Malaria
GMS	Greater Mekong Subregion
IRS	Indoor residual spraying
ITN	Insecticide-treated net
LLIN	Long-lasting insecticidal net
MENLMR	Malaria Elimination Network in Lancang-Mekong Region
MDGs	UN Millennium Development Goals
MMP	Mobile and migrant population
NHC	National Health Commission
NRLN	National Reference Laboratory Network
QA	Quality assurance
RDT	Rapid diagnosis test
TDR	Special Programme for Research and Training in Tropical Diseases
WHO	World Health Organization

Author details

Shaosen Zhang[1,2,3,4,5,6,7,8], Li Zhang[1,2,3,4,5], Jun Feng[1,2,3,4,5], Jianhai Yin[1,2,3,4,5], Xinyu Feng[1,2,3,4,5], Zhigui Xia[1,2,3,4,5], Roger Frutos[6,7], Sylvie Manguin[8] and Shuisen Zhou[1,2,3,4,5]*

*Address all correspondence to: shuisenzhou@126.com

1 National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (China CDC), Shanghai, China

2 National Center for Tropical Diseases Research, Shanghai, China

3 Key Laboratory of Parasite and Vector Biology, Ministry of Health, Shanghai, China

4 National Center for International Research on Tropical Diseases, Ministry of Science and Technology, Shanghai, China

5 WHO Collaborating Center for Tropical Diseases, Shanghai, China

6 IES, University Montpellier, CNRS, Montpellier, France

7 Cirad, UMR 17, Intertryp, Campus international de Baillarguet, Montpellier, France

8 HSM, IRD, CNRS, Univ. Montpellier, Montpellier, France

References

[1] WHO. World Malaria Report. Geneva, Switzerland: World Health Organization; 2017. p. 196

[2] WHO. Global Technical Strategy for Malaria 2016-2030. Geneva, Switzerland: World Health Organization; 2015

[3] Alonso P, Noor AM. The global fight against malaria is at crossroads. Lancet. 2017; **390**(10112):2532

[4] Zhou XN, Xia ZG, Wang RB, Qian YJ, Zhou SS, Utzinger J, Tanner M, Kramer R, Yang WZ. Feasibility and roadmap analysis for malaria elimination in China. Advances in Parasitology. 2014;**86**:21-46

[5] Yin JH, Zhou SS, Xia ZG, Wang RB, Qian YJ, Yang WZ, Zhou XN. Historical patterns of malaria transmission in China. Advances in Parasitology. 2014;**86**:1-19

[6] Hu T, Liu YB, Zhang SS, Xia ZG, Zhou SS, Yan J, Cao J, Feng ZC. Shrinking the malaria map in China: Measuring the progress of the National Malaria Elimination Programme. Infectious Diseases of Poverty. 2016;**5**(1):52

[7] WHO. World Malaria Report 2016. Geneva, Switzerland; 2016. p. 186

[8] Feng J, Zhang L, Zhang SS, Xia ZG, Zhou SS. Malaria epidemiological characteristics in China, 2005-2015. China Tropical Medicine. 2017;**17**(4):325-335

[9] Zhang L, Feng J, Zhang SS, Xia ZG, Zhou SS. Malaria situation in the People's Republic of China in 2015. Chinese Journal of Parasitology and Parasitic Diseases. 2016;**34**(6): 477-481

[10] Zhang L, Zhou SS, Feng J, Fang W, Xia ZG. Malaria situation in the People' s republic of China in 2014. Chinese Journal of Parasitology & Parasitic Diseases. 2015;**33**(5):319-326

[11] Feng J, Xiao H, Zhang L, Yan H, Feng X, Fang W, Xia Z. The *Plasmodium vivax* in China: Decreased in local cases but increased imported cases from Southeast Asia and Africa. Scientific Reports. 2015;**5**:8847

[12] Feng J, Xiao H, Xia Z, Zhang L, Xiao N. Analysis of malaria epidemiological characteristics in the People's Republic of China, 2004-2013. The American Journal of Tropical Medicine and Hygiene. 2015;**93**(2):293-299

[13] Zhang SS, Zhou SS, Zhou ZB, Wang XZ, Jiang WK, Shi WQ, Yang YH, Yin SQ, Li XS, Wang JZ, Li SG, Zhang Y, Zhou XN. Investigation on population density and bionomics of *Anopheles minimus* in China-Myanmar border areas Yunnan province, P.R. China. Chinese Journal of Vector Biology and Control. 2017;**28**(3):216-219,254

[14] Zhang SS, Guo SH, Feng XY, Afelt A, Frutos R, Zhou SS, Manguin S. *Anopheles* vectors in mainland China while approaching malaria elimination. Trends in Parasitology. 2017; **33**(11):889-900

[15] Chen T, Zhang SS, Zhou SS, Wang X, Luo C, Zeng X, Guo X, Lin Z, Tu H, Sun X, Zhou H. Receptivity to malaria in the China-Myanmar border in Yingjiang County, Yunnan Province, China. Malaria Journal. 2017;**16**(1):478

[16] Feng X, Zhang S, Huang F, Zhang L, Feng J, Xia Z, Zhou H, Hu W, Zhou S. Biology, bionomics and molecular biology of *Anopheles sinensis* Wiedemann 1828 (Diptera: Culicidae), main malaria vector in China. Frontiers in Microbiology. 2017;**8**:1473

[17] China Ministry of Health. Action Plan of China Malaria Elimination (2010-2020). Beijing: Ministry of Health and other 12 Ministries in P.R. China; 2010

[18] Tang LH, Gao Q. Malaria Control and Elimination in China. 1st ed. Shanghai: Shanghai Scientific & Technical Publishers; 2013. p. 197

[19] WHO. Malaria Elimination. A Field Manual for Low and Moderate Endemic Countries. Geneva, Switzerland: World Health Organization; 2007

[20] Zhou SS, Zhang SS, Zhang L, Rietveld AE, Ramsay AR, Zachariah R, Bissell K, Van den Bergh R, Xia ZG, Zhou XN, Cibulskis RE. China's 1-3-7 surveillance and response strategy for malaria elimination: Is case reporting, investigation and foci response happening according to plan? Infectious Diseases of Poverty. 2015;**4**:55

[21] Cao J, Sturrock HJ, Cotter C, Zhou S, Zhou H, Liu Y, Tang L, Gosling RD, Feachem RG, Gao Q. Communicating and monitoring surveillance and response activities for malaria elimination: China's "1-3-7" strategy. PLoS Medicine. 2014;**11**(5):e1001642

[22] Bu L. Anti-Malaria Campaigns and the Socialist Reconstruction of China. 1950-1980; 2014

[23] Zhang L, Feng J, Xia ZG. Malaria situation in the People's Republic of China in 2013. Chinese Journal of Parasitology and Parasitic Diseases. 2014;**32**(6):407-413

[24] Xia ZG, Feng J, Zhou SS. Malaria situation in the People's Republic of China in 2012. Chinese Journal of Parasitology and Parasitic Diseases. 2013;**31**(6):413-418

[25] Xia ZG, Yang MN, Zhou SS. Malaria situation in the People's Republic of China in 2011. Chinese Journal of Parasitology and Parasitic Diseases. 2012;**30**(6):419-422

[26] Zhou SS, Wang Y, Li Y. Malaria situation in the People's Republic of China in 2010. Chinese Journal of Parasitology and Parasitic Diseases. 2011;**29**(6):401-403

[27] Zhou S, Li Z, Cotter C, Zheng C, Zhang Q, Li H, Zhou S, Zhou X, Yu H, Yang W. Trends of imported malaria in China 2010-2014: Analysis of surveillance data. Malaria Journal. 2016;**15**:39

[28] Li Z, Zhang Q, Zheng C, Zhou S, Sun J, Zhang Z, Geng Q, Zhang H, Wang L, Lai S, Hu W, Clements AC, Zhou XN, Yang W. Epidemiologic features of overseas imported malaria in the People's Republic of China. Malaria Journal. 2016;**15**:141

[29] Wang D, Li S, Cheng Z, Xiao N, Cotter C, Hwang J, Li X, Yin S, Wang J, Bai L, Zheng Z, Wang S. Transmission risk from imported *Plasmodium vivax* malaria in the China-Myanmar border region. Emerging Infectious Diseases. 2015;**21**(10):1861-1864

[30] Yin JH, Yang MN, Zhou SS, Wang Y, Feng J, Xia ZG. Changing malaria transmission and implications in China towards National Malaria Elimination Programme between 2010 and 2012. PLoS One. 2013;**8**(9):e74228

[31] Li Z, Yang Y, Xiao N, Zhou S, Lin K, Wang D, Zhang Q, Jiang W, Li M, Feng X, Yu J, Ren X, Lai S, Sun J, Fang Z, Hu W, Clements AC, Zhou X, Yu H, Yang W. Malaria imported from Ghana by returning gold miners, China, 2013. Emerging Infectious Diseases. 2015; **21**(5):864-867

[32] Yang HL, Xiao N, Yang YM, Xu JW. Challenges, opportunities and strategies of malaria elimination along China-Myanmar and China- Laos border. China Tropical Medicine. 2017;**17**(4):321-335

[33] WHO. A Framework for Malaria Elimination. Geneva, Switzerland: World Health Organization; 2017

[34] WHO. Eliminating Malaria. Geneva, Switzerland: World Health Organization; 2016

[35] Wang L, Wang Y, Jin S, Wu Z, Chin DP, Koplan JP, Wilson ME. Emergence and control of infectious diseases in China. Lancet. 2008;**372**(9649):1598

[36] Lu G, Liu Y, Beiersmann C, Feng Y, Cao J, Muller O. Challenges in and lessons learned during the implementation of the 1-3-7 malaria surveillance and response strategy in China: A qualitative study. Infectious Diseases of Poverty. 2016;**5**(1):94

[37] WHO. Strategy for Malaria Elimination in the Greater Mekong Subregion: 2015-2030. Geneva, Switzerland: World Health Organization; 2015

[38] WHO. Malaria surveillance, Monitoring & Evaluation: A Reference Manual. Geneva, Switzerland: World Health Organization; 2018

[39] Yin JH, Yan H, Huang F, Li M, Xiao HH, Zhou SS, Xia ZG. Establishing a China malaria diagnosis reference laboratory network for malaria elimination. Malaria Journal. 2015; **14**:40

[40] Shang LY, Gao Q, Liu X, Shen YZ, Huang GQ. Evaluation on the effect of cooperative malaria control in 5 provinces of Central China in 30 years. Journal of Pathogen Biology. 2006;**1**(1):51-53

[41] Zhang HW, Liu Y, Zhang SS, Xu BL, Li WD, Tang JH, Zhou SS, Huang F. Preparation of malaria resurgence in China: Case study of vivax malaria re-emergence and outbreak in Huang-Huai plain in 2006. Advances in Parasitology. 2014;**86**:205-230

[42] Wang RB, Zhang QF, Zheng B, Xia ZG, Zhou SS, Tang LH, Gao Q, Wang LY, Wang RR. Transition from control to elimination: Impact of the 10-year global fund project on malaria control and elimination in China. Advances in Parasitology. 2014;**86**:289-318

[43] Xia ZG, Zhang L, Feng J, Li M, Feng XY, Tang LH, Wang SQ, Yang HL, Gao Q, Kramer R, Ernest T, Yap P, Zhou XN. Lessons from malaria control to elimination: Case study in Hainan and Yunnan provinces. Advances in Parasitology. 2014;**86**:47-79

[44] Xia ZG, Wang RB, Wang DQ, Feng J, Zheng Q, Deng CS, Abdulla S, Guan YY, Ding W, Yao JW, Qian YJ, Bosman A, Newman RD, Ernest T, O'Leary M, Xiao N. China-Africa cooperation initiatives in malaria control and elimination. Advances in Parasitology. 2014;**86**:319-337

[45] Zhang Q, Sun J, Zhang Z, Geng Q, Lai S, Hu W, Clements AC, Li Z. Risk assessment of malaria in land border regions of China in the context of malaria elimination. Malaria Journal. 2016;**15**(1):546

[46] Wang D, Cotter C, Sun X, Bennett A, Gosling RD, Xiao N. Adapting the local response for malaria elimination through evaluation of the 1-3-7 system performance in the China-Myanmar border region. Malaria Journal. 2017;**16**(1):54

[47] Shi B, Zheng J, Qiu H, Yang GJ, Xia S, Zhou XN. Risk assessment of malaria transmission at the border area of China and Myanmar. Infectious Diseases of Poverty. 2017;**6**(1):108

[48] Xu JW, Liu H. The relationship of malaria between Chinese side and Myanmar's five special regions along China-Myanmar border: A linear regression analysis. Malaria Journal. 2016;**15**(1):368

[49] Sriwichai P, Karl S, Samung Y, Kiattibutr K, Sirichaisinthop J, Mueller I, Cui L, Sattabongkot J. Imported *Plasmodium falciparum* and locally transmitted *Plasmodium vivax*: Cross-border malaria transmission scenario in northwestern Thailand. Malaria Journal. 2017;**16**(1):258

[50] Xu C, Wei QK, Li J, Xiao T, Yin K, Zhao CL, Wang YB, Kong XL, Zhao GH, Sun H, Liu X, Huang BC. Characteristics of imported malaria and species of *Plasmodium* involved in Shandong Province, China (2012-2014). The Korean Journal of Parasitology. 2016;**54**(4):407-414

[51] Cao Y, Wang W, Liu Y, Cotter C, Zhou H, Zhu G, Tang J, Tang F, Lu F, Xu S, Gu Y, Zhang C, Li J, Cao J. The increasing importance of *Plasmodium ovale* and *Plasmodium malariae* in a malaria elimination setting: An observational study of imported cases in Jiangsu Province, China, 2011-2014. Malaria Journal. 2016;**15**:459

[52] Feng J, Yan H, Feng XY, Zhang L, Li M, Xia ZG, Xiao N. Imported malaria in China, 2012. Emerging Infectious Diseases. 2014;**20**(10):1778-1780

[53] Feng J, Xia ZG, Vong S, Yang WZ, Zhou SS, Xiao N. Preparedness for malaria resurgence in China: Case study on imported cases in 2000-2012. Advances in Parasitology. 2014;**86**:231-265

[54] Zhang SS, Xia ZG, Yin JH, Yan H, Zhou SS, Li SZ, Zheng X, Huang F, Li M, Chen HT, Wang Q, Zhang L, Liu W, Xiao N, Zhou XN. Analysis report of National Technical Competition for diagnosis of parasitic diseases in 2012: I. Capability analysis of *Plasmodium* detection. Chinese Journal of Parasitology and Parasitic Diseases. 2013;**31**(2):131-134

[55] Rabinovich RN, Drakeley C, Djimde AA, Hall BF, Hay SI, Hemingway J, Kaslow DC, Noor A, Okumu F, Steketee R, Tanner M, Wells TNC, Whittaker MA, Winzeler EA, Wirth

DF, Whitfield K, Alonso PL. malERA: An updated research agenda for malaria elimination and eradication. PLoS Medicine. 2017;**14**(11):e1002456

[56] The malERA Refresh Consultative Panel on Health Systems and Policy Research. malERA: An updated research agenda for health systems and policy research in malaria elimination and eradication. PLoS Medicine. 2017;**14**(11):e1002454

[57] Cibulskis RE, Alonso P, Aponte J, Aregawi M, Barrette A, Bergeron L, Fergus CA, Knox T, Lynch M, Patouillard E, Schwarte S, Stewart S, Williams R. Malaria: Global progress 2000-2015 and future challenges. Infectious Diseases of Poverty. 2016;**5**(1):61

[58] Liu P, Guo Y, Qian X, Tang S, Li Z, Chen L. China's distinctive engagement in global health. Lancet. 2014;**384**(9945):793-804

[59] Tang K, Li Z, Li W, Chen L. China's silk road and global health. Lancet. 2017;**390**(10112): 2595-2601

[60] Sinha S, Medhi B, Sehgal R. Challenges of drug-resistant malaria. Parasite. 2014;**21**:61

[61] Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, Sreng S, Anderson JM, Mao S, Sam B, Sopha C, Chuor CM, Nguon C, Sovannaroth S, Pukrittayakamee S, Jittamala P, Chotivanich K, Chutasmit K, Suchatsoonthorn C, Runcharoen R, Hien TT, Thuy-Nhien NT, Thanh NV, Phu NH, Htut Y, Han KT, Aye KH, Mokuolu OA, Olaosebikan RR, Folaranmi OO, Mayxay M, Khanthavong M, Hongvanthong B, Newton PN, Onyamboko MA, Fanello CI, Tshefu AK, Mishra N, Valecha N, Phyo AP, Nosten F, Yi P, Tripura R, Borrmann S, Bashraheil M, Peshu J, Faiz MA, Ghose A, Hossain MA, Samad R, Rahman MR, Hasan MM, Islam A, Miotto O, Amato R, MacInnis B, Stalker J, Kwiatkowski DP, Bozdech Z, Jeeyapant A, Cheah PY, Sakulthaew T, Chalk J, Intharabut B, Silamut K, Lee SJ, Vihokhern B, Kunasol C, Imwong M, Tarning J, Taylor WJ, Yeung S, Woodrow CJ, Flegg JA, Das D, Smith J, Venkatesan M, Plowe CV, Stepniewska K, Guerin PJ, Dondorp AM, Day NP, White NJ. Tracking resistance to artemisinin C. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. The New England Journal of Medicine. 2014;**371**(5):411-423

[62] Na-Bangchang K, Karbwang J. Emerging artemisinin resistance in the border areas of Thailand. Expert Review of Clinical Pharmacology. 2013;**6**(3):307-322

Chapter 11

Declining Transmission of Malaria in India: Accelerating Towards Elimination

Sunil Dhiman, Vijay Veer and Vas Dev

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.77046

Abstract

India is ecologically vast and has close to a billion-population living at risk of malaria. Given the evidence-based present-day intervention tools and large-scale implementation, India has recorded declining trends in disease transmission from 2 million cases in 2001 to close to a million cases in 2017 and embarked upon malaria elimination in keeping with the Global Technical Strategy by 2030. India is malaria endemic, but transmission intensities varied across its landscape with just few States of the east, central and northeast contributing bulk (80%) of total positive cases. *Plasmodium falciparum* and *P. vivax* are the predominant infections of which there has been steady increase in proportions of the former for constituting >60% of total cases what was 50:50 in 2001, a phenomenon attributed to emerging drug resistance. With the rolling out of the available intervention tools, malaria elimination is foreseeable yet there are multiple challenges which must be addressed to overcome the constraints. We strongly advocate continued disease surveillance and monitoring, universal coverage and intensification of core-interventions for prevention and treatment prioritizing high-risk States, strengthening cross-border collaborations for information sharing and coordinated activities, and above all sustained allocation of resources, creating the enabling environment to end malaria transmission.

Keywords: malaria elimination, epidemiology, *Plasmodium falciparum*, drug-resistance, mosquito vectors, insecticide resistance, Southeast Asia

1. Introduction

The advent of new intervention tools including Noble prize-winning discovery of Artemisinin by Tu Youyou for treatment of malaria combined with large-scale implementation of

insecticide-treated netting materials for vector containment has once again renewed the optimism of malaria elimination globally. Malaria map is shrinking with more than 35 countries certified to be malaria free, and another 21 countries that are likely to reach zero indigenous transmission (categorized as E-2020) are set to be declared malaria free by 2020 [1, 2]. Many more countries are moving forward from control to elimination. The Global Technical Strategy for Malaria 2016–2030 envisages: (i) to reduce global malaria mortality rates and case incidence by at least 90% compared to 2015 levels, (ii) to make at least 35 countries malaria free that reported cases in 2015 and (iii) preventing re-establishment in countries with no indigenous transmission [3]. Among member countries of the Southeast Asia Region of WHO (SEAR), Maldives and Sri Lanka have already been certified malaria free in 2015 and 2016, respectively, and Bhutan is targeting elimination in the foreseeable future. In the past decade, India has registered drastic decrease in cases and have formulated National Framework for Malaria Elimination (2016–2030) in close alignment with the Global Technical Strategy for Malaria, Roll Back Malaria Action (RBM), Investment to defeat Malaria (AIM) and the Asia Pacific Leaders Malaria Alliance (APLMA) for shared experiences and coordinated action to eliminate malaria (zero indigenous cases) throughout the country by 2030 [4]. The said task is set to be accomplished in phased manner with the following objectives: (i) eliminate malaria in all 26 low-to-moderate transmission States/Union Territories (UTs) by 2022, (ii) reduce the case incidence to <1 per 1000 population by 2024 in all States/UTs, (iii) interrupt indigenous transmission throughout the country by 2027 and (iv) prevent re-establishment of local transmission and maintain malaria-free status by 2030 and beyond.

India is historically endemic for both *Plasmodium vivax* and *P. falciparum* malaria and has history of successes and resurgences [5, 6]. Malaria was on the verge of elimination post-independence in 1960s with 0.1 million cases and no death, yet it reared its ugly head again in 1970s with record number of six million cases and many deaths attributed to technical and operational constraints. Transmission is largely seasonal corresponding to rainy season with record of focal disease outbreaks characterized by high rise in cases and attributable deaths. In 2017, India reported 0.84 million cases, the highest disease burden in SEAR member countries of WHO [4, 7]. Almost all Indian States and UTs are reporting cases, which can be broadly stratified into three different categories based on Annual Parasite Incidence (API) per 1000 population, that is, Category—I (total of 15 States/UTs including districts with API < 1) that are targeted for elimination phase, Category—II (total of 11 States/UTs with API < 1 with one or more districts reporting > 1 API) marked for pre-elimination phase and Category—III (total of 10 States/UTs with > 1 API) targeted for intensified control operations. With the rolling out of the present day evidence-based intervention tools, disease transmission is on the steady decline presenting window of opportunity to accelerate toward universal coverage for malaria prevention and treatment. India is a huge country (population 1.3 billion) with majority populous (80%) living at risk of malaria. The task is enormous and daunting. Given the political commitment and National Framework developed by the National Vector Borne Disease Control Programme (NVBDCP) of Government of India, malaria elimination is foreseeable, yet there are multiple challenges which must

http://dx.doi.org/10.5772/intechopen.77046

be addressed to overcome the constraints. In this chapter, we attempt to enumerate some of these issues helping strengthen healthcare services in combating malaria menace enabling elimination by due date.

2. Malaria transmission in India: current distribution and parasite formula

Malaria transmission is heterogenous across Indian landscape for its diverse ecology and multiplicity of disease vectors [8]. Malaria is a serious public health concern and almost all 36 States/UTs are consistently contributing cases, but transmission intensities varied ranging from low-to-moderate (**Table 1**). Among these, north-eastern, eastern and central Indian States consistently contributed 80% of the total disease burden having concentration of cases (API > 10) associated with large forest cover, ethnic tribes, poverty and high rainfall (**Figure 1**). These included States of Odisha (formerly Orissa) and Jharkhand (eastern India), Chhattisgarh and Madhya Pradesh (central India) and Meghalaya and Tripura (northeast India), which together contributed >65% of *P. falciparum* cases. Approximately a billion population of India resides in malaria endemic areas, however, 80% of malaria cases are reported by just 20% of the population living in the forest-fringe, tribal and foothills hard-to-reach areas of the country little aware of disease prevention and access to treatment [9]. These areas are prone to periodic disease outbreaks resulting in flare up of cases and attributable mortality accounting for inter-annual variation in reported morbidity. From the epidemiological data for the past 17 years (2001–2017), disease transmission trends are observed to be declining from two million cases in 2001 to close to a million cases in 2017 (**Figure 2**). However, consequent to the introduction of artemisinin-based combination therapy (ACT) beginning 2010 coupled with insecticide-treated netting materials (ITNs); there has been drastic decrease in cases and deaths. *P. falciparum* and *P. vivax* are the predominant infections of which there has been steady increase in proportions of the former parasite species presently constituting >60% of total cases what was 50:50 in 2001. Every single death was attributed to *P. falciparum*, majority of which were contributed by high-risk States of north-eastern, eastern and central India (**Figure 3**). The distribution of *P. malariae* is patchy, recorded in indigenous tribes of eastern, north-eastern and central India [10–12], but transmission of *P. ovale* except for few sporadic reports could not be clearly ascertained [13–15]. There exists no record of *P. knowlesi* malaria in India making inroads in other Southeast Asian countries [16].

The reported cases and deaths; however, are far from acccurate for disease surveillance that can be best described as fragmented and there is no system in place to capture data from private and public sectors alike, least the asymptomatic cases [17]; WHO estimates are much higher to the tune of >10 million cases and deaths manifold [1]. Nevertheless, the presented data showed disease transmisison trends in relation to existing interventions, and monitoring and evaluation in practice.

No	State/Union Territories	2014			2015			2016			2017		
		Total malaria cases	*Plasmodium falciparum* cases	Deaths	Total malaria cases	*Plasmodium falciparum* cases	Deaths	Total malaria cases	*Plasmodium falciparum* cases	Deaths	Total malaria cases	*Plasmodium falciparum* cases	Deaths
1	Andhra Pradesh	21,077	15,511	0	25,042	18,709	0	23,613	17,443	0	16,913	11,944	0
2	Arunachal Pradesh	6082	2338	9	5088	1714	7	3144	911	2	1538	487	0
3	Assam	14,540	11,210	11	15,557	11,675	4	7826	5686	6	5473	4131	0
4	Bihar	2043	699	0	4006	1286	1	5205	895	0	3175	356	2
5	Chhattisgarh	128,993	108,874	53	144,886	123,839	21	148,220	121,503	61	141,310	115,153	0
6	Goa	824	42	0	651	75	1	742	130	0	653	75	2
7	Gujarat	41,608	6253	16	41,566	7232	7	44,783	6298	6	37,801	3502	2
8	Haryana	4485	45	1	9308	726	3	7866	552	0	6887	904	0
9	Himachal Pradesh	102	1	0	60	1	0	121	19	0	95	9	0
10	Jammu & Kashmir	291	21	0	216	8	0	242	11	0	226	0	0
11	Jharkhand	103,735	46,448	8	104,800	54,993	6	141,414	83,232	15	92,770	42,047	1
12	Karnataka	14,794	1329	2	12,445	1598	0	11,078	1746	0	6529	1118	0
13	Kerala	1751	305	6	1549	400	4	1547	419	2	1194	317	2
14	Madhya Pradesh	96,879	41,638	26	100,597	39,125	24	69,106	22,304	3	46,176	15,554	3
15	Maharashtra	53,385	25,770	68	56,603	31,139	59	23,983	7815	26	18,133	5929	19
16	Manipur	145	72	0	216	119	0	122	58	0	80	22	0
17	Meghalaya	39,168	37,149	73	48,603	43,828	79	35,147	31,867	45	16,433	14,974	11
18	Mizoram	23,145	21,083	31	28,593	24,602	21	7583	5907	9	5710	4978	0

No	State/Union Territories	2014			2015			2016			2017		
		Total malaria cases	*Plasmodium falciparum* cases	Deaths	Total malaria cases	*Plasmodium falciparum* cases	Deaths	Total malaria cases	*Plasmodium falciparum* cases	Deaths	Total malaria cases	*Plasmodium falciparum* cases	Deaths
19	Nagaland	1936	647	2	1527	532	3	828	316	0	394	188	1
20	Orissa	395,035	342,280	89	436,850	369,533	80	449,697	389,332	77	352,140	297,554	25
21	Punjab	1036	14	0	596	13	0	693	8	0	808	12	0
22	Rajasthan	15,118	603	4	11,796	662	3	12,741	1031	5	6837	377	0
23	Sikkim	35	18	0	27	11	0	15	5	0	12	3	0
24	Tamil Nadu	8729	339	0	5587	355	0	4341	242	0	5449	197	0
25	Telangana	5189	4602	0	10,951	10,206	4	3512	2617	1	2688	2170	0
26	Tripura	51,240	49,653	96	32,525	30,074	21	10,546	9545	14	7040	6572	6
27	Uttarakhand	1171	89	0	1466	73	0	961	47	0	532	14	0
28	Uttar Pradesh	41,612	326	0	42,767	371	0	39,238	158	0	32,345	159	0
29	West Bengal	26,484	4981	66	24,208	5775	34	35,236	5928	59	30,008	4632	29
30	A.N. Islands	557	109	0	409	77	0	485	140	0	404	67	0
31	Chandigarh	114	0	0	152	1	1	157	0	0	114	1	0
32	D & N Haveli	669	90	1	418	46	0	375	30	0	297	16	0
33	Daman & Diu	56	4	0	84	18	0	48	7	0	37	4	0
34	Delhi	98	0	0	54	0	0	31	0	0	577	2	0
35	Lakshadweep	0	0	0	4	0	0	2	0	0	1	0	0
36	Puducherry	79	3	0	54	5	1	76	11	0	59	13	0
All India total		1,102,205	722,546	562	1,169,261	778,821	384	1,090,724	716,213	331	840,838	533,481	103

*Source: Ref. [7].

Table 1. Malaria-attributable morbidity and mortality in different States and Union Territories (UTs) of India during 2014–2017*.

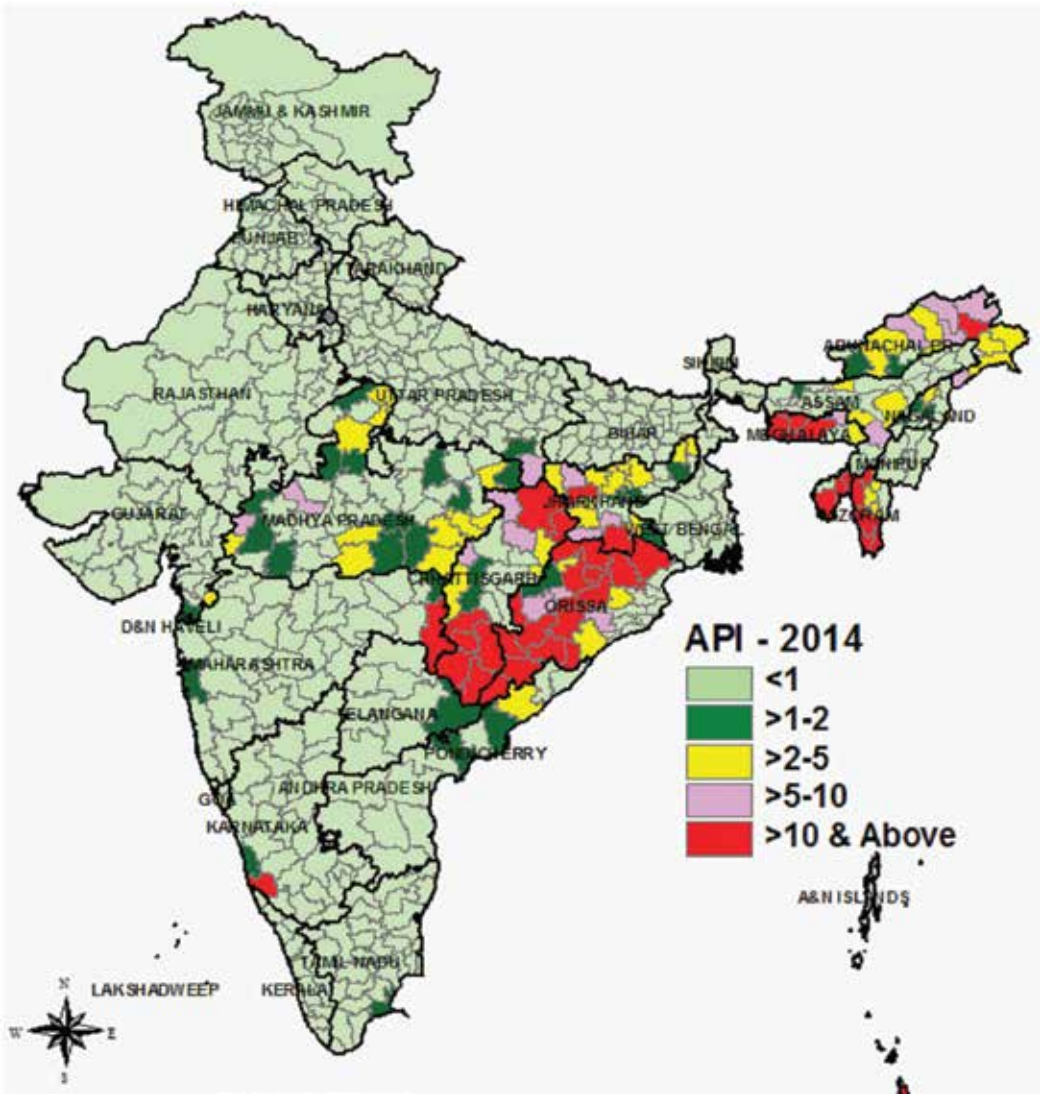


Figure 1. Malaria stratification by Annual Parasite Incidence (API) in Indian States for data based on 2014. API 10 corresponds to 10 confirmed cases per 1000 population. *Source*: Ref. [4].

3. Multiple disease vectors and insecticide resistance

India holds the distinction in malaria epidemiological research for Noble prize-winning discovery that malaria is transmitted by mosquitoes by Sir Ronald Ross on the day of August 20, 1897, and for monumental work on faunistic surveys dating back to 1930s [18]. Of the 58 anopheline species recorded in India [19], six major vector taxa are implicated in malaria transmission, including *Anopheles culicifacies s.l.*, *An. fluviatilis s.l.*, *An. minimus s.l.*, *An. dirus s.l.*, *An. sundaicus s.l.* and *An. stephensi* [20]. All of these, except *An. stephensi*, are species complexes among which members

http://dx.doi.org/10.5772/intechopen.77046

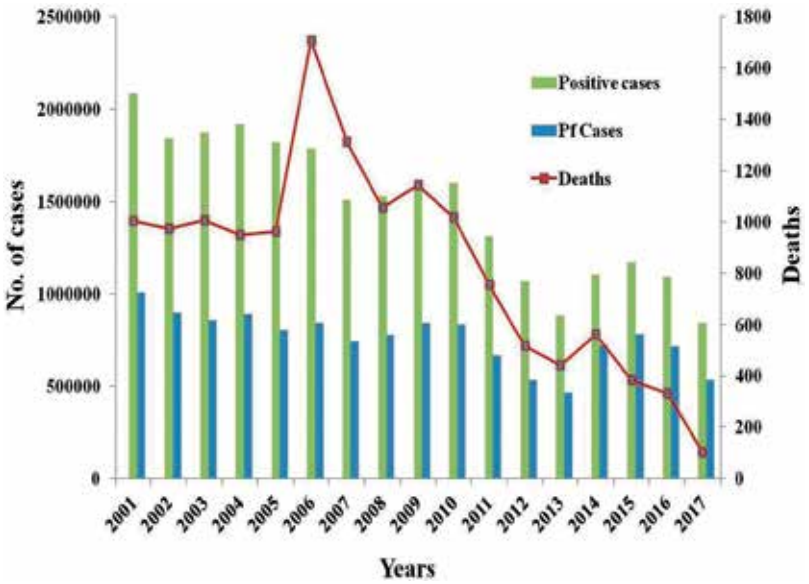


Figure 2. Malaria-attributable morbidity and mortality in India during 2001–2017. Malaria positive cases denote confirmed diagnosis by presence of malarial parasite in finger-prick blood-smears; Pf cases denote positivity for *Plasmodium falciparum;* death cases are attributed to confirmed falciparum malarial infection. *Source*: Ref. [7].

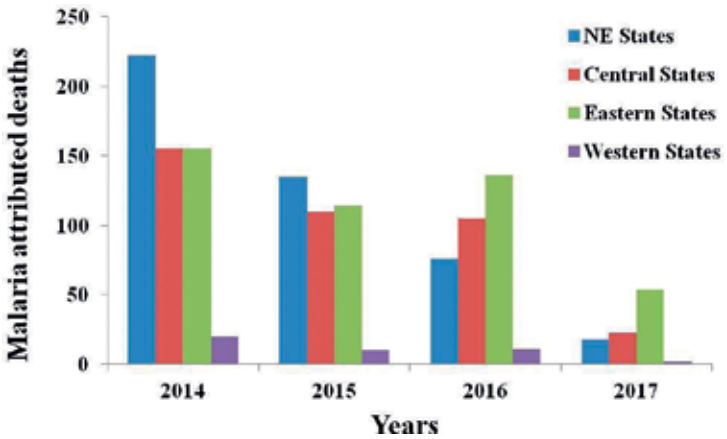


Figure 3. Distribution of malaria-attributed deaths in different geoepidemiological regions of India for data based on 2014–2017. NE refers to group of seven sister States of northeast India including Arunachal Pradesh, Assam, Meghalaya, Manipur, Mizoram, Nagaland and Tripura; Central States include Madhya Pradesh and Chhattisgarh; Eastern States include Bihar, Jharkhand, Odisha (formerly Orissa) and West Bengal; Western States include Maharashtra, Goa, Gujarat and Rajasthan. In the remaining Indian States and Union Territories, death cases were few and far (not shown). *Source*: Ref. [7].

were repeatedly incriminated as vectors evidenced by detection of live sporozoites in salivary glands across range of their distribution [21, 22]. With added tools of molecular taxonomy; however, there have been significant advances in understanding sibling-species composition of these taxa [23–25], distribution and their bionomics helping target species-specific control interventions in place and time (**Table 2**). Among these, *An. culicifacies* is the most widespread and extensively

Anopheles species/ taxa	Sibling species prevalent in India (total identified)	Diagnostic cytotaxonomic/ molecular tools	Breeding habitats	Feeding behavior (peak biting activity)	Resting habitats	Sporozoite infectivity (%)	Insecticide susceptibility status	Distribution range
An. culicifacies s.l.	A,B,C,D and E (5)	Fixed paracentric inversions, PCR based sequencing of 28S-D3 domain; ITS2-PCR-RFLP; rDNA ITS2	Rain water collections, riverine pools, rice fields, seepage water, streams, borrow pits, irrigation channels	Predominantly zoophilic except 'E' (A and B: 22:00–23:00; C and D: 18:00–21:00; no data for E)	Human-dwellings indoors and cattle sheds	Incriminated (0.3–20)	Resistant to DDT, malathion and pyrethroids	Throughout rural India
An. fluviatilis s.l.	S, T, U and Form 'V' (4)	Fixed paracentric inversions; PCR based sequencing of rDNA ITS2; 28S rDNA-D3	Seepage water foothill streams, irrigation channels, river ecology, shallow wells	Sibling species 'S'—highly anthropophilic (20:00–24:00); 'T'—zoophilic	S—human dwellings indoors; T—cattle sheds	Incriminated	Highly susceptible to all residual insecticides	Throughout India except north-eastern States
An. minimus s.l.	*An. minimus s.s.* (3)	rDNA ITS2; 28S rDNA-D3	Perennial foothill seepage water streams	Highly anthropophilic (01:00—04:00)	Human-dwellings indoors	Incriminated (3.0)	Highly susceptible to all residual insecticides	North-eastern of Arunachala Pradesh, Assam, Meghalaya, Manipur, Mizoram, Nagaland, Tripura, and Eastern State of Odisha
An. dirus s.l.	*An. baimaii* (8)	Karyotypic studies, polytene chromosome analysis, gene-enzyme variation, DNA probes, rDNA ITS2; SCAR-PCR	Jungle water pools, Elephant foot-prints	Highly anthropophilic (21:00—24:00)	Exophilic	Incriminated (1.9)	Highly susceptible to all residual insecticides	North-eastern of Arunachala Pradesh, Assam, Meghalaya, Manipur, Mizoram, Nagaland, Tripura
An. sundaicus s.l.	*An. sundaicus* cytotype D (4)	Mitochondrial DNA cytochrome oxidase 1 and cytochrome-b; rDNA ITS2; 28S rDNA-D3	Brackish water including swamps, salt water lagoons, creeks as well as fresh water	Predominantly zoophilic except indoor resting populations (21:00—04:00)	Both indoors and outdoors	Incriminated	Highly susceptible to all residual insecticides	Andaman & Nicobar Islands

Anopheles species/ taxa	Sibling species prevalent in India (total identified)	Diagnostic cytotaxonomic/ molecular tools	Breeding habitats	Feeding behavior (peak biting activity)	Resting habitats	Sporozoite infectivity (%)	Insecticide susceptibility status	Distribution range
An. stephensi	Not applicable		Domestic containers, building construction sites, overhead water storage tanks, underground cement tanks, desert coolers	Predominantly anthropophilic (22:00—24:00)	Endophilic	Incriminated	Resistant to DDT and Malathion	Urban metropolitan cities of India

*Source: Refs. [20, 23–25]; rDNA, ribosomal DNA; SCAR, sequence characterized amplified region; ITS2, internal transcribed spacer 2; PCR, polymerase chain reaction.

Table 2. Bionomics, distribution and sibling-species composition of the dominant mosquito vector taxa of human malaria in India*.

studied for its sibling species composition (A, B, C, D and E), distribution range, seasonal prevalence, larval ecology, feeding and breeding behavior and disease transmission relationships [26]. Species E is the most efficient malaria vector of all having predilection for human host, while species B is a poor vector for its zoophilic characteristics. The other three species (A, C and D) are responsible for local transmission in areas of their predominance [27]. This taxon is the most abundant rural vector in plains of mainland India generating about 65% of cases annually and held responsible for focal disease outbreaks associated with build-up of vector density. It is regarded as highly adaptive species for its diverse breeding habitats and invading new territories in degraded forests of north-eastern India evidenced by records of rising density and incrimination [28–30]. Its control has become a formidable challenge for having grown multi-resistant virtually to all available insecticides including pyrethroids opening new vistas for research on newer interventions that are sustainable, cost-effective and community-based [31–33].

An. fluviatilis complex is just as widespread and have overlapping distribution with *An. culicifacies* throughout India [20]. Among its sibling species, that is, S, T, U and form 'V'; it is species S which is highly anthropophilic and responsible for maintaining hyperendemic malaria predominantly in foothills of eastern India contributing ~15% of reported cases [34]. It shares similar bionomical characteristics with yet another efficient malaria vector species *An. minimus s.s* for breeding in foothill seepage water streams and resting indoors in human dwellings [35]. Both are highly susceptible to residual insecticides. *An. minimus* instead is the most predominant vector species of north-eastern States of India and has long history of disappearance and re-appearance stymying the control authorities. It is reckoned as the most efficient vector species for its high anthropophily (human blood index > 90%) and fulminating focal disease outbreaks taking heavy toll of human lives [36]. It is a perennial species and widely incriminated practically all months of the year with average sporozoite infection rate of 3% [37]. This species exhibits high behavioral plasticity for avoiding sprayed surfaces for weeks and establishing extra-domiciliary transmission in response to indoor residual spraying (IRS). While this species has staged comeback in eastern State of Odisha after lapse of 45 years [38], the populations of *An. minimus* once again are reported diminishing in erstwhile domains of its distribution in northeast India corroborated by evidence of reducing disease transmission [35]. It presents an unprecedented opportunity to strengthen interventions to keep populations of this species at bay helping achieve malaria elimination specific to the region at sub-national level.

Within the *An. dirus* complex, *An. baimaii* is the only vector occurring in India with a wide prevalence in the north-eastern States and has been recorded in high densities and incriminated in range of its distribution [39]. It is just as efficient vector species with strong predilection for human host but distinct from *An. minimus* for its breeding and resting characteristics [40, 41]. It is a forest dweller affecting forest-fringe human settlements along inter-country and inter-state border areas causing devastating disease outbreaks often in conjunction with *An. minimus*; together they contribute 10% of reported cases in the country [42]. Its control has become difficult for peak biting activity during second quartile (21:00–00:00) of the night as well as exophilic resting behavior avoiding sprayed surfaces. Its populations along with that of *An. minimus* are also depleting owing to deforestation and urbanization [29, 30]. However, niche thus vacated by both these species is accessed by *An. culicifacies s.l.* and has established foothold erstwhile recorded in low-density [26, 28]. Among sibling species of the *An. sundaicus* complex, cytotype species D has been characterized with a regional importance presently confined to Andaman and Nicobar Islands [43]. It is a brackish water species, largely zoophilic and susceptible to residual insecticides.

An. stephensi is the only urban vector species breeding in domestic containers and often associated with tropical aggregation of labor at construction sites in metropolitan cities [44]. The species is resistant to multiple insecticides and its control focused on 'source reduction' to contain urban malaria. Due to continued urbanization and associated labor migration, urban malaria is viewed as a growing menace contributing about 10% of cases in the country [5–7].

Besides these dominant vectors, member species of *An. maculatus s.l.*, *An. annularis s.l.* and *An. subpictus s.l.* are also implicated; however, these species are considered of lesser significance for being predominantly zoophilic [45, 46]. Vector control is an integral part of the malaria control strategy in India and huge investment is made annually to contain build-up of disease vectors averting epidemic malaria. What is tantamount to vector control is the entomological surveillance for developing malaria-risk maps, judicious application of insecticides, monitoring insecticide-resistance and residual efficacy, universal coverage for population at risk and continued research for newer interventions, which are community-based and sustainable. We strongly believe that judicious mix of technologies that are situation-specific and doable would help save operational costs in resource-poor settings. The country is in dire need of skilled entomologist/taxonomists (an expertise that is getting scarce) to meet the human resource requirements for control of malaria, and other vector-borne diseases, as well as vector surveillance post-elimination to prevent re-establishment of local transmission in malaria-free territories.

4. Drug-resistant malaria

Of the two prevalent malaria parasite species, the rising proportions of *P. falciparum* is of grave concern for presently constituting >60% of total reported cases in the country [5, 7]. Rising trends of *P. falciparum* are largely attributed to fast emerging drug-resistance over space and time in parallel with phenomenon happening in countries of the Greater Mekong Subregion (GMS) of Southeast Asia [47]. Historically, chloroquine (CQ) was the most commonly used drug for treatment ever since inception of the control program in 1953 for its efficacy and affordability. It had become obsolete since its first report of treatment failure in Assam (northeast India) way back in 1973 [48]. Subsequently, drug-resistant foci had multiplied for which northeast is considered corridor for spread to rest of peninsular India resulting in steady rise in proportions of *P. falciparum* what was 13% in 1978 to 65% in present day malaria [49]. Northeast India shares wide border with Myanmar contiguous with GMS countries which is porous for cross-border migration, facilitating entry of drug-resistant strains enroute to the rest of India and beyond. Malaria transmission along this border is intense vectored by *An. minimus* and *An. baimaii* (the two most efficient vector species), and healthcare access is inadequate resulting in indiscriminate and sub-optimal doses *inter-alia*, poor vector control, illiteracy and treatment-seeking behavior; all contributing to propagation and spread of drug-resistant malaria strains [50]. Chloroquine therapy was subsequently upgraded to sulfadoxine-pyrimethamine (SP) in 2004 as first-line treatment in selected districts reporting CQ-resistant malaria [51]. The therapeutic efficacy of SP was short lived resulting in substantial rise in cases in the following years [52]. It was in 1990s that the development of artemisinin-derivatives raised new hopes for treatment of drug-resistant malaria for its fast acting schizontocidal properties. Initially, artemisinin was used as monotherapy for treatment of severe and complicated clinical malaria, discontinued in 2009 due to high recrudescence

rate and risk of resistance for being commonly prescribed medicine by private practitioners and public sector alike [53]. In 2010, the program adopted ACT by combining it with SP, that is, artesunate + SP (AS + SP) for treatment of every single case of *P. falciparum* malaria in high-risk districts [54]. Mass scale introduction of this combination did result in appreciable transmission reduction in endemic communities formerly intractable. It continues to be in practice throughout India except in the northeast region where it has been replaced by yet another combination therapy, that is, artemether + lumefantrine (AL) in 2013 due to declining efficacy of AS + SP combination drug [55]. The emergence of artemisinin resistance in Southeast Asia [56]; however, is a matter of grave concern for its movement westwards to India for having it detected in Myanmar close to the Indian border in northeast [57]. Genomic studies have already detected mutations in the '*kelch 13*' propeller region (*Pfk 13*) linked to artemisinin resistance in north-eastern States, but to lesser frequency [58]. It is of utmost importance to periodically monitor the therapeutic efficacy of drug-regimen in force for radical cure and keep vigil on circulation of counterfeit drugs. The cost of spread of artemisinin-resistant malaria would be colossal, threatening the control and elimination efforts.

5. *Plasmodium vivax* malaria: the neglected parasite

The Southeast Asian countries contribute most of the vivax cases (58%) in the World of which India is the largest contributor [1]. Historically, much of the control efforts continue to be focused on control of falciparum malaria due to its associated severity and critical illness; the vivax malaria remained a neglected parasite [59]. Paradoxically, control of falciparum malaria is rather measurable in relation to interventions due to development of gametocytaemia 9–10 days post primary infection; instead the formation of gametocytes in vivax malaria is concurrent within few days of initial infection even before the patient seeks treatment permitting uninterrupted transmission. This biological characteristic of vivax malaria along with intrinsic ability of formation of latent hibernating 'hypnozoites' has made control efforts a difficult proposition. Nevertheless, the control of vivax malaria is gaining eminence in the context of malaria elimination across the continents [60].

The magnitude of vivax malaria is huge but grossly underestimated throughout India and continues to be neglected [61]. The transmission and distribution of vivax malaria varied across Indian States/UTs, but large concentrations of cases are occurring in urban metropolitan cities [7]. Although it remains highly susceptible to CQ therapy [62–66], its elimination is one difficult issue owing to latent stage 'hypnozoites' in the liver causing relapses amounting to extended morbidity over months/years. The only available anti-relapse drug 'primaquine (PQ)' does not guarantee radical cure much due to extended therapy over days coupled with poor compliance resulting in repeated episodes [67–69]. In addition, the administration of PQ is associated with several issues including contraindication in special groups, that is, infants, pregnant or lactating mothers, and inborn glucose-6-phosphate dehydrogenase (G6PD) deficiency syndromes due to associated hemolytic anemia; these population groups are excluded from primaquine therapy. There exists no diagnostics for detection of G6PD at point-of-care in the present surveillance system except few laboratories procedure for which

it is time consuming and impractical in field conditions where the problem exists. Further, given the available technologies, the detection of latent hypnozoites and sub-patent parasitemia is presently not built in the disease surveillance.

The primary attack of vivax malaria is invariably associated with acute paroxysm presenting classical symptoms but treatable and rarely fatal [70–72]; although few sporadic cases of CQ-resistant cases have been reported in India and several other countries [73]. There is acute need of alternative 8-aminoquinolines, which are safe and universally applicable for radical cure across all population groups preventing relapses. As of today, we stand ill equipped to tackle this stubborn parasite calling for renewed attack both on parasite and disease vectors in reducing parasite reservoir which is likely to persist for long.

6. Asymptomatic malaria

India is historically endemic for malaria with record of devastating epidemics in the pre-DDT era and varied population groups have been subject to repeated attacks of malarial bouts, resulting in acquired immunity and consequent build-up of asymptomatic sub-patent parasitemia in the endemic communities, serving as infectious reservoir for continued transmission. In India, the surveillance program is aimed at taking blood-smears from those who are either febrile (active surveillance) or presenting themselves (passive surveillance) for malaria diagnosis or treatment. There is no built-in mechanism to detect asymptomatic cases or even low-density/sub-patent parasitemia in the endemic communities. These infections may go undetected with conventional diagnostic techniques leaving them untreated, except for mass-blood surveys/mass-drug administrations that are conducted only to contain epidemics. Asymptomatic malaria is more abundant than assumed and have been reported in different endemic States of India; however, the extent and distribution vary corresponding to transmission intensities [74–77]. Asymptomatic parasitemia is often associated with gametocyte carriage and may persist infectious throughout the year to mosquito vectors. These gametocytes are unable to cause clinical symptoms of malaria, rather ensure the uninterrupted transmission of malaria in the presence of efficient vectors. Asymptomatic cases remain undetected and not accounted for disease incidence amounting to gross underestimates.

Asymptomatic malaria in India remains entrenched in low-socioeconomic groupings living in forest-fringe communities, particularly along inter-border areas (both inter-province and international borders), which are largely inaccessible (marred with insurgent activities), wherein healthcare infrastructure is meager or even non-existent. Such areas are 'hot-spots' for explosive disease outbreaks due to mixing/importation of drug-resistant strains associated with illegal migration more so in the northeast (the gateway to India) that shares wide international border with Myanmar, a member country of GMS (an epicenter of multi-drug resistant malaria) and other WHO SEAR countries. Asymptomatic malaria has been documented for both *P. falciparum* and *P. vivax*, yet the magnitude was much higher for the former parasite species, more so in the winter months/dry-season, for example, in north-eastern State of Assam, for the high-risk districts surveyed, it varied from 7.1 to 31.1% in *P. falciparum* and 0.6–6.1% in *P. vivax* [78]. But on average, 12.8% of afebrile subjects were positive for malaria

compared to 34.4% of those presenting with fever, and gametocyte carriage varied from 1.31 to 2.16%. Major bottleneck in eliminating asymptomatic malaria parasite is the absence of standard guidelines and diagnostic procedures aimed at targeting asymptomatic carriers. Malaria control program in practice is largely limited to the qualitative detection of parasite mostly in febrile human hosts and subsequently treating with suitable drugs; however, no attention is given on follow up for quantification of malaria parasite till radical cure. Moreover, a universally accepted parasite level for categorizing a patient as asymptomatic is also not available. Quantification and treating asymptomatic malaria has become increasingly important and relevant for disrupting transmission, requisite for achieving malaria elimination.

7. Cross-border malaria

India shares vast international border with Nepal, Bhutan and China to the North, Myanmar to the East, Bangladesh to the South and Pakistan to the West. Among these, borders with Myanmar and Bangladesh are of immediate concern for their high endemicity and common disease vectors and ecology in the adjoining vicinity on either side of the border [79, 80]. These border areas are porous for cross-migration and have high forest cover inhabited by indigenous tribes living in impoverished conditions. These communities have poor access to healthcare services and are at high-risk to disease outbreaks attributed to drug-resistant strains originating from the GMS countries. These populations are largely marginalized and just as reluctant to seek treatment amounting to unattended parasite reservoirs. Border with Myanmar in particular is believed to be the corridor for entry of drug-resistant strains to northeast India for onward spread. The detection of artemisinin-resistant malaria in closer proximity to Indian border is seen as threat for making its way to India and beyond, similar to the path that was followed by CQ-resistant malaria [48, 49]. Cross-border malaria transmission from neighboring endemic countries can be daunting task and has regional implications jeopardizing the elimination efforts [81, 82]. For example, most cases reported in Bhutan (a country that is heading for malaria elimination), are imported from adjoining districts of Assam seeking treatment on other side of the border. Labor migration across borders engaged in developmental projects is unstoppable for want of livelihood and there exists every possibility of re-entry of malaria given the similar vectors and ecology. It is important to characterize the imported malaria strains enabling interventions well in place and time to prevent re-establishment of local transmission in declared malaria-free territories [83]. Inter-country coordinated efforts are deemed essential to maintain vigil and strengthening border-posts (entry/exit doors) with capacity to detect and treat malaria in the migrant/itinerant labor force at the earliest available opportunity. In keeping the same mandate, India has joined hands with the Asia Pacific Malaria Elimination Network (APMEN) countries for shared experiences and coordinated action to achieve malaria elimination by 2030 [84]. Mitigating cross-border malaria should be accorded priority in context of malaria elimination.

8. Strengthening health systems

India has a well-structured vector-borne disease control program in place providing logistics support along with guidance and monitoring/evaluation services to malaria endemic States/

UTs [4, 7]. Utilizing the evidence-based intervention tools and large-scale implementation, India has registered notable decline in cases and malaria-attributable deaths in the preceding few years (**Figures 2** and **3**). Among these, roll-out of ACT for treatment of every single case of *P. falciparum* malaria, rapid diagnostics test (RDT) kits for on-the-spot diagnosis and large-scale provision of long-lasting insecticidal nets (LLINs) for vector control have resulted in rich dividends in reducing disease transmission in areas formerly intractable. Malaria threat is seen receding presenting an unprecedented opportunity for upscaling interventions in achieving universal coverage for populations at any risk making elimination an achievable target. However, the logistics requirement is huge for which increased funding from donors (both national and international agencies) and political commitment for sustained allocation of resources is of paramount importance for strengthening healthcare services reaching the outreach population groups for equitable access. A humble beginning has been made under National Health Mission (NHM), which envisages achievement of universal access to equitable, affordable and quality healthcare services both in urban and rural India [85]. Much needed disease surveillance in the country is further strengthened by Integrated Disease Surveillance Programme (IDSP) to detect early warning signals for impending disease outbreaks instituting interventions to thwart the disease onslaught and spread [86]. Both these establishments have helped the program immensely in strengthening laboratory services averting disease outbreaks, as well as human resource development in providing training to State surveillance officers, rapid response teams and other medical and paramedical staffs. However, continuing education program is need of the hour for upgrading skills to keep pace with the changing technologies as well as to fill the void due to attrition of skilled workforce. The induction of Accredited Social Health Activists (ASHA) have proven boon to the program ensuring door-to-door surveillance raising new levels of confidence in the poverty-stricken communities. Host of Non-Governmental Organizations (NGOs)/media coverage have increased the reach of services in remote/inaccessible areas helping combating illness and saving lives. Collectively, communities today stand better informed and clearly benefitted by increased awareness on disease prevention and control.

9. The way forward

In India, some States (Sikkim and Himachal Pradesh) and UTs (Lakshadweep, Daman & Diu and Puducherry) are already reporting <100 cases, while others recorded substantial decrease (>50%) in cases over past few years, for example, Assam and Karnataka (**Table 1**). Given the reducing transmission levels, malaria elimination at sub-national level is seemingly achievable. However, the emergence of artemisinin-resistant malaria and possible spread, coupled with multiple insecticide resistance in disease vectors (**Table 2**), could reverse the gains for which disease surveillance, monitoring and evaluation should be the corner-stone activity. *P. vivax* and asymptomatic malaria continue to be unattended and should be accorded priority for reducing parasite reservoir in the endemic communities. Priority should be accorded for strengthening healthcare services in high-risk States of eastern, central and north-eastern States helping mitigate disease onslaught and deter entry and spread of drug-resistant strains in India. Inter-sectoral linkages with research establishments and medical colleges for developing innovative newer interventions (possibly vaccines), assessing therapeutic efficacy of

antimalarials and upgradation of drug-treatment policy, human resource development and field-evaluation of newer technologies, including innovative vector control approaches, are vital before these are incorporated in the control program. Disease epidemiology is rapidly changing in the face of fast urbanization, deforestation and anomalous weather conditions opening new vistas, which must be watched for targeting interventions in place and time. Mosquito vectors are invading new territories, and adapting to altered ecology establishing outdoor transmission in response to strengthening insecticide interventions, which are largely based on indoor residual applications. We strongly advocate the judicious mix of technologies used in an integrated manner to overcome the challenges of outdoor transmission and growing insecticide resistance threatening the efficacy of present day intervention tools. There remains of scope of newer interventions in Indian geo-epidemiological conditions, namely, eave tubes, attractive sugar baits, nano-synthesized pesticides loaded with microbial- and plant-borne compounds, for trapping adult mosquito vectors and population reduction presently being put to field evaluation in African countries (Beier, personal communication). It is the high time to strengthen the entomological component at the State/Zonal level for monitoring vector densities and insecticide resistance targeting interventions averting impending disease outbreaks. Above all, educating communities and stakeholders on disease prevention and control should be the guiding principle for increased compliance and harmonious action. Increased allocation of resources (both from State and Central assistance), for ensuring universal coverage of interventions, should be given utmost priority in reducing parasite reservoir much below threshold density disrupting transmission [87]. It is time accelerating towards elimination and let there be no complacency at various echelons of operation for keeping disease at bay. Outside Africa, Southeast Asia is the largest contributor of cases and source of spread of drug-resistant malaria for which it is strongly advocated that larger share of global investments in this part of the World would go a long way in alleviating poverty and malaria. In summary, given the enormity of disease burden and myriad of issues, odds are all against, yet concerted efforts should be made in rendering malaria a thing of the past; together we can beat malaria.

Acknowledgements

We are thankful to Professor S. Manguin (Montpellier, France) for inviting us to contribute this article for this compilation, and the Directorate of National Vector Borne Disease Control Programme, Directorate of Health Services, Government of India for grant of permission to data access.

Acronyms

ACT	artemisinin-based combination therapy
AIM	action and investment to defeat malaria

http://dx.doi.org/10.5772/intechopen.77046

AL	artemether lumefantrine
API	annual parasite incidence
APLMA	Asia Pacific Leaders Malaria Alliance
APMEN	Asia-Pacific Malaria Elimination Network
ASHA	Accredited Social Health Activist
AS + SP	artesunate + sulfadoxine-pyrimethamine
CQ	chloroquine
DDT	dichloro-diphenyl-trichloroethane
G6PD	glucose-6-phosphate dehydrogenase
GMS	Greater Mekong Subregion
IDSP	Integrated Disease Surveillance Project
IRS	indoor residual spray
ITN	insecticide treated nets
ITS-2	internal transcribed spacer-2
LLIN	long-lasting insecticidal nets
NGO	Non-Governmental Organization
NHM	National Health Mission
NVBDCP	National Vector Borne Disease Control Programme
PCR	polymerase chain reaction
Pf	*Plasmodium falciparum*
Pfk 13	*Plasmodium falciparum kelch 13*
RBM	roll back malaria
RDT	rapid diagnostic test
r-DNA	ribosomal-DNA
SCAR	Sequence Characterized Amplified Region
SEAR	WHO Southeast Asia Region
SP	sulfadoxine-pyrimethamine
UT	Union Territory
WHO	World Health Organization

Author details

Sunil Dhiman[1]*, Vijay Veer[2] and Vas Dev[3]

*Address all correspondence to: sunildhiman81@gmail.com

1 Defence R&D Establishment (DRDE), Gwalior, Madhya Pradesh, India

2 Defence Research Laboratory, Tezpur, Assam, India

3 Senior Scientist (Retired), formerly with the National Institute of Malaria Research, New Delhi, India

References

[1] World Health Organization (WHO). World Malaria Report 2017. Geneva. Available from: http://www.who.int/malaria/world_malaria_report_2017 [Accessed: 20 February 2018]

[2] Newby G, Bennett A, Larson E, Cotter C, Shretta R, Phillips AA, Feachem RG. The path to eradication: A progress report on the malaria-eliminating countries. Lancet. 2016;**387**:1775-1784

[3] World Health Organization (WHO). Global Technical Strategy 2016-2030. Global Malaria Programme. Geneva: World Health Organization. 30 pp. Available from: http://www.who.int/malaria/areas/global_technical_strategy/en/ [Accessed: 20 February 2018]

[4] National Framework for Malaria Elimination in India 2016-2030. Directorate of National Vector Borne Disease Control Programme (NVBDCP). Ministry of Health & Family Welfare, Government of India. p. 43. Available from: http://www.nvbdcp.gov.in/malaria [Accessed: 20 February 2018]

[5] Sharma VP. Re-emergence of malaria in India. Indian Journal of Medical Research. 1996;**103**:26-45

[6] Sharma VP. Fighting malaria in India. Current Science. 1998;**75**:1127-1140

[7] National Vector Borne Disease Control Programme, Directorate General of Health Services, Ministry of Health & Family Welfare. Government of India. Malaria. Available from: http://www.nvbdcp.gov.in/malaria [Accessed: February 20, 2018]

[8] Das A, Anvikar AR, Cator LJ, Dhiman RC, Eapen A, Mishra N, Nagpal BN, Nanda N, Raghavendra K, Read AF, Sharma SK, Singh OP, Singh V, Sinnis P, Srivastava HC, Sullivan SA, Sutton PL, Thomas MB, Carlton JM, Valecha N. Malaria in India: The centre for the study of complex malaria in India. Acta Tropica. 2012;**121**:267-273

[9] Sharma VP. Continuing challenge of malaria in India. Current Science. 2012;**102**:678-682

[10] Yadav RS, Sharma VP, Ghosh SK, Kumar A. Quartan malaria—An investigation on the incidence of *Plasmodium malariae* in Bisra PHC, District Sundergarh, Orissa. Indian Journal of Malariology. 1990;**27**:85-94

[11] Dev V. *Plasmodium malariae*—A case of quartan malaria in Assam. Journal of Communicable Diseases. 2000;**32**:149-151

[12] Bharti PK, Chand SK, Singh MP, Mishra S, Shukla MM, Singh R, Singh N. Emergence of a new focus of *Plasmodium malariae* in forest villages of district Balaghat, Central India: Implications for the diagnosis of malaria and its control. Tropical Medicine & International Health. 2013;**18**:12-17

[13] Singh R, Jain V, Singh PP, Bharti PK, Thomas T, Basak S, Singh N. First report of detection and molecular confirmation of *Plasmodium ovale* from severe malaria cases in Central India. Tropical Medicine & International Health. 2013;**18**:1416-1420

[14] Prakash A, Mohapatra PK, Bhattacharya DR, Goswami BK, Mahanta J. *Plasmodium ovale:* First case report from Assam, India. Current Science. 2003;**84**:1187

[15] Jambulingam P, Mohapatra SS, Das LK, Das PK, Rajagopal PK. Detection of *Plasmodium ovale* in Koraput district of Orissa State. Indian Journal of Medical Research. 1989;**89**: 115-116

[16] Cox-Singh J. Zoonotic malaria: *Plasmodium knowlesi*, an emerging pathogen. Current Opinion in Infectious Diseases. 2012;**25**:530-536

[17] Dhingra N, Jha P, Sharma VP, Cohen AA, Jotker RM, Rodriguez PS, Bassani DG, Suraweera W, Laxminarayan R, Peto R. Adult and child mortality in India: A nationally representative mortality survey. Lancet. 2010;**376**:1768-1774

[18] Christopher SR. The fauna of British India, including Ceylon and Burma. Diptera, Vol. IV. Family Culicidae, Tribe Anophelini. London: Taylor and Francis; 1933. p. 371

[19] Das BP, Rajagopal R, Akiyama J. Pictorial key to the species of Indian anopheline mosquitoes. Journal of Pure and Applied Zoology. 1990;**2**:131-162

[20] Dev V, Sharma VP. The dominant mosquito vectors of human malaria in India. In: Manguin S, editor. *Anopheles* Mosquitoes: New Insights into Malaria Vectors. Croatia: InTech Open Access; 2013. pp. 239-271. DOI: 10.5772/55215

[21] Rao TR. The Anophelines of India. New Delhi: Indian Council of Medical Research; 1984. p. 596

[22] Nagpal BN, Sharma VP. Indian Anophelines. New Delhi: Oxford & IBH Publishing Co. Pvt. Ltd.; 1995. p. 416. ISBN: 81-204-0929-9

[23] Subbarao SK. Anopheline Species Complexes in South and South-East Asia. Geneva. World Health Organization (WHO); 2007. p. 102. ISBN: 978-92-9022-294-1

[24] Manguin S, Garros C, Dusfour I, Harbach RE, Coosemans M. Bionomics, taxonomy and distribution of the major vector taxa of *Anopheles* subgenus *Cellia* in Southeast Asia: An updated review. Infection, Genetics and Evolution. 2008;**8**:489-503

[25] Sinka ME, Bangs MJ, Manguin S, Chareonviriyaphap T, Patil AP, Temperley WH, Gething PW, Elyazar IRF, Kabaria CW, Harbach RE, Hay SI. The dominant *Anopheles* vectors of human malaria in the Asia-Pacific region: Occurrence data, distribution maps, and bionomics precis. Parasites & Vectors. 2011;**4**:89

[26] Sharma VP, Dev V. Biology and control of *Anopheles culicifacies* Giles 1901. Indian Journal of Medical Research. 2015;**141**:525-536

[27] Subbarao SK. The *Anopheles culicifacies* complex and control of malaria. Parasitology Today. 1988;**4**:72-75

[28] Bhuyan M, Das NG, Chakraborty BC, Talukdar PK, Sarkar PK, Das SC. Role of *Anopheles culicifacies* during an outbreak of malaria in Garubandha PHC, Assam. Journal of Communicable Diseases. 1997;**29**:243-246

[29] Saxena R, Nagpal BN, Singh VP, Srivastava A, Dev V, Sharma MC, Gupta HP, Tomar AS, Sharma S, Gupta SK. Impact of deforestation on known malaria vectors in Sonitpur district of Assam, India. Journal of Vector Borne Diseases. 2014;**51**:211-215

[30] Nasreen A, Nagpal BN, Kapoor N, Srivastava A, Gupta HP, Saxena R, Shamim A, Kumar V, Gupta SK, Singh VP, Dev V, Nanda N, Valecha N. Impact of ecological and climatic changes on vectors of malaria in four North-eastern States of India. Indian Journal of Ecology. 2016;**43**:1-15

[31] Singh OP, Raghavendra K, Nanda N, Mittal PK, Subbarao SK. Pyrethroid resistance in *Anopheles culicifacies* in Surat district Gujarat, West India. Current Science. 2002;**82**:547-550

[32] Raghavendra K, Verma V, Srivastava HC, Gunasekaran K, Sreehari U, Das AP. Persistence of DDT, malathion & deltamethrin resistance in *Anopheles culicifacies* after their sequential withdrawal from indoor residual spraying in Surat district, India. Indian Journal of Medical Research. 2010;**132**:260-264

[33] Sahu SS, Sahu K, Gunasekaran T, Vijayakumar JP. Triple insecticide resistance in *Anopheles culicifacies*: A practical impediment for malaria control in Odisha State, India. Indian Journal of Medical Research. 2015;**142**:59-63

[34] Nanda N, Yadav RS, Subbarao SK, Joshi H, Sharma VP. Studies on *Anopheles fluviatilis* and *Anopheles culicifacies* sibling species in relation to malaria in forested hilly and deforested riverine ecosystems in northern Orissa, India. Journal of American Mosquito Control Association. 2000;**16**:199-205

[35] Dev V, Manguin S. Biology, distribution and control of *Anopheles (Cellia) minimus* in the context of malaria transmission in northeastern India. Parasites & Vectors. 2016;**9**:585

[36] Dev V, Ansari MA, Hira CR, Barman K. An outbreak of *P. falciparum* malaria due to *Anopheles minimus* in Central Assam. Indian Journal of Malariology. 2001;**38**:32-38

[37] Dev V. *Anopheles minimus*: Its bionomics and role in the transmission of malaria in Assam, India. Bulletin of the World Health Organization. 1996;**74**:61-66

[38] Jambulingam P, Sahu SS, Manonmani A. Reappearance of *Anopheles minimus* in Singhbum hills of hills of East-Central India. Acta Tropica. 2005;**96**:31-35

[39] Prakash A, Walton C, Bhattacharyya DR, O'Loughlin SM, Mohapatra PK, Mahanta J. Molecular characterization and species identification of *Anopheles dirus* and *An. minimus* complexes in north-east India using r-DNA ITS-2. Acta Tropica. 2006;**100**:156-161

[40] Dutta P, Bhattacharyya DR, Khan SA, Sharma CK, Mahanta J. Feeding pattern of *Anopheles dirus*, the major vector of forest malaria in North-east India. Southeast Asian Journal of Tropical Medicine and Public Health. 1996;**27**:378-381

[41] Dutta P, Khan SA, Bhattacharyya DR, Khan AB, Sharma CK, Mahanta J. Studies on the breeding habitats of the vector mosquito *Anopheles baimaii* and its relationship to malaria incidence in Northeastern region of India. EcoHealth. 2010;**7**:498-506

[42] Prakash A, Mohapatra PK, Bhattacharyya DR, Sharma CK, Goswami BK, Hazarika NC, Mahanta J. Epidemiology of malaria outbreak (April/May 1999) in Titabor Primary Health Centre, district Jorhat (Assam). Indian Journal of Medical Research. 2000;**111**:121-126

[43] Das MK, Nagpal BN, Sharma VP. Mosquito fauna and breeding habitats of anophelines in Car Nicobar Island, India. Indian Journal of Malariology. 1998;**35**:197-205

[44] Sumodan PK, Kumar A, Yadav RS. Resting behaviour and malaria vector incrimination of *Anopheles stephensi* in Goa, India. Journal of the American Mosquito Control Association. 2004;**20**:317-318

[45] Prakash A, Bhattacharyya DR, Mohapatra PK, Mahanta J. Role of the prevalent *Anopheles* species in the transmission of *Plasmodium falciparum*, and *P. vivax* in Assam State and north-eastern India. Annals of Tropical Medicine & Parasitology. 2004;**98**:559-568

[46] Dhiman S, Bhola RK, Goswami D, Rabha B, Kumar D, Baruah I, Singh L. Polymerase chain reaction detection of human host preference and *Plasmodium* parasite infections in field collected potential malaria vector species. Pathogens and Global Health. 2012;**106**:177-180

[47] Shah NK, Dhillon GPS, Dash AP, Arora U, Meshnick SR, Valecha N. Antimalaria drug resistance of *Plasmodium falciparum* in India: Changes over space and time. Lancet Infectious Diseases. 2011;**11**:57-64

[48] Sehgal PN, Sharma MID, Sharma SL, Gogai S. Resistance to chloroquine in falciparum malaria in Assam State, India. Journal of Communicable Diseases. 1973;**5**:175-180

[49] Sharma VP. Status of drug-resistance in malaria in India. In: Mahajan RC, editor, Multi-drug Resistance in Emerging Re-emerging Diseases. Delhi: Narosa Publications. pp. 191-202

[50] Mohapatra PK, Namchoom NS, Prakash A, Bhattacharya DR, Goswami BK, Mahanta J. Therapeutic efficacy of anti-malarials in *Plasmodium falciparum* malaria in an Indo-Myanmar border area of Arunachal Pradesh. Indian Journal of Medical Research. 2003;**118**:71-76

[51] Dev V, Phookan S, Barman K. Therapeutic efficacies of antimalarial drugs in the treatment of uncomplicated *Plasmodium falciparum* malaria in Assam, north-eastern India. Annals of Tropical Medicine & Parasitology. 2003;**97**:783-791

[52] Dev V, Sharma VP. Hojai. Malaria transmission and disease burden in Assam: Challenges and opportunities. Journal of Parasitic Diseases. 2009;**33**:13-22

[53] Mishra N, Anvikar AR, K Shah NK, Kamal VK, Sharma SK, Srivastava HC, Das K, Pradhan K, Kumar H, Gupta YK, Gupta P, Dash AP, Valecha N. Prescription practices and availability of artemisinin monotherapy in India: Where do we stand? Malaria Journal. 2011;**10**:360

[54] Dev V, Biswas S, Joshi H, Prajapati SK, Valecha N, Dash AP. Safety and efficacy of aretsunate + sulfadoxine-pyrimethamine in the treatment of *Plasmodium falciparum* malaria in northeast India. Parassitologia. 2009;**51**:29-34

[55] Mishra N, Kaitholia K, Srivastava B, Shah NK, Narayan JP, Dev V, Phookan S, Anvikar AR, Rana R, Bharti RS, Sonal GS, Dhariwal AC, Valecha N. Declining efficacy of artesunate plus sulphadoxine-pyrimethamine in northeastern India. Malaria Journal. 2014;**13**:284

[56] Phyo AP, Nkhoma S, Stepniewska K, Ashley EA, Nair S, McGready R, ler Moo C, Al-Saai S, Dondorp AM, Lwin KM, Singhasivanon P, Day NP, White NJ, Anderson TJ, Nosten F. Emergence of artemisinin-resistant malaria on the western border of Thailand: A longitudinal study. Lancet. 2012;**379**:1960-1966

[57] Tun KM, Imwong M, Lwin KM, Win AA, Hlaing TM, Hlaing T, Lin K, Kyaw MP, Plewes K, Faiz MA, Dhorda M, Cheah PY, Pukrittayakamee S, Ashley EA, Anderson TJ, Nair S, McDew-White M, Flegg JA, Grist EP, Guerin P, Maude RJ, Smithuis F, Dondorp AM, Day NP, Nosten F, White NJ, Woodrow CJ. Spread of artemisinin-resistant *Plasmodium falciparum* in Myanmar: A cross-sectional survey of the *K13* molecular marker. Lancet Infectious Diseases. 2015;**15**(4):415-421

[58] Mishra N, Prajapati SK, Kaitholia K, Bharti RS, Srivastava B, Phookan S, Anvikar AR, Dev V, Sonal GS, Dhariwal AC, White NJ, Valecha N. Surveillance of artemisinin resistance in *Plasmodium falciparum* in India using the *kelch13* molecular marker. Antimicrobial Agents and Chemotherapy. 2015;**59**:2548-2553

[59] Joshi H, Prajapati SK, Verma A, Kang S, Carlton JM. *Plasmodium vivax* in India. Trends in Parasitology. 2008;**24**:228-235

[60] Shanks GD. Control and elimination of *Plasmodium vivax*. Advances in Parasitology. 2012;**80**:301-341

[61] Sharma VP, Dev V, Phookan S. Neglected *Plasmodium vivax* malaria in northeastern States of India. Indian Journal of Medical Research. 2015;**141**:546-555

[62] Srivastava HC, Yadav RS, Joshi H, Valecha N, Mallick PK, Prajapati SK, Dash AP. Therapeutic responses of *Plasmodium vivax* and *P. falciparum* to chloroquine, in an area of western India where *P. vivax* predominates. Annals of Tropical Medicine and Parasitology. 2008;**102**:471-480

[63] Rishikesh K, Kamath A, Hande MH, Vidyasagar S, Acharya RV, Acharya V, Belle J, Shastry AB, Saravu K. Therapeutic assessment of chloroquine-primaquine combined regimen in adult cohort of *Plasmodium vivax* malaria from a tertiary care hospital in southwestern India. Malaria Journal. 2015;**14**:310

[64] Nandy A, Addy M, Maji AK, Bandyopadhyay AK. Monitoring the chloroquine sensitivity of *Plasmodium vivax* from Calcutta and Orissa, India. Annals of Tropical Medicine and Parasitology. 2003;**97**:215-220

[65] Ganguly S, Saha P, Guha SK, Das S, Bera DK, Biswas A, Kundu PK, Saha B, Ray K, Maji AK. In vivo therapeutic efficacy of chloroquine alone or in combination with primaquine against *vivax* malaria in Kolkata, West Bengal, India, and polymorphism in *pvmdr1* and *pvcrt-o* genes. Antimicrobial Agents and Chemotherapy. 2013;**57**:1246-1251

[66] Valecha N, Joshi H, Eapen A, Ravinderan J, Kumar A, Prajapati SK, Ringwald P. Therapeutic efficacy of chloroquine in *Plasmodium vivax* from areas with different epidemiological patterns in India and their *Pvdhfr* gene mutation pattern. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2006;**100**:831-837

[67] Adak T, Sharma VP, Orlov VS. Studies on the *Plasmodium vivax* relapse pattern in Delhi, India. American Journal of Tropical Medicine and Hygiene. 1998;**59**:175-179

[68] Yadav RS, Ghosh SK. Radical curative efficacy of five-day regimen of primaquine for treatment of *Plasmodium vivax* malaria in India. Journal of Parasitology. 2002;**88**:1042-1044

[69] Rajgor DD, Gogtay NJ, Kadam VS, Kamtekar KD, Dalvi SS, Chogle AR, Aigal U, Bichile LS, Kshirsagar KKC. Efficacy of 14-day primaquine regimen in preventing relapses in patients with *Plasmodium vivax* malaria in Mumbai, India. Transactions Royal Society Tropical Medicine & Hygiene. 2003;**97**:438-440

[70] Valecha N, Pinto RG, Turner GD, Kumar A, Rodrigues S, Dubhashi NG, Rodrigues E, Banaulikar SS, Singh R, Dash AP, Baird JK. Histopathology of fatal respiratory distress caused by *Plasmodium vivax* malaria. American Journal of Tropical Medicine and Hygiene. 2009;**81**:75-62

[71] Kochar DK, Das A, Kochar SK, Saxena V, Sirohi P, Garg S, Kochar A, Khatri MP, Gupta V. Severe *Plasmodium vivax* malaria: A report on serial cases from Bikaner in Northwestern India. American Journal of Tropical Medicine & Hygiene. 2009;**80**:194-198

[72] Mishra VN, Singh DJ. Cerebral malaria by *Plasmodium vivax*. Journal of Association of Physicians of India. 1989;**37**:411

[73] Dua VK, Kar PK, Sharma VP. Chloroquine resistant 7. *Plasmodium vivax* malaria in India. Tropical Medicine & International Health. 1996;**1**:816-819

[74] Das NG, Dhiman S, Talukdar PK, Goswami D, Rabha B, Baruah I, Veer V. Role of asymptomatic carriers and weather variables in persistent transmission of malaria in an endemic district of Assam, India. Infection Ecology and Epidemiology. 2015;**5**:25442

[75] Ganguly S, Saha P, Guha SK, Biswas A, Kundu PK, Mali AK. High prevalence of asymptomatic malaria in a tribal population of Eastern India. Journal of Clinical Microbiology. 2013;**51**(5):1439-1444

[76] Chourasia MK, Raghavendra K, Bhatt RM, Swain DK, Meshram HM, Meshram JK, Suman S, Dubey V, Singh G, Prasad KM, Kleinschmidt I. Additional burden of

asymptomatic and sub-patent malaria infections during low transmission season in forested tribal villages in Chhattisgarh, India. Malaria Journal. 2017;**16**:320

[77] Laishram DD, Sutton PL, Nanda N, Sharma VL, Sobti RC, Carlton JM, Joshi H. The complexities of malaria disease manifestations with a focus on asymptomatic malaria. Malaria Journal. 2012;**11**:29

[78] Dev V, Phookan S, Sharma VP, Dash AP, Anand SP. Malaria parasite burden and treatment seeking behavior in ethnic communities of Assam, Northeastern India. Journal of Infection. 2006;**52**:131-139

[79] Dev V, Sangma BM, Dash AP. Persistent transmission of malaria in Garo hills of Meghalaya bordering Bangladesh, north-east India. Malaria Journal. 2010;**9**:263

[80] Dhiman S, Goswami D, Rabha B, Gopalakrishnan R, Baruah I, Singh L. Malaria epidemiology along Indo-Bangladesh border in Tripura State, India. The Southeast Asian Journal of Tropical Medicine and Public Health. 2010;**41**(6):1279-1289

[81] Wangdi K, Gatton ML, Kelly GC, Banwell C, Dev V, Clements AC. Malaria elimination in India and regional implications. Lancet Infectious Diseases. 2016;**16**:e214-e224

[82] Wangdi K, Gatton ML, Kelly GC, Clements AC. Cross-border malaria: A major obstacle for malaria elimination. Advances in Parasitology. 2015;**89**:79-102

[83] Dharmawardena P, Premaratne RG, Gunasekera WM, Hewawitarane M, Mendis K, Fernando D. Characterization of imported malaria, the largest threat to sustained malaria elimination from Sri Lanka. Malaria Journal. 2015;**14**:177

[84] Asia Pacific Malaria Elimination Network (APMEN). Elimination 2030—Working Together for Malaria Free Asia Pacific. Available from: http://www.apmen.org [Accessed: 20 February 2018]

[85] National Health Mission. Ministry of Health & Family Welfare, Government of India. Available from: http://www.nhm.gov.in [Accessed: 28 February 2018]

[86] Integrated Disease Surveillance Programme, National Centre for Disease Control, Directorate General of Health Services, Ministry of Health & Family Welfare, Government of India. Available from: http://www.idsp.nic.in [Accessed: 28 February 2018]

[87] Sharma VP. Battling malaria iceberg incorporating strategic reforms in achieving Millennium Development Goals & malaria elimination in India. Indian Journal of Medical Research. 2012;**136**:907-925

Chapter 12

Malaria Transmission in South America—Present Status and Prospects for Elimination

Jan Evelyn Conn, Maria Eugenia Grillet,
Margarita Correa and Maria Anice Mureb Sallum

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.69750

Abstract

Four countries (Brazil, Colombia, Peru, and Venezuela) together contributed ~80% of the 875,000 malaria cases reported in the Latin American region (2016). During the 10-year period (2005–2015) when global malaria incidence was dramatically reduced, Brazil and Colombia were an integral part of this trend, on track to meet the mid-term 2020 goal established by the World Health Organization. In Colombia, since 2015 at the cessation of a five-year globally funded malaria program, both incidence and proportion of *Plasmodium falciparum* infections have increased, mainly due to the budget constraints. Similarly, despite a strong record and major recognition for reducing malaria, in 2017, Brazil has seen a resurgence of malaria cases, but no increase in the proportion of *Plasmodium falciparum* to *P. vivax*. A globally funded malaria control program in Peru from 2005 to 2010 resulted in appreciable reduction in the annual parasitic incidence down to 1/1000 by 2011–2012, but soon after, the annual malaria incidence began to rise and by the end of 2017, there were 53,261 reported cases. To add to Venezuela's political and financial woes, malaria continues to increase, such that, 300,189 cases were reported by the end of week 42, 2017. The only rational pathway to malaria elimination is sustained nation-level financial support that does not fall prey to political vicissitudes.

Keywords: malaria, Brazil, Colombia, Peru, Venezuela, epidemiology, transmission landscape, vector biology, interventions

1. Introduction

Malaria transmission control and eventual elimination is one of the greatest worldwide challenges in public health. The World Health Organization (WHO) has established a

well-delineated and ambitious plan for control and elimination of the disease by 2030 [1], with a mid-term 2020 global target of reduction of at least 40% in malaria case incidence and malaria mortality rate. Significant advances were made in most of the endemic countries in Latin America, particularly from 2000 to 2015 [2], when the incidence of cases declined by 62% (1,181,095 in 2000 to 451,242 in 2015) and malaria-related deaths by 61.2% (410 to 159). The main strategies used have been rapid diagnosis, treatment with artemisinin-based combination therapy (ACT), indoor residual spraying (IRS), and insecticide-treated bednets (ITNs) or long-lasting insecticide-treated nets (LLINs) [3, 4].

However, malaria is still an important public health concern in the whole Neotropical region, more so during 2016, when a substantial increase in case incidence (875,000) was estimated [1]. Of the 18 endemic countries of Latin America, nine showed an increase in cases of more than 20% compared to 2015 [5], whereas the highest percentage increase (36%) of change in case incidence rate took place in 2014–2016. This was mainly due to the situation in Venezuela. In 2016, Venezuela (34.4%) and Brazil (18%) together accounted for more than 50% of the total reported cases, followed by Colombia (15.3%) and Peru (14.3%). According to the WHO report [1], malaria cases in Colombia nearly doubled in 2016 compared to 2015, despite an earlier reduction; in Peru cases have also been rising steadily since 2011, which has resulted in a loss of the gains achieved since 2000. In Venezuela, there has been a persistent increase in cases since 2000 and even more so since 2015 due to economic and political mismanagement [6] and Guyana recorded an increase in the proportion of *P. falciparum* (42%) to *P. vivax* cases (58%), the highest in South America [1, 7]. In contrast, Suriname observed declining malaria transmission trends to near-elimination levels through a rigorous control and education campaign, together with fortuitous flooding that destroyed populations of the primary malaria vector in the interior [8]. In 2016, Suriname reported only seven cases of *P. falciparum* and 69 of *P. vivax* [1].

The malaria landscape in Latin America consists of low transmission interspersed with diverse hot-spots where transmission is spatially and temporally focused [4, 9–11]. At a regional scale, reported malaria cases where the Annual Parasite Index (API) is >100 are concentrated in the municipalities of Bolívar, Delta Amacuro, and Sucre (Venezuela); Acre, Amapá, and Amazonas (Brazil); Amazonas, Antioquia, Chocó, and Vichada (Colombia); and Loreto (Peru) [1]. Nine countries reported zero local *P. falciparum* cases; Bolivia and Guatemala reported <10 cases. Twelve countries (Argentina, Paraguay, Costa Rica, Belize, Mexico, French Guiana, Suriname, Dominican Republic, Honduras, Bolivia, Haiti, and Brazil) are projected to have attained ≥40% reduction in case incidence by 2020, and five (El Salvador, Ecuador, Guatemala, Guyana, and Colombia) are on target for 20–40% reduction [1].

Currently, an estimated 102 million people are living in areas at risk of malaria transmission in Latin America, of which at least 28 million live in high-risk localities (>10 cases/1000 inhabitants). Most malaria cases in South America result from *P. vivax* (69%) infections, followed by *P. falciparum* (27%), and most occur in the Amazon rain forest. Colombia differs from most of its neighbors in having a large proportion of malaria transmission outside the Amazon,

such as the northwest, along the Pacific Coast and in the east, bordering Venezuela [12]. There has been renewed interest in understanding the biology, epidemiology, and the specific challenges of *P. vivax*, particularly since the decline of *P. falciparum* [4, 13–15]. *Plasmodium malariae*, responsible for <1% of cases in this region, is rarely considered in malaria reports, but is likely underestimated because it is difficult to diagnose using microscopy, has a slow growth rate, is generally asymptomatic in humans, and is considered less pathogenic compared with *P. falciparum* and *P. vivax* [2].

To stay on track and advance towards elimination, some of the main challenges in this region, identified by WHO (2017), are a lack of sustainable and predictable international and domestic funding, risks posed by political conflict in malaria endemic zones (e.g., Venezuela), environmental change and anomalous climate patterns [16–19], the emergence of parasite resistance to antimalarials [20–22], and insecticide resistance in mosquito vectors (reviewed in [23, 24]). Additional regional challenges to ongoing efforts to decrease malaria incidence include a significant rise in malaria cases in recent years in Venezuela [6], evidence of submicroscopic and asymptomatic infections [25], increases in peri-urban and gold mining-related malaria [26], and an upsurge in cases of *P. falciparum* in Colombia and Peru [1, 2].

Throughout this chapter, we adopt the new nomenclature proposed for the subfamily Anophelinae by Foster and collaborators [27]. Consequently, *Anopheles* (*Nyssorhynchus*) *darlingi* is herein referred to as *Nyssorhynchus darlingi*. The most important *Nyssorhynchus* vectors involved in this malaria landscape epidemiology are anthropophilic and/or opportunistic and ecologically/behaviorally variable [28]. Patterns of transmission vary regionally, depending on climate, biogeography, ecology, and anthropogenic activities. Transmission is exacerbated by deforestation for timber extraction, agricultural settlements, and mining and development of dams for hydroelectric projects. The creation of breeding sites (such as fish ponds, microdams, forest streams blocked by road construction, and mining pools) [29–31] and spatial mobility of humans, where there is little public health infrastructure (if any), also facilitate transmission in endemic malaria regions and beyond [32–34]. Factors such as infectivity of vectors by *P. vivax* or *P. falciparum* at levels rarely above 1% and heterogeneous entomological inoculation rates (EIRs) combined with low-to-moderate human blood indices (HBI) can result in high-risk for malaria transmission in certain habitats, often associated with anthropogenic change [29, 35, 36]. Inadequate housing protects no one and is a major impediment for reducing and ultimately ending human-mosquito contact [37].

The main objectives of this chapter are: (1) to evaluate the available intervention options that may be generalizable among the main vector species, (2) to determine scenarios where hotspot-specific vector biology and ecological interventions have the best prospects for success, and (3) to propose ways to test and combine current and novel interventions against the diversity of malaria vector species and habitats. This chapter focused on the four countries that together contributed the highest proportion (81.6%) of all reported malaria cases in Latin America in 2016, namely Venezuela, Brazil, Colombia, and Peru [1].

2. Current malaria situation

2.1. Brazil

Brazil had been reporting the highest number of malaria cases in Latin America for many years, but this shifted in 2015. Venezuela, with the growing economic and political crisis, had the dubious distinction of the highest estimated incidence of malaria in the region [38]. Recently, Brazil reported the second highest number of malaria cases (18%), down from 24% of cases in 2015 [1, 38]. Furthermore, Brazil recorded a 76.8% decrease in malaria incidence during 2000–2014 [4], even though transmission was observed to be ongoing in 808 municipalities in 2013 [13]. Nearly all malaria cases (99.5%) in Brazil are reported in the Amazon region, an enormous territory that covers an estimated 60% of Brazil and consists of nine States: Acre, Amazonas, Amapá, Maranhão, Mato Grosso, Pará, Rondônia, Roraima, and Tocantins [4]. The State with the most malaria cases and highest API since 2005 is Acre; the region within Acre with the highest-risk cluster is Vale do Juruá [39] including the municipalities of Cruzeiro do Sul, Mâncio Lima, and Rodrigues Alves that are persistent malaria hot-spots [40]. Other States with API >50 as of 2015 include Amapá, Amazonas, Pará, and Roraima (**Figure 1**).

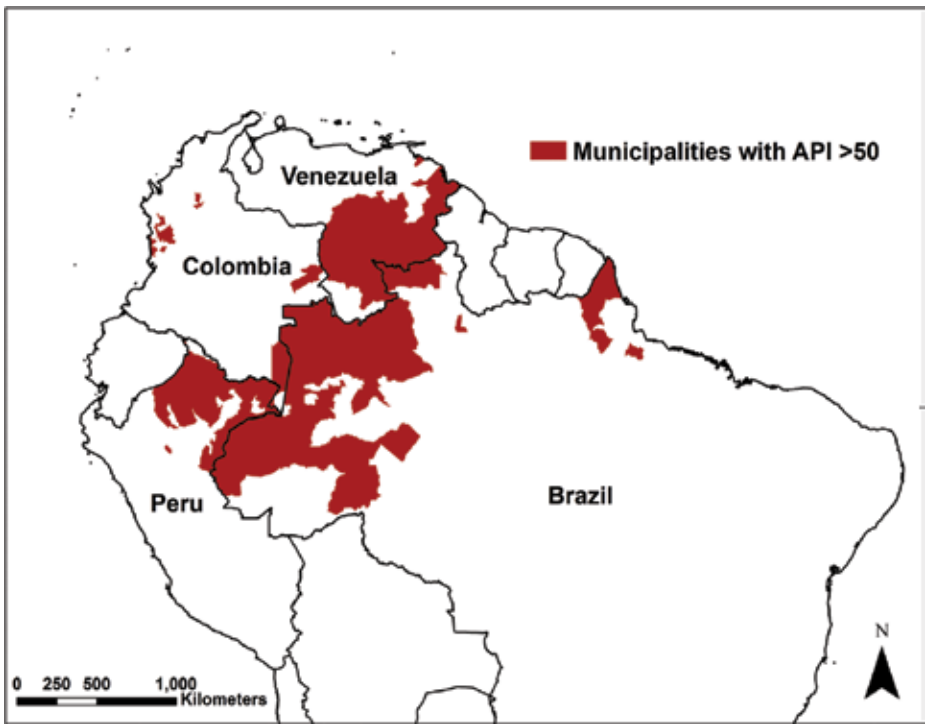


Figure 1. Geographical location of municipalities in Brazil, Colombia, Peru and Venezuela reporting Annual Parasite Index (API) >50 for data based on 2015 [38, 41].

Country	2014		2015		2016		2017	
	Number of malaria positive cases		Number of malaria positive cases		Number of malaria positive cases		Number of malaria positive cases	
	P. vivax	***P. falciparum***	***P. vivax***	***P. falciparum***	***P. vivax***	***P. falciparum***	***P. vivax***	***P. falciparum***
Brazil	117,009	22,234	122,743	15,445	110,343	13,829	172,876	21,017
Colombia	20,129	20,634	21,987	26,061	32,635	49,974	22,405	29,404
Peru	54,819	10,416	49,287	12,569	41,287	15,319	40,564	12,697
Venezuela	62,850	27,843	100,880	35,509	179,554	61,034	246,859	53,330

Note: Source of malaria case numbers 2014–2016 is WHO (2017); 2017 data are from individual Ministry of Health websites from each of the four countries.

Table 1. Number of malaria cases of ***Plasmodium vivax*** and ***P. falciparum*** in Brazil, Colombia, Peru, and Venezuela (2014–2017) [1, 42–45].

Across the Brazilian Amazon, the proportion of *P. falciparum* cases has been declining steadily for several years (**Table 1**), and in 2015, this parasite comprised approximately 11% of all cases, with *P. vivax* responsible for the remaining 89% [4]. In 2015, the Brazilian Ministry of Health (MOH) launched The Plan for Elimination of Malaria in Brazil, which focuses on the elimination of *P. falciparum* [46]. It is comprehensive, but substantial challenges remain: behavioral heterogeneity of the primary vector *Ny. darlingi* means that LLINs are only partially effective; most Amazonian housing structures do not meet criteria for routine IRS application; larviciding is most effective for accessible stagnant water bodies, e.g., fish ponds, especially those associated with hot-spots, but not effective for many natural water bodies, which may be difficult to identify and reach, or for streams and rivers with slow-moving water, which are typical *Ny. darlingi* habitats [47, 48]. By the end of 2016, *P. falciparum* still accounted for 11% of all malaria infections reported, and near the end of 2017, this was 10.8% (**Table 1**). In 2016–2017, Brazil was challenged by malaria resurgence, including in municipalities that were in the prevention phase and others with low malaria transmission. Furthermore, the total number of malaria cases in Brazil has increased from 105,057 cases during the period January 1 to December 31, 2016, to 154,343 cases during the period January 1 to October 31, 2017, an increase of 47% [42].

Some of the roadblocks in reducing and eliminating *P. vivax* include the high frequency of low-density *P. vivax* infections and the difficulty of their diagnosis by microscopy, particularly in areas approaching elimination and the persistence of liver stage hypnozoites that may be responsible for relapses [2, 4, 49]. Peri-urban and urban malaria transmission has been difficult to eliminate in cities such as Manaus (Amazonas State) and Cruzeiro do Sul (Acre State). In 2015, Manaus reported 7300 cases, most of which were acquired during work or other activities in neighboring municipalities, suggesting that interventions need to be focused on the mobile proportion of the human population [13]. Better transmission control is thought to lead to a lower *P. falciparum:P. vivax* ratio, reflecting the rapid and stable reduction of cases in urban settings compared with a lower and more heterogeneous reduction in rural and indigenous areas [13]. In a study based in and around the small cities of Mâncio Lima and Rodrigues Alves, Acre State; three development gradients, i.e., urban-rural, rural-riverine, and housing location were analyzed for multiple households. The lowest risk (OR = 0.55, 1.23–1.12) of

having a household with malaria was along the rural-riverine gradient, the most forested of the three; in contrast, the highest risk (OR = 1.92, 1.03–3.92) was along the urban-rural gradient, where urbanization was associated with roads, basic services, water treatment, electricity from a power grid, and less forest access [40]. This is an interesting and important finding, because malaria is so often assumed to be rural, associated with nearby water bodies and often linked to the forest environment. However, malaria risk is clearly linked with poverty, as another important finding of this study was that malaria risk is higher for poor individuals living in rural areas than those living in urban areas [40]. The poor in urban areas generally are exposed less frequently to biting, infected *Nyssorhynchus* and *Anopheles* mosquitoes, and have better access to health services than the poor in rural areas [40].

A valuable epidemiological tool was developed in 2010 to identify malaria outbreaks via an automated algorithm [50]. Use of the algorithm aimed to mobilize local control managers to act as rapidly as possible and they identified *P. vivax* as the primary causative pathogen for nearly all outbreaks, most of which occur in low or interrupted transmission areas where the likelihood of reintroduction is high. In 2014 and 2015, as many 112 and 111 outbreaks were identified, respectively [13]. The effectiveness of this tool has not been validated but it demonstrated usefulness in transmission reduction, which could lead to widespread adoption in Brazil.

2.2. Colombia

In 2016, Colombia recorded 83,227 cases, the third highest number in Latin America, which comprised 15.3% [1, 41]. Thus, malaria continues to be a serious public health problem and transmission is heterogeneous, presenting zones of low unstable transmission with endemic-epidemic patterns including various hot-spots [12]. From 2000 to 2014, Colombia made solid gains against malaria (50–75% reduction in cases), mainly due to interventions such as diagnostic health posts and vector control. However, these gains have been undermined since the Colombia Malaria Project ended in 2015; case numbers doubled between 2015 and 2016 [41].

For the past decade, *P. vivax* accounted for approximately 70% of reported cases, with the remainder exclusively *P. falciparum* [12]. However, in 2016, this proportion shifted alarmingly in favor of *P. falciparum* constituting 60% of reported cases [1, 41, 43, 51, 52]. This parasite species predominates along the Pacific Coast, one of the endemic hot-spots, where there is a high occurrence of Colombian Afro-descendant individuals who are Duffy-negative [53].

Taken together, eight Colombia Departments accounted for 90.8% of all the 2016 noncomplicated malaria cases. These are Chocó, Nariño, and Cauca (western Colombia), Antioquia and Córdoba (northwestern), Guainía and Vichada (central-eastern along the border with Venezuela), and Amazonas (southeastern). Among various Departments, Chocó was worst affected and contributed 53% of all reported cases during 2014–2015 [38]. Nevertheless, up to the 49th epidemiologic week of 2017, Chocó registered a lower proportion of cases (30.7% [43]) compared with the same period in 2015, because several health posts ceased reporting due to national, State, and municipal budgetary constraints with the closure of the Colombia Malaria Project (2015). In the Departments of Arauca and Guajira in eastern Colombia, bordering Venezuela, there was an increase in cases compared to the average number registered during 2012–2016. Of the 860 non-autochthonous cases reported overall, most (76.7%) were *P. vivax* and nearly all (93.1%) were from Venezuelan patients [43].

Malaria transmission in Colombia has mainly been rural, but a recent study indicated that between 2008 and 2012, urban and peri-urban malaria transmission described as endemic, unstable and of low intensity, occurred in many municipalities in the Pacific Coast and a few in eastern Colombia [53]. However, the authors indicated that a serious limitation was not having a clear consensus on the definition of urban and peri-urban. Nevertheless, there appears to be a trend of decreasing rural and a concurrent progressive increase of urban malaria. Possible explanations of this phenomenon are human migration resulting from ongoing-armed conflict, illegal mining, or illicit crop activities, and the movement of asymptomatic carriers.

In western and northwestern Colombia, with the existing healthcare and disease prevention programs, gold-mining (mostly illegal) has played an important role in the maintenance of malaria as shown by public health surveillance data based on 2010–2013 [26]. This study showed that gold-mining was predominant in seven Colombian Departments that contributed 89.3% (270,753 cases) of the national malaria cases during this period; of which, 31.6% of the cases were from mining areas. The worst of these were located in Antioquia, Córdoba, and Buenaventura municipalities in Valle del Cauca.

Vector control interventions in Colombia rely on the use of insecticides, larvicides, and ITNs [54] to reduce human-vector contact. Some research groups focused on mosquito vector biology aiming to provide baseline information for the development and implementation of appropriate vector control interventions by the evaluation of ecology and biology of vector species, improved species identification, spatio-temporal distribution, biting behavior and preferences, and natural infection by *Plasmodium* [55–60].

A comprehensive early warning system, as part of the Integrated National Adaptation Pilot project and the Integrated Surveillance and Control System at the municipality level, has been implemented in four pilot sites in Colombia, where it showed promise, providing new data on malaria incidence and seasonality, vector species presence and abundance, entomological indices and feeding frequencies, climate variables, human population information, and some data on vector control activities [61]. Limitations that remained included the scarcity and difficulty of accessing cultural qualitative and quantitative factors and the limited preparedness of State and municipal health authorities to implement malaria dynamic models [61].

2.3. Peru

The most recent WHO data showed that Peru reported an estimated 14.3% of all malaria cases in the region for 2016; this amounted to 56,606 cases, of which 73% were *P. vivax* [1]. This estimate has been rising fairly steadily since 2010–2011, ever since cessation of the international financial support provided by the Global Fund Malaria Project "PAMFRO" that had successfully reduced the annual incidence to <1 case/1000 inhabitants for 2010 and 2011 [62]. After 2011, there was a surprisingly rapid malaria resurgence, hypothesized to be due to: (1) budgetary constraints; (2) the perception that malaria was under control; and (3) a concurrent regional dengue epidemic in Loreto [63]. Transmission may have been worsened due to the historic Loreto flood of 2011–2012 that inundated and damaged many riverine communities [62]. During the period between 2002 and 2013, 79% of cases were *P. vivax* and 21% *P. falciparum* [11]. A worrisome trend has been the recent increase in the proportion of *P. falciparum* in 2016 (27%) and 2017 (24%) (**Table 1**).

Numerous malaria endemic riverine and highway villages exist near the Iquitos-Nauta highway and along the Itaya and Nanay Rivers to the south and west of Iquitos. Inhabitants of two of these villages, Lupuna and Cahuide, took part in a cross-sectional survey in January 2013 (off-peak malaria season), with census data taken in mid-2012. One substantial determination was that prevalence of *P. vivax* and *P. falciparum* was many times higher by packed red blood cell (PRBC)-PCR compared with microscopy (25 vs. 3.6% and 5 vs. 0.2%, respectively) [33]. Routine surveillance, using the more sensitive PCR detection method and treatment that includes individuals with very low parasitemia who maintain local transmission even during the off-peak malaria season, acting as potential parasite reservoirs, could be an effective addition to prompt diagnosis and treatment to further reduce malaria regionally. In addition, the overall heterogeneous distribution patterns of *P. vivax* and *P. falciparum* differ sharply in Lupuna and Cahuide, i.e., *P. vivax* is transmitted more locally within villages and *P. falciparum* is more often acquired at a distance, related to occupation, and transported on a regional basis [33].

Most years, between 90 and 95% of all malaria cases and 99.4% of *P. falciparum* are reported from Loreto Department, in northern Amazonian Peru [64]. In 2017, this amounted to 50,702 cases (96.2% of those across Peru); there were also small foci in Amazonas State (822 cases in 2017), west of Loreto, and in San Martin (415 cases in 2017), south of Loreto [64]. There was a serious *P. vivax* outbreak in the gold-mining region of the southern Amazon, in Madre de Dios and neighboring Ucayali until about 2011 [65], but only 6 cases were reported in Madre de Dios and 79 in Ucayali in 2017 [64]. In Tumbes and Piura, along the northwestern coast, malaria has greatly diminished and what remains is epidemic, sporadic, and peri-urban, likely the result of reintroduction [64, 66–68].

Loreto Department comprises an estimated 30% of Peruvian territory and there are about one million inhabitants [69]. Malaria transmission is highly seasonal, coinciding mainly with the heavy rainy season (January to June) and Andean snowmelt, that together increase river levels up to 10 m, causing major fluctuations in the abundance of the main regional malaria vector *Ny. darlingi* [70, 71]. Most malaria infections are found in rural and remote villages whose inhabitants live along the Amazon River, and its many tributaries, in enclosed or partially enclosed wooden houses [62, 72]. There has been increasing recognition, beginning with a ground-breaking study [73], of hyperendemic foci linked to occupational activities (such as timber extraction, farming, and charcoal production) and human mobility [33].

2.4. Venezuela

Whereas the continent achieved a significant decline in malaria-related morbidity (62%) and mortality (61%) between 2000 and 2015 as part of the implementation of the Global Malaria Action Plan 2008–2015 [41], Venezuela, in contrast, was the alarming exception in the region, displaying an unprecedented 365% increase in malaria cases between 2000 and 2015 [6]. In 2016 alone, 240,588 malaria cases were officially reported [1], whereas by the end of 2017, this number had increased to 300,189 total cases [45]. Astonishingly, the number of cases reported in 2017 in Venezuela is higher than that reported in the last 29 years (1988–2016) [74].

Economic and political mismanagement have precipitated a general collapse of Venezuela's health system creating an ongoing humanitarian crisis with severe social consequences [75, 76]. Consequently, a malaria epidemic has been fueled by financial constraints that

prevented the procurement of malaria commodities (insecticides, drugs, diagnostic supplies, mosquito nets, etc.), epidemiological surveillance, reporting activities, vector-control and disease-treatment efforts, high internal human migration associated with illegal gold mining, and underlying malnutrition due to a general lack of provision and implementation of services. In 2016, *P. vivax* malaria accounted for 76% of all cases, followed by *P. falciparum* (18%), *P. malariae* (<1%), and *P. vivax*/*P. falciparum* mixed (6%) infections [1].

Although *P. falciparum* malaria occurs mostly in the lowland rain forests of the Venezuelan Guayana region, *P. vivax* malaria is endemic in the coastal plains and savannas, as well as the lowland Guayana forests [17]. Currently, an estimated 80% of malaria in Venezuela is associated with gold mining areas in the forest ecosystem of the southeastern region, where local transmission is maintained in few but persistent disease hot-spots by *Ny. darlingi* and *Ny. albitarsis* s.l. ([77–79]; Grillet unpublished). Infection Rates (IR) of *Ny. albitarsis* s.l. and *Ny. darlingi* collected during 2009–2012 in Sifontes, Bolivar State, were very high: 5.4 and 4.0%, respectively [80]. Gold mining extraction activities substantially reduce forest vegetation cover, which seems to favor aquatic vector habitat production, especially for *Ny. albitarsis* s.l. ([79]; Grillet unpublished). Mining activities in turn result in highly mobile human populations that migrate in search of jobs, working, and sleeping outdoors, exposed to continuous mosquito biting for long periods of time. Many of these economic migrants are previously unexposed to *Plasmodium* and some of them return to nonendemic malaria regions, e.g., near the capital Caracas, with circulating gametocytes, reintroducing *Plasmodium* to areas where malaria had been eliminated previously [81]. Although, most disease transmission in Venezuela has been rural, recent observations suggest a significant change in the landscape epidemiology of malaria since 2013—urban and peri-urban malaria transmission are now associated with some cities close to Caracas [Grillet unpublished]. Finally, case spillover has overloaded frontier health care infrastructure in Brazil and Colombia where in 2016, 78 and 81%, respectively, of imported malaria cases originated from Venezuela [2]. The continued upsurge of malaria in Venezuela threatens to become uncontrollable, jeopardizing the hard-won gains in the Americas' elimination agenda and global malaria targets.

For decades, Venezuela was a leader in vector control and public health policies in Latin America, especially after being the first WHO-certified country to eliminate malaria in much of its territory in 1961 as a result of a very aggressive, vertical malaria control campaign [82]. This campaign consisted of the interruption of malaria transmission through systematic and integrative infection and vector control. Additionally, the program included the detailed knowledge of malaria microepidemiology (at local level, case management, consisting of diagnosis, patient treatment, and mass drug administration), mapping malaria cases, malaria health information system updated weekly, community participation through volunteer community health workers, application of larvicides, and sanitary engineering such as housing improvement and water management. This public health success helped to galvanize interest in global elimination [82]. The Venezuelan approach for malaria elimination in the past differs little from current prevention, control and elimination, except that it was implemented in an epidemiological landscape where insecticide and parasite resistance were absent, political will was significant, and government support was very strong. Vector control and case prevention require long-term investment and sustainability without which it is difficult to envision elimination as a viable outcome.

3. The main malaria vectors

3.1. *Nyssorhynchus darlingi*

The most widespread and dominant malaria vector in the Amazon region is *Nyssorhynchus darlingi* (**Figure 2**) [27, 28, 84, 86]. Localities where *Ny. darlingi* has been formally incriminated by ELISA or other molecular techniques are shown in **Figure 3**, although the full distribution of *Ny. darlingi* extends from southern Mexico through northern Argentina [84]. This species shares several characteristics with invasive species (e.g., *Aedes albopictus*) and other primary malaria vectors such as *An. gambiae* s.s., including fast growth, phenotypic plasticity, rapid reproduction, moderate-high dispersal ability, ecological competence, and association with humans [28, 104–106]. In Loreto Department, Peru, since *Ny. darlingi* reinvaded, or re-expanded its range into the peri-Iquitos area about 1998 [107], it has spread along numerous Peruvian river drainages to the north and west [70, 108]. In Brazil and Peru, it is ranked the number one vector [4, 29, 109]; in Colombia, it is one of three main vectors, the other two being *Ny. albimanus* and *Ny. nuneztovari* [87, 110, 111]; and in Venezuela, it shares top billing with *Ny. albitarsis* s.l. [77, 78, 80]. A recent review highlights the very low insecticide resistance in *Ny. darlingi* detected in the Neotropics, i.e., one population in Choco, western Colombia is resistant to DDT, permethrin, lambda-cyhalothrin, and deltamethrin [23].

The distribution in Brazil includes the lowlands of the Amazonian biome, the Cerrado, and the southern Atlantic forest [84, 112, 113]. *Nyssorhynchus darlingi* is adaptable and flexible in its behavior: exophagic and endophagic; anthropophilic and opportunistic; though generally exophilic [28, 71, 97, 114]. The standard entomological indices range widely across its distribution [71, 80, 96, 97, 103, 114]. One frequently recognized characteristic of *Ny. darlingi* is the speed with which it colonizes deforested Amazonian patches and a variety of anthropogenic water bodies such as gold mining pools, brick-making depressions, wells, cisterns, and fishponds, as well as natural breeding site types linked to rivers or flooded forest [29, 60, 111, 115, 116]. Its adaptation to novel environments may lead to increased vectorial capacity and survival, as well as greater risk of malaria transmission [117, 118]. The most likely drivers of *Ny. darlingi* divergence at a macro-geographic scale, across its broad distribution, are biogeographic or geographic boundaries and Pleistocene environmental changes [113, 119]. At a regional scale, isolation-by-distance has been shown to influence population structure [120], whereas at a micro-geographic scale, current local environmental conditions have a marked effect [113, 119–122].

In Colombia, *Ny. darlingi* is distributed on either side of the Andes mountain range in lowland regions characterized by biogeographical and ecological heterogeneity [111]. West of the Andes, in the Urabá-Bajo Cauca and Alto Sinú (UCS) region, *Ny. darlingi* is the most common *Nyssorhynchus* species, exhibits endo and exophagy, is infected with *P. vivax*, and maintains transmission even at low abundance [60, 87, 111]. In most localities included in this study, the peak biting activity of *Ny. darlingi* was after 20:00 or 21:00 h when people conduct indoor and/or outdoor activities increasing the risk of vector-human contact. East of the Andes [111] and in southern Colombia, peak biting activity is at sunset [92] when no one is protected under ITNs. The dominance of *Ny. darlingi* in most of northwestern Colombian localities seems to be favored by ecological perturbations resulting from various human activities, such as alluvial mining, livestock, small-scale rice

http://dx.doi.org/10.5772/intechopen.69750

Figure 2. Distribution of *Nyssorhynchus darlingi* (denoted by white dots). Map made in Google Earth Pro [83] using data from the Malaria Atlas Project [84, 85].

Figure 3. Localities (denoted by yellow dots) where the primary malaria vector *Nyssorhynchus darlingi* has been reported infected with *Plasmodium vivax* or *Plasmodium falciparum* incriminated by molecular methods during 2005 to 2017 [8, 35, 59, 60, 73, 80, 87–103]. Map made in Google Earth Pro [83, 84].

production, and forest fragment landscapes [60]. Vector control strategies that include ITNs are recommended for containment of *Ny. darlingi* populations [60, 87, 111, 123].

Studies on the genetic structure of *Ny. darlingi* in Colombia have shown that at the micro-geographic scale, in northwestern Colombia, *Ny. darlingi* is characterized by low genetic differentiation and high gene flow [123, 124]. The environmental heterogeneity that is a hallmark of this malaria endemic region does not reach a threshold to impact the population structure of *Ny. darlingi* [124]. A comprehensive genetic study that evaluated *Ny. darlingi* throughout its distribution in Colombia found that at a macro-geographic scale, differentiation into two main groups, west and east of the Andes, was most likely influenced by the Andes; at a micro-geographic scale, differentiation was partly the result of isolation by resistance, probably due to ecological differences, with significant impact on its population structure. In the current malaria scenario in Colombia and considering that Anophelinae mosquitoes adapt to climate and environmental changes, population studies should contribute to the development and implementation of vector control interventions and monitor their effectiveness in important malaria endemic regions of Colombia where *Ny. darlingi* maintains transmission.

Within Peru, only in the peri-Iquitos region of Loreto Department has the genetic structure of *Ny. darlingi* been evaluated, initially using Random Amplified Polymorphic DNA-PCR, that detected substantial homogeneity [125]. When populations from highway and riverine habitats were compared over a decade later using microsatellite markers, two highly admixed subpopulations were detected in each of nine villages [35]. The second major finding was that the 2012–2014 population of *Ny. darlingi* [35] had replaced that of the 2006 [126] and both of these subpopulations had the signature of a recent expansion. The source of the replacement population is unknown, although a broad analysis of microsatellite data across South America suggests that it most likely comes from western Brazil [35].

In Venezuela, *Ny. darlingi* is found in the lowland tropical rainforest, in the southern part of the country (Amazonas and Bolivar States), the piedmont ecoregion characterized by high rainfall and tropical forests in Trujillo State, western Venezuela, and in the llanos in central-western Venezuela, a subregion of the savanna ecoregion [127]. There is very little population structure in Venezuelan *Ny. darlingi* based on isozymes, RAPDs, ITS2 sequences [86], but more sensitive molecular markers, or whole genomes, might detect micro-geographic differences among the diverse ecoregions.

3.2. *Nyssorhynchus albimanus*

Nyssorhynchus albimanus is a malaria vector [27] characterized by ecological adaptability and a widespread, mostly coastal lowland, Neotropical distribution (**Figure 4**) [128]. Its presence usually coincides with areas that experience two annual rainy seasons, precipitation greater than 1000 mm, high relative humidity and a monthly variation in temperature between 22° and 29°C [127, 129, 130]. Despite its absence in Brazil, in Colombia, *Ny. albimanus* constitutes one of the main vectors in rural and peri-urban areas below 400–500 m, predominating along the Colombian Caribbean and Pacific Coasts and on the Island of San Andres [130–133]. These regions have different levels of *Plasmodium* transmission and the importance of *Ny. albimanus* also differs [133]. The Pacific is a humid tropical forest and one of the rainiest regions globally; in contrast, the Caribbean tropical forest is drier and hotter [134]. Malaria cases increase in

http://dx.doi.org/10.5772/intechopen.69750

Figure 4. Distribution of *Nyssorhynchus albimanus* (denoted by white dots). Map made in Google Earth Pro [83] using data from the Malaria Atlas Project [84, 85].

relation to ENSO patterns and cycles, particularly those transmitted by *Ny. albimanus* along the Pacific Coast of Colombia [61].

The availability of suitable breeding sites determines distribution and abundance of *Ny. albimanus* [130], a species that can thrive in fresh and brackish water, natural habitats (animal tracks, lakes, streams, and wells), and anthropogenic ones (rice fields, lagoons, and mining excavations, among others) [130, 135]. Behaviorally, *Ny. albimanus* is mainly zoophilic, exophagic, and exophilic; yet it can be anthropophilic, depending on local circumstances and abundance [130]. It is also known to be endophagic in local malaria hot-spots along the Pacific Coast, i.e., the urban sector of Buenaventura. The main outdoor biting time is 19:00–23:00 h, when many inhabitants are outside, and therefore exposed to biting and *Plasmodium* transmission [130]. As a vector of *P. falciparum* and *P. vivax*, *Ny. albimanus* has been incriminated in the Pacific region [133] and a new species from the southern Pacific Coast, *Ny. albimanus* B, detected by mitochondrial *COI* sequences, was infected with *P. falciparum* [57]. Despite the high abundance of *Ny. albimanus* in the Caribbean region, no infected specimens were detected [136].

Population genetic studies of *Ny. albimanus* in Colombia confirm its status of a single taxon throughout its distribution, with low population structuring and little genetic differentiation [137]. Two broader studies that included samples from Nicaragua to Ecuador, both nuclear and mitochondrial markers, found evidence for geographic structuring [138] and population contraction across Panama followed by an east-west expansion [139]. Under the hypothesis that malaria vectors are exposed to control pressures and environmental alterations that may lead to genotypic and phenotypic variation, genetic (microsatellite) and phenotypic (wing trait) data

in populations of *Ny. albimanus* from the Pacific and Caribbean, despite a significant effect of environmental factors on wing traits, support a regional metapopulation of *Ny. albimanus* [132].

In Peru, *Ny. albimanus* is restricted to the Tumbes region of the northern coast, where it transmits *P. vivax* at the end of the hot rainy season. Local insecticide application, mostly in rice fields, lead to extreme levels of insecticide resistance [23]. A series of meetings and decisions between southern Ecuador and northern Peru health personnel resulted in a highly successful control program that employed a wide array of interventions such that autochthonous malaria was eliminated in El Oro, Ecuador in 2011 and in Tumbes, Peru in 2012 [135].

In Venezuela, *Ny. albimanus* is distributed along the coast and the margins of Valencia Lake, south of Maracay, although it does not appear to contribute to malaria transmission locally [127, 140]. It was found to be as abundant as the known coastal vector *Ny. aquasalis* in Aragua State, northcentral Venezuela, where both species had similar peak biting times during the early evening and were collected biting outdoors [141].

3.3. *Nyssorhynchus albitarsis* s.l.

The *Albitarsis* Complex comprises at least eight species [142] that extend across Central and South America and some Caribbean islands (**Figure 5**). The difficulty of their morphological differentiation complicates recognition of their role(s) in malaria transmission, an important aspect for the implementation of targeted and effective vector control strategies [143]. Three species are known vectors: *Ny. deaneorum*, *Ny. janconnae*, and *Ny. marajoara.* The latter is important regionally in *Plasmodium* transmission in central and eastern Brazil, where its distribution includes Amapá, Mato Grosso, Pará, and Rôndonia [84, 142]. Its role in transmission rivals that of *Ny. darlingi* in some habitat types such as peri-urban Macapá City, Amapá [144] and along the Rio Matapi, Amapá [88]. An entomological survey during an outbreak in western French Guiana, in an illegal gold mining area, detected a high *P. vivax* infectivity rate (6.4%) in specimens of *Ny. marajoara* [99]. An ecological niche model, based on current and future (2070), distributions of *P. falciparum*, *Ny. darlingi*, all species of the *Albitarsis* Complex, climate, biome and topography, projected that, whereas climate change would reduce suitable habitat for *Ny. darlingi,* both *Ny. marajoara* and *Ny. deaneorum* are expected to expand southward, thereby increasing their likely role in *P. falciparum* transmission by the projected date of 2070 [19].

In Colombia, only a few species, in particular *Ny. marajoara,* have been identified morphologically in this complex [90, 145–147] and implicated in urban transmission [145]. This species is thought to be widespread in this country [110]. However, a detailed analysis of many Colombian specimens, identified molecularly, did not detect any individual *Ny. marajoara* [147], in agreement with Ruiz-Lopez et al. [142], whose study indicated that *Ny. marajoara* is restricted to the central-eastern and western regions of Brazil and is most likely absent in Colombia. Further studies need to be done on this vector to better frame its geographic distribution.

Albitarsis Complex species appear to be uncommon in Peru but this could reflect a general lack of *Nyssorhynchus* taxon sampling and molecular identification, particularly outside the Amazon region of Loreto.

Although there are several published reports of *Ny. marajoara* as an important regional malaria vector in Bolivar State, Venezuela, along with *Ny. darlingi* [77, 78, 148], a different species,

http://dx.doi.org/10.5772/intechopen.69750

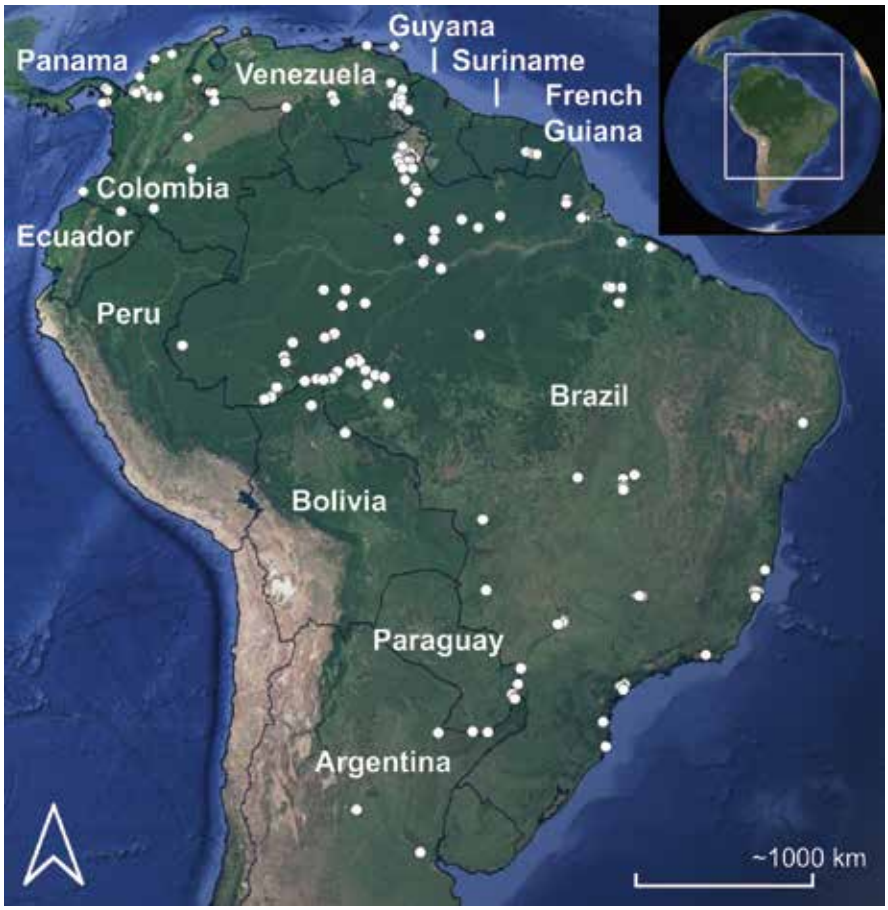


Figure 5. Distribution of the *Albitarsis* Complex (denoted by white dots). Map made in Google Earth Pro [83] using data from the Malaria Atlas Project [84, 85].

Ny. albitarsis F in the *Albitarsis* Complex [142], was identified from the Caura Basin, Bolivar State [96]. In the most recent publication from the malaria hot-spot Sifontes, the specimens infected by *Plasmodium* are referred to only as *Ny. albitarsis* s.l. [80]. Hopefully, the correct species identities and distribution will soon be determined in this very crucial Venezuelan hot-spot.

3.4. The *Nuneztovari* Complex

The *Nuneztovari* Complex, extending through much of northern South America, includes *Ny. nuneztovari* (**Figure 6**), *Nyssorhynchus dunhami*, and *Nyssorhynchus goeldii* [149]. Like the *Albitarsis* Complex, species in the *Nuneztovari* Complex are similar morphologically and difficult to identify accurately. Scarpassa and collaborators [150] presented strong molecular evidence that additional species exist in Brazil and briefly reviewed the role of *Ny. nuneztovari* as a malaria vector in five Amazonian States. *Nyssorhynchus nuneztovari* is restricted to Colombia and western Venezuela, *Ny. goeldii* to Amazonian Brazil, and *Ny. dunhami* to central-western Brazil, Colombia and Amazonian Peru [71, 150]. It is difficult to evaluate the identification of

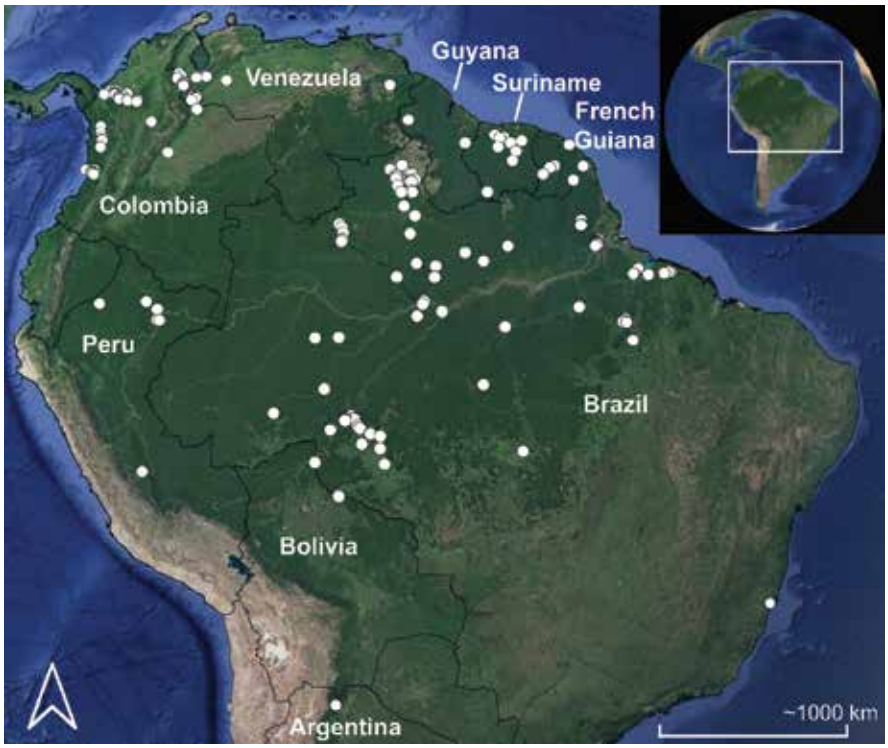


Figure 6. Distribution of the Nuneztovari Complex (denoted by white dots). Map made in Google Earth Pro [83] using data from the Malaria Atlas Project [84, 85].

these species in earlier publications, because distributions of *Ny. nuneztovari* and *Ny. goeldii* overlap, as do those of *Ny. goeldii* and *Ny. dunhami* [149, 150].

In Colombia, *Ny. nuneztovari* is an important malaria vector on both sides of the Andes presenting morphological, behavioral, and genetic heterogeneity throughout the country [151, 152]. In northwestern Colombia, it was found to be the most prevalent species, confirming an earlier study [58], and showed endo and exophagic behavior [152]. It was naturally infected by *P. vivax* VK247 [60, 87], positive for *P. vivax* VK210, and VK247 in the Pacific Coast [103]. In eastern Colombia, there are no recent reports of *Ny. nuneztovari* infected with *Plasmodium*, but more importantly, there is a lack of investigation of malaria outbreaks along the frontier with Venezuela with no record of species identification and vector incrimination. Because of the humanitarian crisis in Venezuela, the numbers of malaria cases have increased dramatically since 2010 [1, 153]. In the most recent study of Colombian *Ny. nuneztovari*, it was reported to be abundant and dominant in localities where anthropogenic activities such as livestock, fish-farming, and small to medium-scale agriculture were common, attributed to its adaptability to environmentally impacted habitats [152]. Common larval habitats were artificial fishponds and wetlands, particularly in the west and northwest [58, 152].

Regionally, this species shows the highest biting activity after 20:00 h, which suggests high transmission risk when people are at home, but not necessarily under nets. ITNs could be one component of an effective vector control intervention. In a locality in the northeast, Tibú,

contiguous with Venezuela, peak biting of *Ny. nuneztovari* was after 21:00 h. This population differed genetically from other Colombian populations and its behavior was similar to *Ny. nuneztovari* from Venezuela. The populations exhibited endo and exophagic behavior in all localities and the results of the study indicated that region-specific interventions on both sides of the Andes would be most effective [152]. EIR values detected for Colombian *Ny. nuneztovari* were 3.5–3.6 in the northwest and 7.2 in the west. The highest value was in Buenaventura, on the Pacific Coast, where *Ny. albimanus* is considered the primary vector [133], but, according to the new study, *Ny. nuneztovari* also has a role in transmission in peri-urban Buenaventura [152].

In Peru, *Ny. nuneztovari* has been detected in five Departments: Pasco, Junín, Loreto, Ucayali, and Madre de Dios [154] and its presence confirmed in Loreto [155]. It may have a role in local malaria transmission, but remains unexplored. *Nyssorhynchus nuneztovari* is known as an important regional vector in western Venezuela where it occurs in seven States [156]. It was first identified morphologically in Bolivar State by Moreno et al. [157], from the malaria hot-spot of Sifontes municipality and was found infected by *P. vivax* (0.52%) [80]. It has also been found to be as abundant as *Ny. darlingi* in the Lower Caura River Basin, Bolivar State, where it was mostly active at sunset, although biting also throughout the night. Nevertheless, it was not detected infected by *Plasmodium* (although *Ny. darlingi* was), so the latter is more important in relation to malaria risk in the Caura River area [96].

4. Conclusions and recommendations

As discussed by Packard [37], for sustainable malaria control, focusing on decreasing incidence towards elimination, effective measures need to be considered, including those related to human ecology. Examples include a significant improvement in living and housing conditions, redesigning of anthropogenic landscapes from those that favor mosquito vectors to a remodeled landscape that is both adequate for humans and inadequate for vector mosquitoes. The sustainability and success of a malaria control program depends on a combination of diagnosis of human infection, treatment with anti-malarial drugs, and vector control. Moreover, proposed changes will need to be maintained such that the malaria baseline will not be affected by either interruption or disruption of a control program [1]. It would be sensible to include malaria control in the One Health Program, to align it with the elimination of extreme poverty, a goal of the global sustainable development program.

The recent elimination of malaria on the Peru-Ecuador border was a successful strategy and included strengthening surveillance and treatment, resource sharing, the use of operational research to inform policy, and novel interventions [135]. The current program depends on prompt, effective diagnosis and treatment with no charge, community personnel trained to collect blood smears from febrile persons within their communities, case reporting to a national surveillance system that includes a five-category case definition (indigenous, imported, introduced, induced, and cryptic), active foci and case investigations, mapping and elimination of larval habitats, and the use of ITNs and LLINs. This could serve as a model for the current situation along the Venezuelan border with its neighbors, Colombia and Brazil. One very important aspect of this program is that it took 20 years to achieve its goals [135].

Worldwide, some of the innovations adopted for prevention, control, and eventual elimination of malaria transmission during the past ~10 years have included the development and deployment of LLINs [158, 159], the completion and exploration of many mosquito and parasite genomes [160–163], major progress on genome editing in vector mosquitoes [164–166], new interventions such as house eaves [167] and push-pull systems [168], and better evaluation of larval source management (LSM) as a potential component of integrated control management systems [169]. Global policies and recommendations provide a useful framework and roadmap guided by the Global Technical Strategy of Malaria Control and Elimination (2016–2025), a reconsideration of the vectorial capacity formula for elimination [170] and the Plan for Elimination of Malaria in Brazil (UN/OMS 2015; [4]).

During the same 10-year timeframe, several novel tools and strategies have been envisaged that focus on the Neotropical malaria control and eradication landscape: (1) successful colonization of the main malaria vector *Ny. darlingi* [171, 172]; (2) development of predictive models on climate change scenarios for Neotropical malaria vectors and *Plasmodium* [18, 19]; and (3) collection of baseline larval habitat characteristics in malaria endemic regions that can guide larval source reduction [29, 48, 58, 173] and may prove effective as part of a broader array of vector interventions in certain landscape types such as abandoned gold mining pools [174] and possibly commercial fish ponds [31].

The most serious challenge to malaria eradication in South America from the viewpoint of vector control is that most vector species are primarily exophilic, often exophagic, and frequently bite early in the evening. Therefore, it is essential to determine and monitor the local biting behavior of a mosquito vector species.

Identified gaps in vector interventions throughout South American endemic areas are:

1. Sustained funding for vector surveillance and intervention;
2. Ongoing training programs for vector biologists and promoting community participation;
3. Use of species distribution models to map potential distribution and epidemiology to focus interventions and planning;
4. New efforts to control exophagic vectors and targeting aquatic stages should be part of integrated control and elimination programs that prioritize hot-spots;
5. More accurate and timely identification of transmission in hot-spots;
6. Routine evaluation of application strategies and insecticide resistance.

Acknowledgements

We thank Sara Bickersmith, New York State Department of Health, Albany, NY, USA and Catharine Prussing, Department of Biomedical Sciences, State University of New York-Albany,

NY, USA for creating the maps. Financial support was provided by US National Institutes of Health (NIH) NIH grant R01 AI110112 to JEC and ICEMR grant U19 AI089681 to J.M. Vinetz, COLCIENCIAS Colombia (Project code No. 596-2013) and Estrategia para la Sostenibilidad de Grupos de Investigación 2016–2107, Universidad de Antioquia, code No. ES84160123 to MMC, and FAPESP Grant no. 2014/26229-7 to MAMS. MEG thanks the Council for International Exchange of Scholars (US Department of State) for a Fulbright Scholar Fellowship to visit the Wadsworth Center, NYS Department of Health, Albany, NY, USA.

Conflict of interest

The authors declare no conflict of interest.

List of abbreviations

ACT	artemisinin-based combination therapy
API	annual parasite index
COI	cytochrome c oxidase I
DDT	dichlorodiphenyltrichloroethane
EIR	entomological inoculation rate
ELISA	enzyme-linked immunosorbent assay
ENSO	El Niño-Southern oscillation
HBI	human blood index
IR	infection rate
IRS	indoor residual spraying
ITN	insecticide-treated net
ITS2	internal transcribed spacer 2
LLIN	long-lasting insecticide-treated net
LSM	larval source management
MOH	Ministry of Health
PAHO	Pan American Health Organization
PCR	polymerase chain reaction
RAPD	random amplified polymorphic DNA
WHO	World Health Organization

Author details

Jan Evelyn Conn[1,2]*, Maria Eugenia Grillet[5], Margarita Correa[4] and Maria Anice Mureb Sallum[3]

*Address all correspondence to: jan.conn@health.ny.gov

1 The Wadsworth Center, New York State Department of Health, Albany, New York, USA

2 Department of Biomedical Sciences, School of Public Health, University at Albany (State University of New York), Albany, New York, USA

3 Department of Epidemiology, Faculty of Public Health, University of São Paulo, São Paulo, SP, Brazil

4 Molecular Microbiology Group, School of Microbiology, University of Antioquia, Medellín, Colombia

5 Laboratory of Vector Biology, Institute of Zoology and Tropical Ecology, Faculty of Sciences, Central University of Venezuela, Caracas, Venezuela

References

[1] WHO. World Malaria Report 2017. Geneva. Switzerland: World Health Organization; 2017

[2] Recht J, Siqueira AM, Monteiro WM, Herrera SM, Herrera S, Lacerda MVG. Malaria in Brazil, Colombia, Peru and Venezuela: Current challenges in malaria control and elimination. Malaria Journal. 2017;**16**(1):273

[3] Flores W, Chang J, Barillas E. Rapid assessment of the performance of malaria control strategies implemented by countries in the Amazon subregion using adequacy criteria: Case study. Malaria Journal. 2011;**10**:379

[4] Ferreira MU, Castro MC. Challenges for malaria elimination in Brazil. Malaria Journal. 2016;**15**(1):284

[5] Alonso P, Noor AM. The global fight against malaria is at crossroads. Lancet. 2017; **390**(10112):2532-2534

[6] Grillet ME, Villegas L, Oletta JF, Tami A, Conn JE. Malaria in Venezuela requires response. Science. 2018 Feb 2;**359**(6375):528. PubMed PMID: 29420282. Epub 2018/02/09. eng

[7] WHO. World Malaria Report 2015, Geneva. Switzerland: World Health Organization; 2015

[8] Hiwat H, Mitro S, Samjhawan A, Sardjoe P, Soekhoe T, Takken W. Collapse of *Anopheles darlingi* populations in Suriname after introduction of insecticide-treated nets (ITNs); malaria down to near elimination level. The American Journal of Tropical Medicine and Hygiene. 2012;**86**(4):649-655

[9] Grillet ME, Barrera R, Martinez JE, Berti J, Fortin MJ. Disentangling the effect of local and global spatial variation on a mosquito-borne infection in a neotropical heterogeneous environment. The American Journal of Tropical Medicine and Hygiene. 2010; **82**(2):194-201

[10] da Silva-Nunes M, Moreno M, Conn JE, Gamboa D, Abeles S, Vinetz JM, et al. Amazonian malaria: Asymptomatic human reservoirs, diagnostic challenges, environmentally driven changes in mosquito vector populations, and the mandate for sustainable control strategies. Acta Tropica. 2012;**121**:281-291

[11] Soto-Calle V, Rosas-Aguirre A, Llanos-Cuentas A, Abatih E, DeDeken R, Rodriguez H, et al. Spatio-temporal analysis of malaria incidence in the Peruvian Amazon region between 2002 and 2013. Scientific Reports. 2017;**7**:40350

[12] Padilla JC, Uribe GÁ, Araújo RM, Narváez PC, Valencia SH. Epidemiology and control of malaria in Colombia. Memórias do Instituto Oswaldo Cruz. 2011;**106**(Suppl 1):14-22

[13] Siqueira AM, Mesones-Lapouble O, Marchesini P, Sampaio VS, Brasil P, Tauil PL, et al. *Plasmodium vivax* landscape in Brazil: Scenario and challenges. The American Journal of Tropical Medicine and Hygiene. 2016;**95**(6 Suppl):87-96

[14] Molina Gomez K, Caicedo MA, Gaitan A, Herrera-Varela M, Arce MI, Vallejo AF, et al. Characterizing the malaria rural-to-urban transmission interface: The importance of reactive case detection. PLoS Neglected Tropical Diseases. 2017;**11**(7):e0005780

[15] Rovira-Vallbona E, Contreras-Mancilla JJ, Ramirez R, Guzmán-Guzmán M, Carrasco-Escobar G, Llanos-Cuentas A, et al. Predominance of asymptomatic and sub-microscopic infections characterizes the *Plasmodium* gametocyte reservoir in the Peruvian Amazon. PLoS Neglected Tropical Diseases. 2017;**11**(7):e0005674

[16] Poveda G, Rojas W, Quinones ML, Velez ID, Mantilla RI, Ruiz D, et al. Coupling between annual and ENSO timescales in the malaria-climate association in Colombia. Environmental Health Perspectives. 2001;**109**(5):489-493

[17] Grillet ME, El Souki M, Laguna F, Leon JR. The periodicity of *Plasmodium vivax* and *Plasmodium falciparum* in Venezuela. Acta Tropica. 2014;**129**:52-60

[18] Alimi TO, Fuller DO, Qualls WA, Herrera SV, Arevalo-Herrera M, Quinones ML, et al. Predicting potential ranges of primary malaria vectors and malaria in northern South America based on projected changes in climate, land cover and human population. Parasites & Vectors. 2015;**8**:431

[19] Laporta GZ, Linton YM, Wilkerson RC, Bergo ES, Nagaki SS, Sant'Ana DC, et al. Malaria vectors in South America: Current and future scenarios. Parasites & Vectors. 2015;**8**:426

[20] de Santana Filho FS, Arcanjo AR, Chehuan YM, Costa MR, Martinez-Espinosa FE, Vieira JL, et al. Chloroquine-resistant *Plasmodium vivax*, Brazilian Amazon. Emerging Infectious Diseases. 2007;**13**(7):1125-1126

[21] Price RN, von Seidlein L, Valecha N, Nosten F, Baird JK, White NJ. Global extent of chloroquine-resistant *Plasmodium vivax*: A systematic review and meta-analysis. The Lancet Infectious Diseases. 2014;**14**(10):982-991

[22] Marques MM, Costa MR, Santana Filho FS, Vieira JL, Nascimento MT, Brasil LW, et al. *Plasmodium vivax* chloroquine resistance and anemia in the western Brazilian Amazon. Antimicrobial Agents and Chemotherapy. 2014;**58**(1):342-347

[23] Quinones ML, Norris DE, Conn JE, Moreno M, Burkot TR, Bugoro H, et al. Insecticide resistance in areas under investigation by the international centers of excellence for malaria research: A challenge for malaria control and elimination. The American Journal of Tropical Medicine and Hygiene. 2015;**93**(3 Suppl):69-78

[24] Galardo AK, Povoa MM, Sucupira IM, Galardo CD, Santos RL. *Anopheles darlingi* and *Anopheles marajoara* (Diptera: Culicidae) susceptibility to pyrethroids in an endemic area of the Brazilian Amazon. Revista da Sociedade Brasileira de Medicina Tropical. 2015;**48**(6):765-769

[25] Rosas-Aguirre A, Guzman-Guzman M, Gamboa D, Chuquiyauri R, Ramirez R, Manrique P, et al. Micro-heterogeneity of malaria transmission in the Peruvian Amazon: A baseline assessment underlying a population-based cohort study. Malaria Journal. 2017;**16**(1):312

[26] Castellanos A, Chaparro-Narváez P, Morales-Plaza CD, Alzate A, Padilla J, Arévalo M, et al. Malaria in gold-mining areas in Colombia. Memórias do Instituto Oswaldo Cruz. 2016;**111**:59-66

[27] Foster PG, de Oliveira TMP, Bergo ES, Conn JE, Sant'Ana DC, Nagaki SS, et al. Phylogeny of Anophelinae using mitochondrial protein coding genes. Royal Society Open Science. 2017 Nov;**4**(11):170758

[28] Hiwat H, Bretas G. Ecology of *Anopheles darlingi* root with respect to vector importance: A review. Parasites & Vectors. 2011;**4**:177

[29] Vittor AY, Pan W, Gilman RH, Tielsch J, Glass G, Shields T, et al. Linking deforestation to malaria in the Amazon: Characterization of the breeding habitat of the principal malaria vector, *Anopheles darlingi*. The American Journal of Tropical Medicine and Hygiene. 2009;**81**:5-12

[30] de Barros FS, Tadei WP, Arruda ME, Honorio NA. On the use of classic epidemiological formulae for estimating the intensity of endemic malaria transmission by vectors in the Amazon. Neotropical Entomology. 2012;**41**(5):426-434

[31] dos Reis IC, Honorio NA, Barros FS, Barcellos C, Kitron U, Camara DC, et al. Epidemic and endemic malaria transmission related to fish farming ponds in the Amazon frontier. PLoS One. 2015;**10**(9):e0137521

[32] Perkins TA, Garcia AJ, Paz-Soldan VA, Stoddard ST, Reiner RC, Vazquez-Prokopec G, et al. Theory and data for simulating fine-scale human movement in an urban environment. Journal of the Royal Society, Interface. 2014;**11**(99):1-2. Article Number: 20140642

[33] Carrasco-Escobar G, Gamboa D, Castro MC, Bangdiwala SI, Rodriguez H, Contreras-Mancilla J, et al. Micro-epidemiology and spatial heterogeneity of *P. vivax* parasitaemia in riverine communities of the Peruvian Amazon: A multilevel analysis. Scientific Reports. 2017;**7**(1):8082

[34] Angelo JR, Katsuragawa TH, Sabroza PC, de Carvalho LA, Silva LH, Nobre CA. The role of spatial mobility in malaria transmission in the Brazilian Amazon: The case of Porto Velho municipality, Rondonia, Brazil (2010-2012). PLoS One. 2017;**12**(2):e0172330

[35] Lainhart W, Bickersmith S, Nadler K, Moreno M, Saavedra M, Chu VM, et al. Evidence for temporal population replacement and the signature of ecological adaptation in a major Neotropical malaria vector in Amazonian Peru. Malaria Journal. 2015;**14**(1):375

[36] Tucker Lima JM, Vittor A, Rifai S, Valle D. Does deforestation promote or inhibit malaria transmission in the Amazon? A systematic literature review and critical appraisal of current evidence. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences. 2017;**372**(1722)

[37] Packard RM. The making of a tropical disease: A short history of malaria. Emerging Infectious Diseases. 2008;**14**(10):1679

[38] WHO. World Malaria Report 2016. Geneva: World Health Organization; 2016

[39] Kohara Melchior LA, Chiaravalloti Neto F. Spatial and spatio-temporal analysis of malaria in the state of acre, western Amazon, Brazil. Geospatial Health. 2016;**11**(3):443

[40] Lana RM, Riback TIS, Lima TFM, da Silva-Nunes M, Cruz OG, Oliveira FGS, et al. Socioeconomic and demographic characterization of an endemic malaria region in Brazil by multiple correspondence analysis. Malaria Journal. 2017;**16**(1):397

[41] Pan American Health Organization. Report on the Situation of Malaria in the Americas, 2000-2015. 2017

[42] Ministério da Saúde. Boletim Malária Região Amazônica. www.saude.gov.br/malaria: Ministério da Saúde; 2018 [cited 2018 Jan 4]

[43] Instituto Nacional de Salud (INS). Instituto Nacional de Salud, Boletín epidemiológico Semanal. Estadísticas del sistema de vigilancia en salud pública- SIVIGILA, Casos totales en la Semana Epidemiológica 52 y acumulados del año, Subdirección de Vigilancia y Control en Salud Pública. Bogotá: Instituto Nacional de Salud; 2017

[44] Ministerio de Salud. Centro Nacional de Epidemiologia, Prevención y Control de Enfermedades—MINSA (*) Hasta la SE 51 del 2017. Lima, Peru: Ministerio de Salud Perú; 2017

[45] Ministerio del Poder Popular para la Salud (MPPS). Ministerio del Poder Popular para la Salud. Caracas, Venezuela. 2018. http://www.vicepresidencia.gob.ve/index.php/tag/ministerio-del-poder-popular-para-la-salud/ [cited January 5 2018]

[46] Lima ID, Lapouble OM, Duarte EC. Time trends and changes in the distribution of malaria cases in the Brazilian Amazon region, 2004-2013. Memórias do Instituto Oswaldo Cruz. 2017;**112**(1):8-18

[47] Manguin S, Roberts DR, Andre RG, Rejmankova E, Hakre S. Characterization of *Anopheles darlingi* (Diptera: Culicidae) larval habitats in Belize, Central America. Journal of Medical Entomology. 1996 Mar;**33**(2):205-211. PubMed PMID: 8742522. Epub 1996/03/01. eng

[48] Rufalco-Moutinho P, Schweigmann N, Bergamaschi DP, Mureb Sallum MA. Larval habitats of *Anopheles* species in a rural settlement on the malaria frontier of Southwest Amazon, Brazil. Acta Tropica. 2016;**164**:243-258

[49] Vitor-Silva S, Siqueira AM, de Souza Sampaio V, Guinovart C, Reyes-Lecca RC, de Melo GC, et al. Declining malaria transmission in rural Amazon: Changing epidemiology and challenges to achieve elimination. Malaria Journal. 2016;**15**(1):266

[50] Braz RM, Duarte EC, Tauil PL. Characteristics of malaria epidemics in the municipalities of the Brazilian Amazon, 2010. Cadernos de Saúde Pública. 2013;**29**(5):935-944. Caracterizacao das epidemias de malaria nos municipios da Amazonia Brasileira em 2010

[51] Instituto Nacional de Salud (INS). Boletín epidemiológico Semanal. Estadísticas del sistema de vigilancia en salud pública- SIVIGILA, Casos totales en la Semana Epidemiológica 52 y acumulados del año, Subdirección de Vigilancia y Control en Salud Pública. 2015. 2015

[52] Instituto Nacional de Salud (INS). Instituto Nacional de Salud, Boletín epidemiológico Semanal. Estadísticas del sistema de vigilancia en salud pública- SIVIGILA, Casos totales en la Semana Epidemiológica 52 y acumulados del año, Subdirección de Vigilancia y Control en Salud Pública. 2016

[53] Padilla JC, Chaparro PE, Molina K, Arevalo-Herrera M, Herrera S. Is there malaria transmission in urban settings in Colombia? Malaria Journal. 2015;**14**:453

[54] Colombian Malaria Project. Colombian Malaria Project report—years 4 and 5, 2013-2014 [web]. Bogotá 2017 [cited Nov 2017]. Available from: http://www.ins.gov.co/temas-de-interes/Paginas/memorias.aspx

[55] Gomez GF, Correa MM. Discrimination of Neotropical *Anopheles* species based on molecular and wing geometric morphometric traits. Infection, Genetics and Evolution. 2017;**54**:379-386

[56] Lopez-Rubio A, Suaza-Vasco J, Marcet PL, Ruiz-Molina N, Caceres L, Porter C, et al. Use of DNA barcoding to distinguish the malaria vector *Anopheles neivai* in Colombia. Zootaxa. 2016;**4175**(4):377-389

[57] Ahumada ML, Orjuela LI, Pareja PX, Conde M, Cabarcas DM, Cubillos EF, et al. Spatial distributions of *Anopheles* species in relation to malaria incidence at 70 localities in the highly endemic Northwest and South Pacific Coast regions of Colombia. Malaria Journal. 2016;**15**(1):407

[58] Conde M, Pareja PX, Orjuela LI, Ahumada ML, Duran S, Jara JA, et al. Larval habitat characteristics of the main malaria vectors in the most endemic regions of Colombia: Potential implications for larval control. Malaria Journal. 2015;**14**(1):476

[59] Jiménez IP, Conn J, Brochero H. Preliminary biological studies on larvae and adult *Anopheles* mosquitoes (Diptera: Culicidae) in Miraflores, a malaria endemic locality in Guaviare department, Amazonian Colombia. Journal of Medical Entomology. 2014; **51**(5):1002-1009

[60] Naranjo-Diaz N, Rosero DA, Rua-Uribe G, Luckhart S, Correa MM. Abundance, behavior and entomological inoculation rates of anthropophilic anophelines from a primary Colombian malaria endemic area. Parasites & Vectors. 2013;**6**:61

[61] Ruiz D, Ceron V, Molina AM, Quinones ML, Jimenez MM, Ahumada M, et al. Implementation of malaria dynamic models in municipality level early warning systems in Colombia. Part I: Description of study sites. The American Journal of Tropical Medicine and Hygiene. 2014;**91**(1):27-38

[62] Rosas-Aguirre A, Gamboa D, Manrique P, Conn JE, Moreno M, Lescano AG, et al. Epidemiology of *Plasmodium vivax* malaria in Peru. The American Journal of Tropical Medicine and Hygiene. 2016;**95**(6 Suppl):133-144

[63] Rosas-Aguirre A, Speybroeck N, Llanos-Cuentas A, Rosanas-Urgell A, Carrasco-Escobar G, Rodriguez H, et al. Hotspots of malaria transmission in the Peruvian Amazon: Rapid assessment through a parasitological and serological survey. PLoS One. 2015; **10**(9):e0137458

[64] MINSA. Ministerio de Salud del Perú: Sala de Situación de Salud: Epidemiológica N° 52-2017. 2017

[65] Sanchez JF, Carnero AM, Rivera E, Rosales LA, Baldeviano GC, Asencios JL, et al. Unstable malaria transmission in the southern Peruvian Amazon and its association with gold mining, Madre de Dios, 2001-2012. The American Journal of Tropical Medicine and Hygiene. 2017;**96**(2):304-311

[66] Rosas-Aguirre A, Llanos-Cuentas A, Speybroeck N, Cook J, Contreras-Mancilla J, Soto V, et al. Assessing malaria transmission in a low endemicity area of North-Western Peru. Malaria Journal. 2013;**12**:339

[67] MINSA. Ministerio de Salud del Perú. Tendenica y situación de las enfermedades sujetas a vigilancia epidemiológica: malaria. Boletin Epidemiologico. 2015;**24**:975-986

[68] Quispe AM, Llanos-Cuentas A, Rodriguez H, Clendenes M, Cabezas C, Leon LM, et al. Accelerating to zero: Strategies to eliminate malaria in the Peruvian Amazon. The American Journal of Tropical Medicine and Hygiene. 2016;**94**(6):1200-1207

[69] Instituto Nacional de Estadisticas e Informatica. Estimaciones y Proyecciones de Población Total por Sexo de las Principales Ciudades 2000-2015. Lima, Peru; 2012

[70] Reinbold-Wasson DD, Sardelis MR, Jones JW, Watts DM, Fernandez R, Carbajal F, et al. Determinants of *Anopheles* seasonal distribution patterns across a forest to periurban gradient near Iquitos, Peru. The American Journal of Tropical Medicine and Hygiene. 2012;**86**(3):459-463

[71] Moreno M, Saavedra MP, Bickersmith SA, Lainhart W, Tong C, Alava F, et al. Implications for changes in *Anopheles darlingi* biting behaviour in three communities in the peri-Iquitos region of Amazonian Peru. Malaria Journal. 2015;**14**:290

[72] Chuquiyauri R, Paredes M, Penataro P, Torres S, Marin S, Tenorio A, et al. Socio-demographics and the development of malaria elimination strategies in the low transmission setting. Acta Tropica. 2012;**121**(3):292-302

[73] Parker BS, Paredes Olortegui M, Penataro Yori P, Escobedo K, Florin D, Rengifo Pinedo S, et al. Hyperendemic malaria transmission in areas of occupation-related travel in the Peruvian Amazon. USA: Malaria Journal. 2013;**12**:178

[74] Pan American Health Organization. Malaria in the Bolivarian Republic of Venezuela. Washington D.C., USA. 2017

[75] Hotez PJ, Basanez MG, Acosta-Serrano A, Grillet ME. Venezuela and its rising vector-borne neglected diseases. PLoS Neglected Tropical Diseases. 2017;**11**(6):e0005423

[76] Hotez PJ. Ten failings in global neglected tropical diseases control. PLoS Neglected Tropical Diseases. 2017;**11**(12):e0005896

[77] Moreno JE, Rubio-Palis Y, Paez E, Perez E, Sanchez V. Abundance, biting behaviour and parous rate of anopheline mosquito species in relation to malaria incidence in gold-mining areas of southern Venezuela. Medical and Veterinary Entomology. 2007;**21**(4):339-349

[78] Moreno JE, Rubio-Palis Y, Paez E, Perez E, Sanchez V, Vaccari E. Malaria entomological inoculation rates in gold mining areas of Southern Venezuela. Memórias do Instituto Oswaldo Cruz. 2009;**104**(5):764-768

[79] Moreno JE, Rubio-Palis Y, Sanchez V, Martinez A. Caracterización de hábitats larvales de anofelinos en el municipio Sifontes del estado Bolívar, Venezuela. Boletin de Malariologia y Salud Ambiental LV. 2015;**2**:117-131

[80] Orm SA, Moreno JE, Carrozza M, Acevedo P, Herrera F. *Plasmodium spp*. infection rates for some *Anopheles spp*. from Sifontes municipality, Bolívar State, Venezuela. Boletin de Malariologia y Salud Ambiental 2017;LVII(1):17-25

[81] Casey N. Hard times in Venezuela breed malaria as desperate flock to mines. New York Times. 2016

[82] Griffing SM, Villegas L, Udhayakumar V. Malaria control and elimination, Venezuela, 1800s -1970s. Emerging Infectious Diseases. 2014;**20**(10):1697-1704

[83] Google Earth. Latin America: 4°59′43″S 66°33′57″W. Available from: https://earth.google.com/web: GOOGLE EARTH v 9.2.52.4; [Accessed: February 23, 2018]

[84] Sinka ME, Rubio-Palis Y, Manguin S, Patil AP, Temperley WH, Gething PW, et al. The dominant *Anopheles* vectors of human malaria in the Americas: Occurrence data, distribution maps and bionomic precis. Parasites & Vectors. 2010 Aug 16;**3**:72. PubMed PMID: 20712879. Pubmed Central PMCID: Pmc2936890. Epub 2010/08/18. eng

[85] Hay SI, Snow RW. The malaria atlas project: Developing global maps of malaria risk. PLoS Medicine. 2006;**3**(12):e473

[86] Manguin S, Wilkerson RC, Conn JE, Rubio-Palis Y, Danoff-Burg JA, Roberts DR. Population structure of the primary malaria vector in South America, *Anopheles darlingi*, using isozyme, random amplified polymorphic DNA, internal transcribed spacer 2, and morphologic markers. The American Journal of Tropical Medicine and Hygiene. 1999;**60**(3):364-376

[87] Gutierrez LA, Gonzalez JJ, Gomez GF, Castro MI, Rosero DA, Luckhart S, et al. Species composition and natural infectivity of anthropophilic *Anopheles* (Diptera: Culicidae) in the states of Cordoba and Antioquia, northwestern Colombia. Memórias do Instituto Oswaldo Cruz. 2009;**104**(8):1117-1124

[88] Galardo AK, Arruda M, D'Almeida Couto AA, Wirtz R, Lounibos LP, Zimmerman RH. Malaria vector incrimination in three rural riverine villages in the Brazilian Amazon. The American Journal of Tropical Medicine and Hygiene. 2007;**76**(3):461-469

[89] Martins-Campos KM, Pinheiro WD, Vitor-Silva S, Siqueira AM, Melo GC, Rodrigues IC, et al. Integrated vector management targeting *Anopheles darlingi* populations decreases malaria incidence in an unstable transmission area, in the rural Brazilian Amazon. Malaria Journal. 2012;**11**:351

[90] Jimenez P, Conn JE, Wirtz R, Brochero H. *Anopheles* (Diptera: Culicidae) vectors of malaria in Puerto Carreno municipality, Vichada, Colombia. Biomedica. 2012;**32**(Suppl 1): 13-21

[91] Jiménez IP, Conn JE, Brochero H. Malaria Vectors in San José del Guaviare, Orinoquia, Colombia. Journal of the American Mosquito Control Association. 2014;**30**(2):91-98

[92] Ahumada ML, Pareja PX, Buitrago LS, Quinones ML. Biting behavior of *Anopheles darlingi* root, 1926 (Diptera: Culicidae) and its association with malaria transmission in Villavicencio (Meta, Colombia). Biomédica. 2013;**33**(2):241-250

[93] Dusfour I, Issaly J, Carinci R, Gaborit P, Girod R. Incrimination of *Anopheles (Anopheles) intermedius* Peryassu, *An. (Nyssorhynchus) nuneztovari* Gabaldon, *An. (Nys.) oswaldoi* Peryassu as natural vectors of *Plasmodium falciparum* in French Guiana. Memórias do Instituto Oswaldo Cruz. 2012 May;**107**(3):429-432

[94] Hiwat H, Issaly J, Gaborit P, Somai A, Samjhawan A, Sardjoe P, et al. Behavioral heterogeneity of *Anopheles darlingi* (Diptera: Culicidae) and malaria transmission dynamics along the Maroni River, Suriname, French Guiana. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2010;**104**(3):207-213

[95] Girod R, Roux E, Berger F, Stefani A, Gaborit P, Carinci R, et al. Unravelling the relationships between *Anopheles darlingi* (Diptera: Culicidae) densities, environmental factors and malaria incidence: Understanding the variable patterns of malarial transmission in French Guiana (South America). Annals of Tropical Medicine and Parasitology. 2011;**105**(2):107-122

[96] Rubio-Palis Y, Bevilacqua M, Medina DA, Moreno JE, Cardenas L, Sanchez V, et al. Malaria entomological risk factors in relation to land cover in the lower Caura River basin, Venezuela. Memórias do Instituto Oswaldo Cruz. 2013 Apr;**108**(2):220-228

[97] Moreno M, Saavedra MP, Bickersmith SA, Prussing C, Michalski A, Tong Rios C, et al. Intensive trapping of blood-fed *Anopheles darlingi* in Amazonian Peru reveals unexpectedly high proportions of avian blood-meals. PLoS Neglected Tropical Diseases. 2017;**11**(2):e0005337

[98] Montoya C, Bascunan P, Rodriguez-Zabala J, Correa MM. Abundance, composition and natural infection of *Anopheles* mosquitoes from two malaria-endemic regions of Colombia. Biomédica. 2017;**37**:98-105

[99] Pommier de Santi V, Girod R, Mura M, Dia A, Briolant S, Djossou F, et al. Epidemiological and entomological studies of a malaria outbreak among French armed forces deployed at illegal gold mining sites reveal new aspects of the disease's transmission in French Guiana. Malaria Journal. 2016;**15**:35

[100] Vezenegho SB, Adde A, Pommier de Santi V, Issaly J, Carinci R, Gaborit P, et al. High malaria transmission in a forested malaria focus in French Guiana: How can exophagic *Anopheles darlingi* thwart vector control and prevention measures? Memórias do Instituto Oswaldo Cruz. 2016;**111**(9):561-569

[101] Pommier de Santi V, Dusfour I, de Parseval E, Lespinet B, Nguyen C, Gaborit P, et al. Risk of daytime transmission of malaria in the French Guiana rain forest. Medecine et sante tropicales. 2017;**27**(1):111-112. Risque de transmission diurne du paludisme en foret guyanaise

[102] Herrera-Varela M, Orjuela LI, Penalver C, Conn JE, Quinones ML. *Anopheles* species composition explains differences in *Plasmodium* transmission in La Guajira, northern Colombia. Memórias do Instituto Oswaldo Cruz. 2014;**109**(7):955-959

[103] Naranjo-Diaz N, Altamiranda M, Luckhart S, Conn JE, Correa MM. Malaria vectors in ecologically heterogeneous localities of the Colombian Pacific region. PLoS One. 2014;**9**(8):e103769

[104] Cohuet A, Harris C, Robert V, Fontenille D. Evolutionary forces on *Anopheles*: What makes a malaria vector? Trends in Parasitology. 2010;**26**(3):130-136

[105] Kaufman MG, Fonseca DM. Invasion biology of *Aedes japonicus japonicus* (Diptera: Culicidae). Annual Review of Entomology. 2014;**59**:31-49

[106] Lounibos LP, Kramer LD. Invasiveness of *Aedes aegypti* and *Aedes albopictus* and vectorial capacity for Chikungunya virus. The Journal of Infectious Diseases. 2016;**214** (suppl 5):S453-s458

[107] Aramburu Guarda J, Ramal Asayag C, Witzig R. Malaria reemergence in the Peruvian Amazon region. Emerging Infectious Diseases. 1999;**5**(2):209-215

[108] Schoeler GB, Flores-Mendoza C, Fernandez R, Davila JR, Zyzak M. Geographical distribution of *Anopheles darlingi* in the Amazon Basin region of Peru. Journal of the American Mosquito Control Association. 2003;**19**(4):286-296

[109] Vittor AY, Gilman RH, Tielsch J, Glass G, Shields T, Lozano WS, et al. The effect of deforestation on the human-biting rate of *Anopheles darlingi*, the primary vector of falciparum malaria in the Peruvian Amazon. The American Journal of Tropical Medicine and Hygiene. 2006;**74**:3-11

[110] Gonzalez R, Carrejo N. Introducción al estudio taxonómico de *Anopheles* de Colombia Claves y notas de distribución. 2nd ed. Cali; Programa Editorial Universidad de Valle; 2009. 260 pp. Epub 2

[111] Naranjo-Diaz N, Conn JE, Correa MM. Behavior and population structure of *Anopheles darlingi* in Colombia. Infection, Genetics and Evolution. 2016;**39**:64-73

[112] Pedro PM, Sallum MAM. Spatial expansion and population structure of the neotropical malaria vector, *Anopheles darlingi* (Diptera: Culicidae). Biological Journal of the Linnean Society. 2009;**97**(4):854-866

[113] Emerson KJ, Conn JE, Bergo ES, Randel MA, Sallum MAM. Brazilian *Anopheles darlingi* (Diptera: Culicidae) clusters by major biogeographical region. PLoS One. 2015;**10**(7): e0130773

[114] Zimmerman RH, Galardo AK, Lounibos LP, Arruda M, Wirtz R. Bloodmeal hosts of *Anopheles* species (Diptera: Culicidae) in a malaria-endemic area of the Brazilian Amazon. Journal of Medical Entomology. 2006;**43**(5):947-956

[115] Rejmankova E, Rubio-Palis Y, Villegas L. Larval habitats of anopheline mosquitoes in the upper Orinoco, Venezuela. Journal of Vector Ecology. 1999;**24**(2):130-137

[116] Gil LH, Tada MS, Katsuragawa TH, Ribolla PE, da Silva LH. Urban and suburban malaria in Rondonia (Brazilian western Amazon) II. Perennial transmissions with high anopheline densities are associated with human environmental changes. Memórias do Instituto Oswaldo Cruz. 2007;**102**(3):271-276

[117] Moutinho PR, Gil LH, Cruz RB, Ribolla PE. Population dynamics, structure and behavior of *Anopheles darlingi* in a rural settlement in the Amazon rainforest of acre, Brazil. Malaria Journal. 2011;**10**:174

[118] Conn JE, Ribolla PE. Ecology of *Anopheles darlingi*, the primary malaria vector in the Americas and current nongenetic methods of vector control. In: Adelman ZN, editor. Genetic Control of Malaria and Dengue. Oxford: Academic Press; 2015. pp. 81-102

[119] Loaiza JR, Bermingham E, Sanjur OI, Scott ME, Bickersmith SA, Conn JE. Review of genetic diversity in malaria vectors (Culicidae: Anophelinae). Infection, Genetics and Evolution. 2012;**12**(1):1-12

[120] Scarpassa VM, Conn JE. Population genetic structure of the major malaria vector *Anopheles darlingi* (Diptera: Culicidae) from the Brazilian Amazon, using microsatellite markers. Memórias do Instituto Oswaldo Cruz. 2007;**102**(3):319-327

[121] Angella AF, Salgueiro P, Gil LH, Vicente JL, Pinto J, Ribolla PE. Seasonal genetic partitioning in the neotropical malaria vector, *Anopheles darlingi*. Malaria Journal. 2014;**13**:203

[122] Campos M, Conn JE, Alonso DP, Vinetz JM, Emerson KJ, Ribolla PE. Microgeographical structure in the major Neotropical malaria vector *Anopheles darlingi* using microsatellites and SNP markers. Parasites & Vectors. 2017;**10**(1):76

[123] Gutiérrez LA, Gomez GF, Gonzalez JJ, Castro MI, Luckhart S, Conn JE, et al. Microgeographic genetic variation of the malaria vector *Anopheles darlingi* root (Diptera: Culicidae) from Cordoba and Antioquia, Colombia. The American Journal of Tropical Medicine and Hygiene. 2010;**83**(1):38-47

[124] Altamiranda-Saavedra M, Conn JE, Correa MM. Genetic structure and phenotypic variation of *Anopheles darlingi* in Northwest Colombia. Infection, Genetics and Evolution. 2017;**56**:143-151

[125] Pinedo-Cancino V, Sheen P, Tarazona-Santos E, Oswald WE, Jeri C, Vittor AY, et al. Limited diversity of *Anopheles darlingi* in the Peruvian Amazon region of Iquitos. The American Journal of Tropical Medicine and Hygiene. 2006;**75**(2):238-245

[126] Mirabello L, Vineis JH, Yanoviak SP, Scarpassa VM, Povoa MM, Padilla N, et al. Microsatellite data suggest significant population structure and differentiation within the malaria vector *Anopheles darlingi* in central and South America. BMC Ecology. 2008;**8**:3

[127] Rubio-Palis Y, Zimmerman RH. Ecoregional classification of malaria vectors in the neotropics. Journal of Medical Entomology. 1997;**34**(5):499-510

[128] Faran ME. Mosquito studies (Diptera, Culicidae) XXXIV. A revision of the *Albimanus* section of the subgenus *Nyssorhynchus* of *Anopheles*. Contributions of the American Entomological Institute. 1980;**15**:1-215

[129] Loaiza JR, Scott ME, Bermingham E, Rovira J, Conn JE. Evidence for pleistocene population divergence and expansion of *Anopheles albimanus* in southern central America. The American Journal of Tropical Medicine and Hygiene. 2010;**82**(1):156-164

[130] Montoya-Lerma J, Solarte YA, Giraldo-Calderon GI, Quinones ML, Ruiz-Lopez F, Wilkerson RC, et al. Malaria vector species in Colombia: A review. Memórias do Instituto Oswaldo Cruz. 2011;**106**(Suppl 1):223-238

[131] Olano V, Brochero H, Sáenz R, Quiñones M, Molina J. Mapas preliminares de la distribución de especies de *Anopheles* vectores de malaria en Colombia. Biomédica. 2001; **21**:402-408

[132] Gómez GF, Márquez EJ, Gutiérrez LA, Conn JE, Correa MM. Geometric morphometric analysis of Colombian *Anopheles albimanus* (Diptera: Culicidae) reveals significant effect of environmental factors on wing traits and presence of a metapopulation. Acta Tropica. 2014;**135**:75-85

[133] Gutiérrez LA, Naranjo N, Jaramillo LM, Muskus C, Luckhart S, Conn JE, et al. Natural infectivity of *Anopheles* species from the Pacific and Atlantic regions of Colombia. Acta Tropica. 2008;**107**:99-105

[134] Instituto Geografico Agustin Codazzi. Bogota: Atlas de Colombia; 2002:167. Epub 5. http://www2.igac.gov.co/igac_web/contenidos/plantilla_general_titulo_contenido.jsp?idMenu=328

[135] Krisher LK, Krisher J, Ambuludi M, Arichabala A, Beltran-Ayala E, Navarrete P, et al. Successful malaria elimination in the Ecuador-Peru border region: Epidemiology and lessons learned. Malaria Journal. 2016;**15**(1):573

[136] Cáceres DC, De La Hoz F, Nicholls S, DeAntonio R, Velandia MP, Olano V, et al. Brote de malaria en La Guajira, 1 de diciembre de 1999 a 1 de febrero de 2000. Biomédica. 2000;**20**:152-161

[137] Gutiérrez LA, Naranjo NJ, Cienfuegos AV, Muskus CE, Luckhart S, Conn JE, et al. Population structure analyses and demographic history of the malaria vector *Anopheles albimanus* from the Caribbean and the Pacific regions of Colombia. Malaria Journal. 2009;**8**:259

[138] Molina-Cruz A, de Merida AM, Mills K, Rodriguez F, Schoua C, Yurrita MM, et al. Gene flow among *Anopheles albimanus* populations in central America, South America, and the Caribbean assessed by microsatellites and mitochondrial DNA. The American Journal of Tropical Medicine and Hygiene. 2004 Sep;**71**(3):350-359. PubMed PMID: 15381818. Epub 2004/09/24. eng

[139] Loaiza JR, Scott ME, Bermingham E, Sanjur OI, Wilkerson R, Rovira J, et al. Late Pleistocene environmental changes lead to unstable demography and population divergence of *Anopheles albimanus* in the northern Neotropics. Molecular Phylogenetics and Evolution. 2010;**57**(3):1341-1346

[140] Lounibos LP, Conn JE. Malaria vector heterogeneity in South America. American Entomologist. 2000;**46**(4):238-249

[141] Rubio-Palis Y. Evaluation of light traps combined with carbon dioxide and 1-octen-3-ol to collect anophelines in Venezuela. Journal of the American Mosquito Control Association. 1996 Mar;**12**(1):91-96. PubMed PMID: 8723264. Epub 1996/03/01. eng

[142] Ruiz-Lopez F, Wilkerson RC, Conn JE, McKeon SN, Levin DM, Quinones ML, et al. DNA barcoding reveals both known and novel taxa in the *Albitarsis* group (*Anopheles: Nyssorhynchus*) of Neotropical malaria vectors. Parasites & Vectors. 2012;**5**:44

[143] WHO. World Malaria Report 2012. Geneva: WHO; 2012

[144] Conn JE, Wilkerson RC, Segura MN, de Souza RT, Schlichting CD, Wirtz RA, et al. Emergence of a new neotropical malaria vector facilitated by human migration and changes in land use. The American Journal of Tropical Medicine and Hygiene. 2002;**66**(1):18-22

[145] Brochero HL, Rey G, Buitrago LS, Olano VA. Biting activity and breeding sites of *Anopheles* species in the municipality Villavicencio, meta, Colombia. Journal of the American Mosquito Control Association. 2005;**21**(2):182-186

[146] Brochero HH, Li C, Wilkerson RC. A newly recognized species in the *Anopheles (Nyssorhynchus) albitarsis* complex (Diptera: Culicidae) from Puerto Carreno, Colombia. The American Journal of Tropical Medicine and Hygiene. 2007;**76**(6):1113-1117

[147] Gomez G, Jaramillo L, Correa MM. Wing geometric morphometrics and molecular assessment of members in the *Albitarsis* complex from Colombia. Molecular Ecology Resources. 2013;**13**(6):1082-1092

[148] Moreno JE, Rubio-Palis Y, Martinez AR, Acevedo P. Evolución espacial y temporal de la malaria en el municipio Sifontes del estado Bolívar, Venezuela. 1980-2013. Boletin de Malariologia y Salud Ambiental. 2014;**54**(2):236-249

[149] Foster PG, Bergo ES, Bourke BP, Oliveira TM, Nagaki SS, Sant'Ana DC, et al. Phylogenetic analysis and DNA-based species confirmation in *Anopheles (Nyssorhynchus)*. PLoS One. 2013;**8**(2):e54063

[150] Scarpassa VM, Cunha-Machado AS, Saraiva JF. Evidence of new species for malaria vector *Anopheles nuneztovari* sensu lato in the Brazilian Amazon region. Malaria Journal. 2016;**15**:205

[151] Gómez G, Cienfuegos A, Gutiérrez L, Conn J, Correa M. Análisis morfológico y molecular evidencia problemas al identificar *Anopheles nuneztovari* (Diptera: Culicidae) por claves dicotómicas. Revista Colombiana de Entomologia. 2010;**36**:68-75

[152] Naranjo-Díaz N, Sallum MAM, Correa MM. Population dynamics of *Anopheles nuneztovari* in Colombia. Infection, genetics and evolution. Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases. 2016;**45**:56-65

[153] Welle D. WHO: Cases of Malaria are Triggered in Venezuela and Colombia, for Different Reasons. 2017. Available from: www.dw.com: Deutsche Welle; http://p.dw.com/p/2oUld [Accessed: January 18, 2018]

[154] Calderón G, Fernández R, Valle J. Especies de la fauna anofelina, su distribucion y algunas consideraciones sobre su abundancia e infectividad en el Peru. Revista Peruana de Epidemiología. 1995;**8**(1):5-23

[155] Matson R, Rios CT, Chavez CB, Gilman RH, Florin D, Sifuentes VL, et al. Improved molecular technique for the differentiation of neotropical anopheline species. The American Journal of Tropical Medicine and Hygiene. 2008;**78**(3):492-498

[156] Rubio-Palis Y. *Anopheles (Nyssorhynchus)* de Venezuela. Taxonomía, bionomía, ecología e importancia médica. Maracay, Venezuela: Publicaciones de la Dirección de Malariologia; 2000

[157] Moreno JE, Rubio-Palis Y, Sanchez V, Mariany D. Primer registro de *Anopheles (Nyssorhynchus) nuneztovari* Gabaldón,1940 (Diptera: Culicidae) en el estado Bolívar, Venezuela y sus implicaciones eco-epidemiológicas. Entomotropica. 2004;**19**(1):55-58

[158] Gatton ML, Chitnis N, Churcher T, Donnelly MJ, Ghani AC, Godfray HC, et al. The importance of mosquito behavioural adaptations to malaria control in Africa. Evolution. 2013;**67**(4):1218-1230

[159] Koenker HM, Yukich JO, Mkindi A, Mandike R, Brown N, Kilian A, et al. Analysing and recommending options for maintaining universal coverage with long-lasting insecticidal nets: The case of Tanzania in 2011. Malaria Journal. 2013;**12**:150

[160] Neafsey DE, Galinsky K, Jiang RH, Young L, Sykes SM, Saif S, et al. The malaria parasite *Plasmodium vivax* exhibits greater genetic diversity than *Plasmodium falciparum*. Nature Genetics. 2012;**44**(9):1046-1050

[161] Neafsey DE, Waterhouse RM, Abai MR, Aganezov SS, Alekseyev MA, Allen JE, et al. Mosquito genomics. Highly evolvable malaria vectors: The genomes of 16 *Anopheles* mosquitoes. Science. 2015;**347**(6217):1258522

[162] Love RR, Steele AM, Coulibaly MB, Traore SF, Emrich SJ, Fontaine MC, et al. Chromosomal inversions and ecotypic differentiation in *Anopheles gambiae*: The perspective from whole-genome sequencing. Molecular Ecology. 2016;**25**(23):5889-5906

[163] Auburn S, Bohme U, Steinbiss S, Trimarsanto H, Hostetler J, Sanders M, et al. A new *Plasmodium vivax* reference sequence with improved assembly of the subtelomeres reveals an abundance of pir genes. Wellcome Open Research. 2016;**1**:4

[164] Hammond A, Galizi R, Kyrou K, Simoni A, Siniscalchi C, Katsanos D, et al. A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*. Nature Biotechnology. 2016;**34**:78-83

[165] Alphey L. Can CRISPR-Cas9 gene drives curb malaria? Nature Biotechnology. 2016; **34**(2):149-150

[166] Reegan AD, Ceasar SA, Paulraj MG, Ignacimuthu S, Al-Dhabi NA. Current status of genome editing in vector mosquitoes: A review. Bioscience Trends. 2017;**10**(6):424-432

[167] Waite JL, Lynch PA, Thomas MB. Eave tubes for malaria control in Africa: A modelling assessment of potential impact on transmission. Malaria Journal. 2016;**15**(1):449

[168] Menger DJ, Omusula P, Holdinga M, Homan T, Carreira AS, Vandendaele P, et al. Field evaluation of a push-pull system to reduce malaria transmission. PLoS One. 2015;**10**(4):e0123415

[169] Tusting LS, Thwing J, Sinclair D, Fillinger U, Gimnig J, Bonner KE, et al. Mosquito larval source management for controlling malaria. Cochrane Database of Systematic Reviews. 2013;**8**:Cd008923

[170] Brady OJ, Godfray HC, Tatem AJ, Gething PW, Cohen JM, McKenzie FE, et al. Vectorial capacity and vector control: Reconsidering sensitivity to parameters for malaria elimination. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2016; **110**(2):107-117

[171] Moreno M, Tong C, Guzman M, Chuquiyauri R, Llanos-Cuentas A, Rodriguez H, et al. Infection of laboratory-colonized *Anopheles darlingi* mosquitoes by *Plasmodium vivax*. The American Journal of Tropical Medicine and Hygiene. 2014;**90**(4):612-616

[172] Villarreal-Trevino C, Vasquez GM, Lopez-Sifuentes VM, Escobedo-Vargas K, Huayanay-Repetto A, Linton YM, et al. Establishment of a free-mating, long-standing and highly productive laboratory colony of *Anopheles darlingi* from the Peruvian Amazon. Malaria Journal. 2015;**14**:227

[173] Pinault LL, Hunter FF. Characterization of larval habitats of *Anopheles albimanus, Anopheles pseudopunctipennis, Anopheles punctimacula,* and *Anopheles oswaldoi* s.L. populations in lowland and highland Ecuador. Journal of Vector Ecology. 2012;**37**(1):124-136

[174] Galardo AK, Zimmerman R, Galardo CD. Larval control of *Anopheles (Nyssorhynchus) darlingi* using granular formulation of *Bacillus sphaericus* in abandoned gold-miners excavation pools in the Brazilian Amazon rainforest. Revista da Sociedade Brasileira de Medicina Tropical. 2013;**46**(2):172-177

Chapter 13

Malaria Eradication in the European World: Historical Perspective and Imminent Threats

Evangelia-Theophano Piperaki

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76435

Abstract

Malaria was introduced to Europe from the southeast during the Neolithic period and subsequently became established throughout the continent, due to the combination of favorable geomorphological and climatic conditions with the presence of adequately sized human and competent vector populations. *Plasmodium vivax*, *P. malariae* and *P. falciparum* all occurred in various areas of the continent, transmitted by numerous *Anopheles* species, mainly *An. atroparvus* in the northwest, *An. labranchiae* and *An. sacharovi* in the south. The height of malaria endemicity in the Early Modern Age was followed by decline in the twentieth century, particularly in the northwest, owing mainly to man-made contraction of vector breeding sites and improvement of living standards. Eradication was accomplished in 1974 through widespread drug treatment and residual insecticide spraying. Since then, despite the sustained presence of competent vectors and numerous malaria cases imported by travelers and immigrants, autochthonous transmission has been sporadic in Europe, probably due to prompt diagnosis and treatment afforded by robust healthcare services. Current and projected climatic conditions are conducive to malaria transmission, particularly vivax malaria, in several areas of Southern Europe. Moreover, the continuing immigration crisis may facilitate the buildup of an infectious parasite reservoir in the area. Although malaria resurgence is currently unlikely particularly in northwest Europe, it is of crucial importance to maintain disease awareness, diagnostic and clinical competence and robust public health infrastructure for surveillance and vector control to diminish the possibility of malaria transmission in Europe's most vulnerable areas.

Keywords: malaria, Europe, history, resurgence, immigration, mosquito vectors

1. Introduction

Malaria, the most notorious parasitic disease of mankind, has accompanied humanity probably since prehistoric times and has shaped the course of human history over the centuries. It is still a major infectious disease today, causing more deaths by far than all other parasitic diseases combined, while nearly half of the current human population lives in areas of ongoing transmission. According to the World Health Organization (WHO) in 2016, 216 million malaria cases occurred worldwide (uncertainty range 196–263 million) resulting in 445,000 deaths. At present, 90% of all cases and 91% of all malaria deaths (of which 70% are in children under 5 years) occur in the WHO African Region [1]. Five species of the parasitic protozoan *Plasmodium* cause malaria: *P. falciparum*, *P. vivax P. ovale, P. malariae* and *P. knowlesi.* The ecology of human malaria species is closely linked to geomorphology and climatic conditions, particularly temperature and rainfall, which affect both the vector species and the parasites themselves, while malaria epidemiology is influenced by additional factors relating to the human host, such as population density and susceptibility to infection. Ronald Ross developed the original mathematical models for malaria transmission in the early twentieth century and his equations were refined by George McDonald and several mathematicians and other scientists for over 70 years. These models expanded further using epidemiological and entomological data from field studies, eventually evolving into the Ross-MacDonald theory of mosquito-borne disease transmission [2]. These equations still form the basis of our understanding of malaria transmission and, despite some rather simplifying assumptions they contain, they epitomize its most important determinants. In one of them MacDonald defined the concept now called the basic reproduction number R_0 as the expected number of secondary infections resulting from a single infection in a completely susceptible population. He denoted it as follows [3]:

$$R_0 = ma^2 bp^n / -r \ln p \tag{1}$$

where m is defined as *Anopheles* mosquito abundance relative to the human population, a describes the inclination of a vector to bite a human host, b represents the proportion of infective mosquito bites, p is defined as the probability the mosquito will survive a day, n is defined as the duration of the extrinsic incubation period, that is, time needed for parasite development within the mosquito and r is the recovery rate of the human host. If R_0 is greater than one, the number of infected people increases, and if it is less than one, that number declines. In terms of these models, one can surmise that for malaria to invade and remain established in a certain area, where geomorphology, temperature and precipitation favor parasite, and vector survival and development, an adequately sized susceptible human population must coexist with efficient malaria vectors. Efficiency depends on mosquito abundance, life span, predilection for feeding on human host and susceptibility to infection by the various *Plasmodium* species. These determining factors for malaria risk in a given area were described in detail within the definitions of receptivity, vector infectivity and vulnerability. Each of these factors and their respective roles in the establishment and the eradication of malaria from the European World are briefly discussed.

Several *Anopheles* species capable of transmitting malaria exist in Europe. Species belonging to the *Anopheles maculipennis* Subgroup (Diptera, Culicidae) are widely distributed and exhibit

variable susceptibility to infection by *Plasmodium* spp., due to differences in behavioral patterns and feeding preferences. This subgroup includes 10 species among which 3 are important malaria vector species, that is, *An. atroparvus* (van Thiel 1927) in most of western, northern and central Europe, *An. labranchiae* (Falleroni 1926) and *An. sacharovi* (Favre 1903) in the south and southeast. Other species, occurring in Europe that are considered as malaria vectors of minor importance, include *An. messeae, An. maculipennis s.s. and An. melanoon* of the Maculipennis Subgroup, while some are of little significance such as *An. algeriensis, An. claviger, An. plumbeus, An. superpictus* to cite a few [4, 5]. The most important geographical features regarding malaria ecology in Europe were the plains and coastal marshes in all areas of the continent. Southern Europe is comprised of mountainous peninsulas, separated by stretches of sea that make up the Mediterranean. In each of these, mountain ranges divide plains, where water from rainfall tended to collect and frequently stagnate, forming marshes on its way to the sea. Coastal marshlands also existed all around the North Sea basin while there are numerous river valleys and flood plains in Central and Northwest Europe. European climatic conditions, particularly the extreme seasonality of temperate climate, presented a substantial challenge for *Plasmodium* species during their gradual spread across the continent, although the distribution of *P. vivax* in Europe until the twentieth century extended as north as Southern Finland [6]. Optimal temperature ranges for parasite development within the mosquito are 16–33 and 18–33°C for *P. vivax* and *P. falciparum,* respectively [7]. Evidently, European winter temperatures were not conducive to parasite development. Temperate *Plasmodium* strains circumvented this problem by employing biological mechanisms to ensure survival and maintain transmission during the winter. *P. vivax* relies primarily on dormancy in the human liver in the form of hypnozoites, whereas *P. malariae* on prolonged incubation and persistent low-level parasitemia for years or even decades. An additional opportunity for continued parasite survival and transmission was the overwintering of female mosquitoes indoors, in the warmth of animal shelters and human dwellings.

2. Introduction, rise and decline of malaria in Europe

It is most likely that malaria (caused by *P. vivax, P. malariae* and *P. falciparum*) was introduced into the area around the Mediterranean and thence to the rest of Europe during the Neolithic period (4000–3000 BC). *P. falciparum's* histidine-rich protein 2 was detected in Egyptian mummies from 3200 BC confirming its presence in the outskirts of the Mediterranean world in the fourth millennium BC, suggesting the known contact between Egypt and Greece in the Early Bronze Age as one possible route of its introduction to Europe. Another possibility is that all three species of *Plasmodium* made the crossing within human hosts, as populations of Neolithic farmers migrated to Europe from the Near East, the wetlands and plains of today's Israel, Palestine, Syria and Jordan, where agriculture is believed to have developed [8].

Attempts to trace the history of malaria in ancient Greece before the fifth century BC are plagued by shortage of evidence and confounded by difficulties in its interpretation. There is an interesting passage in Homer's Iliad in which Achilles hurrying to avenge Patroclus' death is compared to the bright star that rises in late July (Sirius) and heralds the arrival of fever (greek "πυρετός") for wretched mortals. Although impossible to prove that this is an early reference

to malaria, the association with the harvest calls to mind the name "aestivo-autumnal fever" frequently used for *P. falciparum* malaria by doctors many centuries later. However, the clearest references from historic sources that can be attributed to malaria are those to fevers with tertian and quartan periodicities. Hippocrates in the fifth and fourth centuries BC and Celsus in the early first century AD described two types of tertian fever (tertian and semitertian) with symptoms corresponding to vivax and falciparum malaria respectively and a quartan fever corresponding to infection by *P. malariae*. The association with swamps was also common knowledge since the time of Hippocrates' treatise "Airs, Waters, Places" although the connection with mosquitoes was not made, possibly because of the existence of regions where there was no malaria although mosquitoes were abundant. *P. vivax* was always the most important species in the area, as its life cycle characteristics and adaptations were well suited for transmission in the seasonal climate of the Mediterranean, but malaria due to *P. falciparum* and `*P. malariae* occurred as well, unlike *P. ovale* which is absent from the Mediterranean region. Vivax malaria appeared in spring and early summer, followed by falciparum malaria, which peaked in late summer and autumn. Periodic epidemics were also observed in areas of endemicity. Malaria was well known at the time of the Roman Empire. The Pontine Marshes, a 30 km^2 marshland area to the southeast of Rome in central Italy, was a haven for the disease for almost two millennia. The word "malaria" itself, meaning "bad air" (mal'aria) is Italian in origin and reflects the miasmatic theory on the nature of the disease. Julius Caesar, who had suffered from quartan fever himself when he was young, had ambitious plans to drain the Pontine Marshes and claim the land for agriculture, a feat that was finally accomplished by Mussolini, on the eve of World War II. Eminent Byzantine physicians, such as Oribasius and Paul of Aegina, provide information on fevers during the Middle Ages in the Eastern Roman Empire (Byzantium) [9].

P. vivax and *P. malariae* gradually made their way to the north-west and the north of the continent, probably through France and Spain. Little is known about the first centuries of the first millennium AD, but there can be no doubt that malaria was present in northwest Europe from the middle Ages until the nineteenth century. During the medieval climatic optimum, near the end of the twelfth century, increased temperatures and associated rise in sea level probably favored the spread of malaria northward. The coastal flood plains and marshlands in the Iberian Peninsula, France, England, the Netherlands, North Germany, Denmark, Scandinavia and Russia were most affected. A significant vector for coastal areas was the zoophilic *An. atroparvus*, a widely distributed species. Once established on the coast, malaria strains could move inland along river valleys, floodplains and swamps, possibly transmitted by inland mosquito species, such as *An. messeae*. Assessment of disease incidence and mortality from historical records is difficult. Nevertheless, evidence that malaria was the likely cause of fever in European coastal marshes can be gathered from accounts of disease symptoms, such as intermittent fevers (tertian and quartan), anemia and splenomegaly and by its seasonal pattern. Such descriptions exist in Anglo-Saxon medical texts dating back to the ninth century and can be found in accounts from north Germany, Denmark and the Netherlands. *P. malariae* is believed to have been endemic along the river banks of the Rhine, Rhone and Danube, along the eastern and southeastern English coast, and in the Netherlands and as far north as Sweden and Finland in the thirteenth century [10]. It appears that falciparum malaria was

rarely encountered in northwest Europe and when it did, it was probably imported from the south and could not really take root, as its causative agent, *P. falciparum* did not survive within the human host for more than a year and was poorly adapted to local mosquito species.

Malaria reached the height of its endemicity in northwest and north Europe in the Early Modern Age (late fifteenth and early sixteenth centuries), mainly due to high population densities in coastal areas and a variety of human interventions, like the construction of embankments, floodgates, canals and harbors. Surprisingly, the increase in disease frequency occurred despite a general drop in temperature, which began halfway into the sixteenth century, lasted for 150–200 years and was so pronounced that it was termed the Little Ice Age. The area of malaria distribution was the greatest it had ever been at the end of the nineteenth and the beginning of the twentieth centuries. At that time, malaria was highly prevalent in southern Europe while its northernmost limit ran along the 64° N parallel from central England to southern Norway, central Sweden and Finland and Northern European Russia.

The disappearance of malaria from Europe progressed from northwest to southeast and was the result of various contributing factors, including environmental changes, ecological and social developments, introduction of effective treatment and concerted human control efforts. One of the most important factors in lowering the prevalence of malaria is considered to have been the habitat separation of humans from cattle [6]. Malaria transmission in Europe has always been predominantly unstable, due to environmental, climatic and vector biology factors including strong zoophilic behavior. In such an epidemiological setting, it was relatively easy for human interventions to tip the fragile human-parasite balance against the parasite, leading to disease elimination.

The drainage, reclamation of swampland and improvements in water management in the fields drastically reduced the availability of mosquito breeding sites resulting in reduced mosquito populations in many areas. Agricultural innovations led to better human overall health and an increase in animal populations. Animals were fed during the winter and kept in stables, byres and pigsties. These animal shelters and their occupants proved much more attractive for the main vector of malaria in most of Europe, the zoophilic *An. atroparvus*, diverting mosquitoes from the nearby human dwellings. Improvements in house construction and living standards played a significant role as well. Buildings where human living quarters and animal shelters were found together progressively disappeared. This human-domestic animal habitat separation played a major role in the decrease of malaria transmission. Finally, the extensive use of cinchona bark brought to Europe in the 1600s and the introduction of quinine in the 1820s further moderated disease transmission.

Nevertheless, in the beginning of the twentieth century, malaria still plagued a significant part of Europe, particularly the south. At the end of the nineteenth century, malaria was affecting approximately 10% of the Italian population, with annual cases in the range of two million and about 15,000–20,000 deaths per year [11]. The situation was somewhat worse in Greece. During his visit in 1906, Ronald Ross was surprised to discover that 65% of the children in the particularly malarious area around lake Copais in Voiotia were suffering from the disease [12]. The Greek-Turkish War in Asia Minor in 1922 and the tragic events that led to a backwave of 1,300,000 Greek refugees were followed by an increase in the outbreaks and the death toll of malaria in the late 1920s. A communication from Greece to the WHO reported that, in

the 1930s, *P. falciparum*, *P. vivax* and *P. malariae* all occurred in the country; the annual attack rate was estimated at 15–30% of the total population, and the mortality rate was 73.7 deaths per 100,000 inhabitants. Treatment required approximately 30 tons of quinine each year [13]. In 1924, the League of Nations established the Malaria Commission to conduct research and strategize the control of malaria. The socioeconomic devastation and mass displacements caused by World War II interrupted the implementation of national elimination programs and destroyed environmental engineering works that had reduced transmission, setting back malaria control efforts. Control interventions, drug therapy and insecticide spraying resumed successfully in the late 1940s, and the World Health Organization certified the achievement of malaria eradication in Hungary in 1963, followed by Spain in 1964, Bulgaria in 1965, Poland and Romania in 1967, the Netherlands and Italy in 1970, Yugoslavia and mainland Portugal in 1973. In 1975, the last focus of indigenous malaria reported from Macedonia in Greece had been extinguished and Europe was malaria free for the first time in history.

3. Malaria in Europe: current situation

Europe, in the most prevalent definition of the term, is distinct from the area currently designated "the WHO European Region" which comprises all countries of the European Union (EU), the Balkans, South Caucasus and Central Asia and the Russian Federation, Israel and Turkey (53 countries in all), some of which do not belong to Europe geographically. The European Centre for Disease Prevention and Control (ECDC) issues annual epidemiological reports on malaria in Europe based on data retrieved from the European Surveillance System (TESSy) that collects, analyzes and disseminates data on communicable diseases that generally originate from national surveillance systems. Malaria is a notifiable disease in the EU and its reporting is compulsory in 24 countries, voluntary in France and Belgium and "not specified" in the United Kingdom (UK). Active disease surveillance is in place only in the Czech Republic, Slovakia, the UK, and in high-risk areas in Greece. The latest available data on the number of malaria cases in EU and European Economic Area (EEA) countries reported to the ECDC are shown in **Table 1**. Nearly all malaria cases (99.9%) are currently imported by international travelers and immigrants [14, 15]. Of the 31 European countries reporting to the ECDC the highest number of confirmed cases in 2014 and 2015 were reported from France (n = 2299 and 2500) and the United Kingdom (n = 1510 and 1397, respectively) (**Table 1**). Imported malaria in these countries was mainly linked to travel to West Africa, particularly for the purpose of visiting friends and relatives residing in countries and European territories endemic for malaria, such as Mayotte and French Guiana [15]. The causative species depends on the area the parasite is imported from and the largest proportion is identified as *P. falciparum* [16]. Similarly, the incidence and species distribution in refugees and immigrants reflects the local epidemiology in their country of origin and along the migration route they followed. Notably, many immigrants prefer to remain unnoticed by the authorities until they reach the country they intend to request asylum from, and therefore a significant proportion of malaria cases in this population, possibly even half of them are thought to remain unreported [17]. Permanent foreign residents that have settled in European countries and regularly return to their malaria endemic country of origin

Countries	2011		2012		2013		2014		2015	
	Cases confirmed (reported)	Rate**	Cases confirmed (reported)	Rate	Cases confirmed (reported)	Rate	Cases confirmed (reported)	Rate	Cases confirmed (reported)	Rate
Austria	7	0.1	28	0.3	42	0.5	68	0.8	81	0.9
Belgium	184	1.7	206	1.9	253	2.3	235	2.1	276	2.5
Bulgaria	8	0.1	16	0.2	8	0.1	10	0.1	20	0.3
Croatia	—	—	23	0.5	0	0.0	6	0.1	7	0.2
Cyprus	6	0.7	1	0.1	3	0.3	8	0.9	3	0.4
Czech Republic	28	0.3	25	0.2	27	0.3	30	0.3	29	0.3
Denmark	—	—	—	—	—	—	—	—	—	—
Estonia	1	0.1	6	0.5	3	0.2	3	0.2	4	0.3
Finland	33	0.6	46	0.9	38	0.7	39	0.7	39	0.7
France	1891	—	1851	—	2165	—	2299	—	2500	—
Germany	0 (562)	0 (0.7)	0 (547)	0 (0.7)	0 (637)	0 (0.8)	—	—	0 (1063)	0
Greece	92	0.8	95	0.9	25	0.2	38	0.3	84	0.8
Hungary	10	0.1	5	0.1	5	0.1	15	0.2	12	0.1
Iceland	—	—	—	—	—	—	—	—	—	—
Ireland	61	1.3	65	1.4	71	1.5	79	1.7	82	1.8
Italy	—	—	—	—	—	—	—	—	—	—
Latvia	4	0.2	3	0.1	4	0.2	6	0.3	1	0.1
Liechtenstein	—	—	—	—	—	—	—	—	—	—
Lithuania	3	0.1	6	0.2	8	0.3	5	0.2	8	0.3
Luxembourg	3	0.6	7	1.3	4	0.7	3	0.5	1	0.2
Malta	1	0.2	2	0.5	5	1.2	3	0.7	7	1.6
Netherlands	253	1.5	194	1.2	162	1.0	276	1.6	340	2.0
Norway	30	0.6	37	0.7	72	1.4	120	2.3	94	1.8
Poland	14	0.0	21	0.1	36	0.1	19	0.0	29	0.1
Portugal	67	0.6	71	0.7	117	1.1	144	1.4	194	1.9
Romania	40	0.2	32	0.2	43	0.2	47	0.2	30	0.2
Slovakia	1	0.0	6	0.1	4	0.1	5	0.1	0	0.0
Slovenia	6	0.3	7	0.3	3	0.1	7	0.3	5	0.2
Spain	405	0.9	421	0.9	518	1.1	688	1.5	706	1.5
Sweden	95	1.0	85	0.9	119	1.2	354	3.7	250	2.6
United Kingdom	1677	2.7	1378	2.2	1501	2.3	1510	2.3	1397	2.2

Countries	2011		2012		2013		2014		2015	
	Cases confirmed (reported)	Rate**	Cases confirmed (reported)	Rate	Cases confirmed (reported)	Rate	Cases confirmed (reported)	Rate	Cases confirmed (reported)	Rate
EU/EEA	4920 (5482)	0.8 (1.0)	4637 (5184)	0.7 (0.9)	5236 (5873)	0.8 (1.0)	6017	1.0	6199	1.0

*ECDC = European Centre for Disease Prevention and Control.

**Rate denotes number of cases per 100,000 population. Reporting in France is voluntary and surveillance coverage is not nationwide.

Note: • 99.9 and 99.8% of cases for which travel information was provided were travel related in 2014 and 2015 respectively.

Source: [14, 15].

Table 1. Malaria cases in the European Union (EU) and European economic area (EEA) reported to the ECDC* during 2011–2015: confirmed cases (reported cases) and rate per 100,000 population.

to visit friends and relatives (also known as VFRs) are currently the most significant high-risk population for malaria importation, for geographic and behavioral reasons. Specifically, they visit endemic areas frequently, often stay in rural areas with poor health infrastructure for longer periods than tourists do, do not usually seek pre-travel medical advice and have poor compliance with malaria chemoprophylaxis and protection measures. *P. falciparum* and much less so *P. ovale* and *P. malariae* are usually imported from sub-Saharan Africa, particularly West Africa, whereas *P. vivax* from Asia and areas of South America. *P. falciparum* is usually detected shortly after the patient's arrival, due to its prominent clinical presentation, whereas *P. vivax* and *P. malariae* might remain undetected for a significant amount of time. Obviously, the possibility that individuals infected with malaria may remain undetected for several months after arrival to Europe could be a significant risk factor for local transmission, particularly regarding *P. vivax* for which competent vectors are still widely distributed across the continent.

Europe has been considered malaria free since 1975, as was the rest of the WHO European Region at that time, except for Turkey. However, in the late 1980s and early 1990s, autochthonous malaria transmission chiefly due to *P. vivax* resumed in the Transcaucasian countries, the Central Asian republics and less so in the Russian Federation, most likely due to mass population movements, socio-economic challenges, agricultural and developmental schemes and the neglect of malaria prevention and control services. The Roll Back Malaria (RBM) Initiative in 1998 and the Tashkent declaration for "The move from malaria control to elimination" in 2005 [18] seem to have successfully reached their targets. According to WHO, the WHO European Region reported zero indigenous malaria cases in 2015, thus achieving its set goal of disease elimination [19]. As far as the European continent itself is concerned, since the late 1990s, sporadic autochthonous malaria cases occurred in several countries, caused by infection of local mosquitoes by travelers or immigrants from endemic regions. Locally transmitted malaria cases have been reported in Spain [20], Germany [21], the Netherlands [22], France [23], Italy [24] and Greece [25]. More recently, 5 cases were reported to the ECDC as locally acquired in 2014, 7 in 2015, 10 cases in 2016 and 17 in 2017 recorded from 8 different countries (**Table 2**). Epidemiology and modes of transmission included congenital transmission from mothers

infected in an endemic country, induced malaria following a transplant from a donor who had traveled to an endemic country, introduced malaria due to residence near imported cases and "suitcase malaria" [26–28]. Among the locally acquired cases in 2017 included a fatal case of falciparum malaria in a four-year-old diabetic girl in Italy. Epidemiological investigations identified hospitalization of this case along with two other patients infected with *P. falciparum*

Year	Total cases	Country (no. of cases)	Parasite species	Mode of transmission-epidemiology
2014	5	France (2)	Unspecified	Undocumented residents, travel to endemic region possible
		Spain (3)	*P. falciparum*	Congenital (mother originally from Equatorial Guinea)
			P. malariae	Induced (kidney transplant from traveler to Equatorial Guinea)
			P. vivax	Introduced (residence near imported case)
2015	7	Greece (6)	*P. vivax*	Mosquito-borne, autochthonous in receptive rural areas, presence of patients from endemic countries in the area
		Belgium (1)	*P. falciparum*	"Suitcase malaria"
		Netherlands (1)	*P. vivax*	Congenital (mother: an Eritrean refugee)
2016	10	Greece (6)	*P. vivax*	Mosquito-borne, introduced
		France (2)	Unspecified	Mosquito-borne, introduced or airport malaria
		Spain (1)	Unspecified	
		Lithuania (1)	Unspecified	
2017	17 (until 15/12/2017)	France (2)	*P. falciparum*	Mosquito-borne, in the area where *P. falciparum* malaria imported from Burkina Faso occurred
		Greece (7)	*P. vivax* (6)	Mosquito-borne, introduced
			P. falciparum (1)	Nosocomial, mosquito-borne or iatrogenic (not transfusion), patient recently hospitalized in ward where a patient was treated for *P. falciparum* malaria.
		Italy (5)	*P. falciparum* (1)	Mosquito-borne or nosocomial, fatal, 4 years old diagnosed with diabetes mellitus; two patients infected with *P. falciparum* were hospitalized in the same ward
			P. falciparum (4)	Mosquito-borne, patients originally from Africa
		UK (3)	*P. vivax*	Mosquito-borne, contracted in Northern Cyprus

Source: [26–28].

Table 2. Locally acquired malaria cases in Europe during 2014–2017.

in the same ward. Another case of potentially nosocomial *P. falciparum* transmission was reported from northwest Greece.

4. Risk of malaria re-emergence in Europe

The possibility of malaria re-emergence in Europe in the face of climatic and demographic changes was renewed in the 2000s partly aroused by reports of epidemics in neighboring Turkey and central Asia. The risk of malaria introduction in a given area, also known as its malariogenic potential, depends on three characteristics: receptivity, vector infectivity and vulnerability [29].

Receptivity depends on the presence of a competent vector, and ecological and climatic conditions, conducive to vector survival and proliferation. Vectorial capacity is determined by mosquito population density, life span, feeding preferences and duration of parasite development (sporogony). Several *Anopheles* species capable of transmitting malaria are still abundant across Europe. The geographical distribution of the European dominant malaria vector species and their main bionomical characteristics are shown is **Figure 1** and **Table 3**, respectively. The most widely distributed belong to the *Anopheles maculipennis* Subgroup. Historically, *A. atroparvus* was the primary malaria vector in most of northern, western and central Europe [30]. It occurs along the coast of the Atlantic Ocean, from south Sweden to Portugal and Spain, around the Baltic Sea and variably in central Europe and the Balkans. Its distribution in Europe has contracted with the disappearance of coastal marshlands and increasing water pollution. *An. labranchiae* and *An. sacharovi* were the most important malaria vectors in southern Europe, the Balkans, Italy and Greece. *An. labranchiae* has been reported from Corsica, the coastal areas of Italy, Sardinia, Sicily, the Istrian Peninsula and the Dalmatian Coast of Croatia; it also occurs in North Africa. Once endemic in southern coastal Spain, *An. labranchiae* has since disappeared after abandonment of rice cultivation and desiccation of wetlands. *An. sacharovi* is encountered in Greece, the Balkans, south Russia, Turkey and the Middle East [6, 31, 32]. Other species considered malaria vectors of minor importance are also currently encountered in Europe. Some belong to the *An. maculipennis* Subgroup (*An. messeae, An. maculipennis s.s. An. melanoon*); others included *An. algeriensis, An. claviger, An. hyrcanus, An. plumbeus, An. superpictus*. The eradication campaigns of the twentieth century led to severe reduction of *Anopheles* numbers but failed to achieve complete eradication. Over time, in certain areas, *Anopheles* populations recovered to initial levels giving rise to the phenomenon known as "anophelism without malaria" which essentially signifies the presence of *Anopheles* mosquitoes in formerly malarious areas of Europe where malaria no longer occurs.

The climate of south European countries around the Mediterranean Sea, characterized by mild-wet winters and hot-dry summers, is suitable for malaria transmission, whereas in northern Europe ambient temperatures permit outdoor parasite development only during the summer. To the extent that climate change can be predicted with any degree of certainty, it is

http://dx.doi.org/10.5772/intechopen.76435

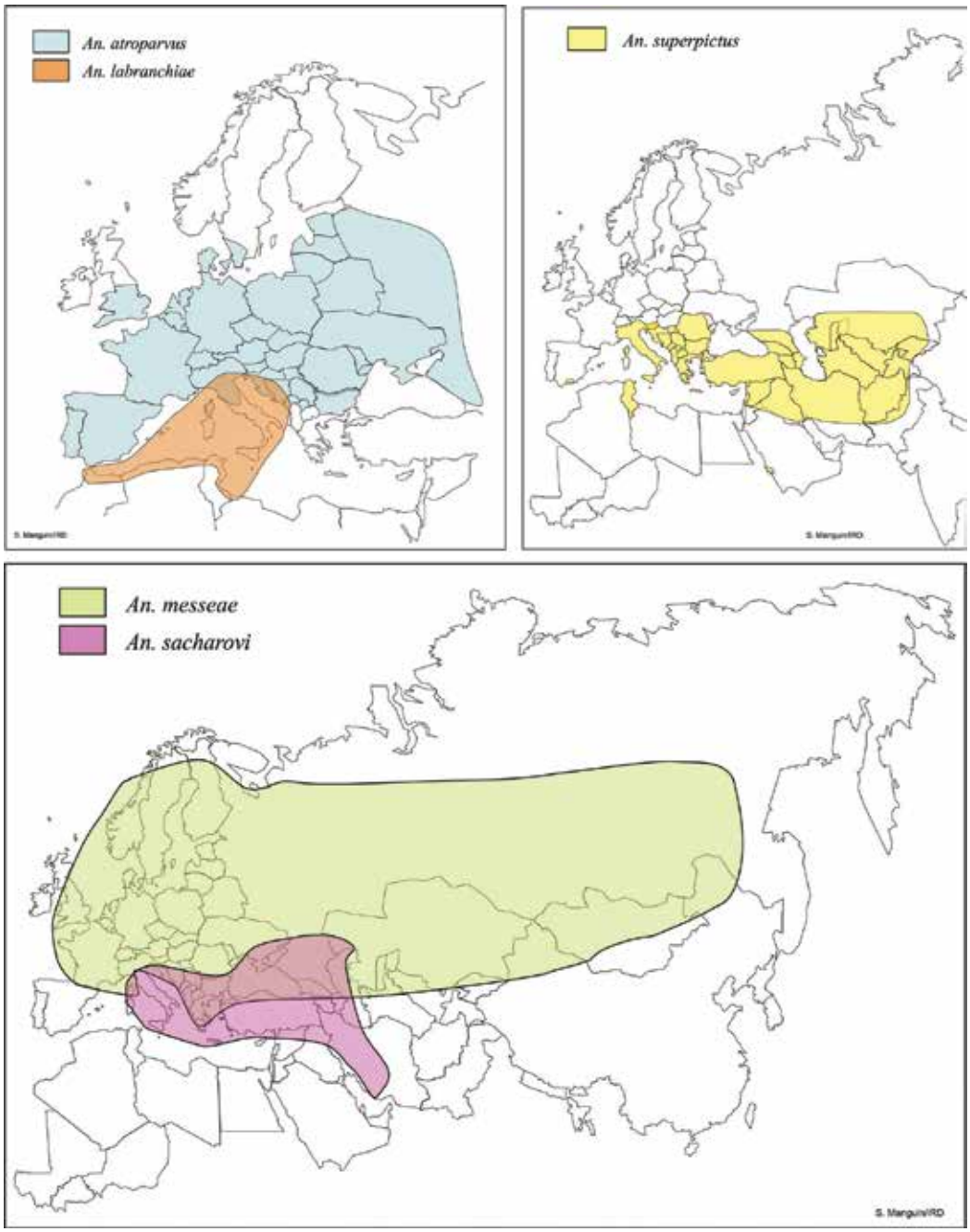


Figure 1. Geographical distribution of dominant malaria vector species in Europe. A: *An. atroparvus* (blue), *An. labranchiae* (orange), B: *An. superpictus* (yellow), C: *An. messeae* (green) and *An. sacharovi* (purple). Source: updated maps from Ref. [6].

Species	Breeding habitats	Feeding	Resting habits	Overwintering	Susceptibility to *P. falciparum*
***An. atroparvus* van Thiel**	Fresh water, brackish and tolerates saltwater. Marshes, ditches, ground pools, river margins, streams, rock pools, rice fields, even used tyres, sun-exposed habitats.	Opportunistic feeder: mostly zoophilic, but anthropophilic too, Exophagic.	Exophilic	Hibernation of female incomplete (periodic feeding), gonotrophic disassociation (without oviposition)	Refractory to Asian and African *P. falciparum* but competent in supporting a European strain
***An. labranchiae* Falleroni**	Mainly freshwater habitats, occasionally brackish water and lagoons. Warmer environment than *An. atroparvus*. Sunlit rock holes, pits, ditches, drains, canals, slow flowing streams/ rivers, ground pools, ponds, lakes, rice fields.	Opportunistic: zoophilic, but anthropophilic too	Mostly exophilic	Hibernation of female incomplete (with occasional blood feeding without ovipositioning) and complete (without feeding and nongonoactive)	Refractory to tropical *P. falciparum* strains Historical evidence of natural infection with European strains Experimental evidence of infection with some strains of African *P. falciparum*
***An. messeae* Falleroni**	Shaded, clear, still or slow flowing fresh water, Lake margins, marshes, swamps, ditches.	Mostly zoophilic, exophagic	Endophilic	Hibernation of female with full diapause, do not feed during the winter	Refractory to tropical *P. falciparum* strains
***An. sacharovi* Favre**	Brackish and fresh still or flowing water. Sunlit sites with aquatic vegetation such as swamps, marshes, river margins, springs, seepages, pools, ditches, irrigation canals, small water collections.	Opportunistic feeder but mainly anthropophilic; exophagic and endophagic	Endophilic	Hibernation of female incomplete (periodic feeding), gonotrophic disassociation (without oviposition)	Essentially refractory to tropical P. falciparum strains (inconclusive experimental results) Historical evidence of natural infection with European strains
***An. superpictus* Grassi**	Brackish and fresh still or flowing water in full sunlit. Small pools in river beds, irrigation canals, storage tanks, rice fields, ditches, borrow pits.	Mostly zoophilic and exophagic	Mostly endophilic	No information	No information

Source: [6, 32].

Table 3. Bionomical characteristics of the European dominant malaria vector species.

expected to encompass changes in temperature, precipitation and the intensity and frequency of extreme weather phenomena. By the end of the twenty-first century, with continuing temperature increase, fewer cold and more frequent hot temperature extremes are projected to occur on daily and seasonal timescales, while heat waves are likely to last longer and occur more often. It is estimated that many regions will probably experience more frequent and intense extreme precipitation events [33]. Occurrence of higher temperatures for longer periods during the summer may increase chances for malaria transmission in certain previously inhospitable areas. Malaria endemicity however is not simply a matter of the right temperature. Climate change is but one element in a complex epidemiological setting and other components such as human activity are probably more important determinants.

Infectivity reflects the vector competence to replicate and transmit a particular *Plasmodium* species or strain. Replication is assessed by the presence of oocysts in the mosquito midgut and capability to transmit is determined by the presence of sporozoites in its salivary glands. European *Anopheles* exhibit variable sensitivity to *Plasmodium* strains from malaria endemic regions. Members of the *An. maculipennis* Subgroup have been found capable of developing *P. vivax* sporozoites following an infected blood meal, but competence is difficult to evaluate given the absence of reliable *P. vivax* gametocyte culture. On the whole, although there is substantial knowledge on the vectorial potential of numerous tropical and subtropical mosquito species, corresponding data on European indigenous species are scarce [34]. Earlier studies have shown European *An. atroparvus* and *An. labranchiae* populations to be refractory to infection by tropical strains of *P. falciparum*, although not universally [35–38]. *An. labranchiae* has been an important malaria vector in the central and western Mediterranean, where both *P. vivax* and *P. falciparum* occurred in the past, and there is historical data from the early twentieth century confirming the existence of naturally infected *Anopheles* in the area, however, without specifying the species. A recent experimental study reported that *An. maculipennis s.l.* from Corsica were successfully infected with the NF54 African strain of *P. falciparum*; furthermore, sporozoites were detected in the salivary glands of some mosquitoes, indicating they were capable of transmission, albeit with very low competence [32]. *An. labranchiae* is thought to have been involved in autochthonous transmission of vivax malaria in southern Italy and possibly in Corsica in 2011 and 2006, respectively [39, 23].

A species that has recently become the focus of increasing attention is *An. plumbeus* (Stephens 1828). It is widely distributed all over Europe (except in the far north regions), the Middle East and North Africa. *An. plumbeus* was originally known as a dendrolimnic species, encountered in forests and breeding almost exclusively in tree holes. Recent reports indicate that it has been adapting to human-made habitats, such as abandoned animal shelters, artificial water containers, septic tanks, sewage ditches, rainwater and liquid manure pits, and that it is becoming increasingly common in suburban and urban environments. It overwinters as an egg or larva, has a relatively long-life span (up to 2 months) and is an avid biter of reptiles, birds and mammals, while some populations have exhibited high anthropophily [30]. Experimental studies have shown it to be a competent vector for both *P. vivax* and *P. falciparum* [40–42]. Furthermore, it has been implicated in indigenous vivax and falciparum

malaria transmission in England and Germany, respectively [21, 43]. Its potential role as a vector under changing climatic conditions and availability of infected human reservoir can only be speculated at present.

Vulnerability depends on the introduction and maintenance of a human reservoir that can transmit the parasite gametocytes to the mosquito populations. A patient suffering from malaria becomes infective for mosquitoes upon the appearance of *Plasmodium* gametocytes in the peripheral blood. In *P. vivax* malaria, gametogenesis occurs early in the course of the infection, within 3 days from the onset of clinical disease, whereas in *P. falciparum* malaria gametocytes usually appear 10 days after blood invasion. Therefore, a patient with vivax malaria is usually infective to mosquitoes frequently even before presenting for medical assistance. The additional delays in diagnosis and treatment, which are common in non-endemic countries, allow more time for transmission of the parasite to the local *Anopheles* populations.

In the past few years, Europe has witnessed a dramatic increase in the number of refugees and migrants, which peaked in 2015, with 1,257,000 asylum applications, double that of the previous year, a trend that continued in 2016 and 2017 [44]. Recently, a substantial increase in the incidence of vivax malaria has been recorded in refugees seeking asylum in European countries. More specifically, from spring 2014 to summer 2015, 37 cases of vivax malaria were diagnosed in newly arrived Eritrean refugees in Germany. Notably, their treatment was complicated by relapses due to difficulties in procuring primaquine for hypnozoite eradication, as the drug was not licensed in Germany [45]. During the same time, 105 malaria cases were recorded in Eritrean refugees in Sweden, of which 84 were due to *P. vivax* [46]. It is speculated that the refugees contracted the disease either at home or somewhere along their route from Eritrea through Ethiopia and Sudan. Interestingly, a cluster of 15 vivax malaria cases in Eritrean refugees was observed in 2010 in Israel [47].

According to data from the European Agency for the Management of Operational Cooperation at the External Borders of the Member States of the European Union (Frontex), a truly explosive increase occurred in 2015 at the Eastern Mediterranean route with 885,386 migrants arriving in Europe through Turkey, compared to 50,830 the previous year. These originated mainly from Syria, followed by Afghanistan and Somalia, and landed on Greek Islands, primarily Lesbos [48].

At present, it is not feasible to make specific, valid predictions as to where malaria might re-emerge, based on existing data. This occurrence will probably be determined by numerous factors besides vector presence, abundance and susceptibility to infection, *inter alia* possible climatic changes in the future, human interventions and population movements among others, none of which can be predicted with any degree of certainty. Recent experience has shown that indigenous cases and outbreaks in Europe typically occur around immigrants or travelers from endemic areas. Neither the settlement location nor the duration of stay of the various migrant populations is predictable, particularly in view of the uncertainty of the current migration crisis. Notably, the potential repercussions of an infectious human reservoir build-up in a previously endemic area conducive to malaria transmission were illustrated by a malaria outbreak experienced in Greece in recent years. Since malaria eradication in Greece in 1974, cases were mostly imported, with a few sporadic reports of autochthonous transmission in 1991, 1999 and 2000 [49]. Since 2009,

however, locally acquired *P. vivax* malaria cases and clusters began to appear consistently almost every year, peaking in the 2011 outbreak, when 42 cases were reported from several foci around the country [50]. Five potential malaria vectors including *An. sacharovi* occur in Greece whereas the country's geomorphology and climate allow for temporary and permanent mosquito breeding sites. The period from May to October has been established as the most favorable for mosquito infectivity and transmission in the area. An important contributing factor for increased vulnerability and malaria occurrence was the presence of migrant farm workers from Pakistan and Afghanistan in the affected areas. Many of these resided in poor housing conditions, situated close to mosquito breeding sites. Moreover, if they fell ill they were often reluctant to utilize the freely available healthcare services, due to their frequently illegal status, thus increasing the chance of parasite transmission to local *Anopheles* populations [50]. *P. vivax* isolates from the affected areas were genotyped revealing a number of different strains [51]. Furthermore, there was indication of sustained transmission for two consecutive years at least in one focus; an observation that has truly unsettling implications in light of the current financial depression and immigration crisis the country is facing [51]. The Greek public health authorities initiated control efforts that focused on training of medical professionals to ensure early detection and treatment, vector control, surveillance, active case detection and public education. Finally, a mass drug administration program to immigrants living in the affected areas was also implemented [52]. The CDC has recently issued guidelines for the presumptive pre-departure treatment of asymptomatic malaria in refugees from sub-Saharan Africa [53]. Similar measures could be implemented for immigrants arriving in Europe from malaria endemic countries, which should include screening for malaria among the newly arrived to prevent clinical malaria in this population and curtail the possibility of transmission to local mosquitoes. An additional measure one should take into account when considering malaria prevalence in immigrants, is the existence of asymptomatic individuals with sub-microscopic *P. falciparum* parasitaemia, and the fact that asymptomatic carriers of *Plasmodium vivax* liver hypnozoites are impossible to detect with any of the currently available methods.

There is no common malaria treatment policy currently adopted by all European countries. Treatment regimens are based on WHO recommendations and vary from country to country, occasionally even between centers within the same country. There is extensive heterogeneity in the management of imported falciparum malaria in Europe for which discussions toward a consensus for management standardization of malaria might be beneficial [54]. *P. falciparum* susceptibility to antimalarials is not assessed in the laboratory; rather it is extrapolated based on the geographical origin of the infecting strain, and national or WHO recommendations are followed accordingly [55, 56]. The issue of antimalarial drug resistance does not constitute an imminent threat for Europe. If any, it might constitute a threat to individual patient health, chiefly when imported *P. falciparum* is involved, but given that disease prevalence in Europe is extremely low (even taking immigrants into account), this issue currently has no public health relevance.

Regarding the susceptibility to insecticides of European putative and confirmed malaria vector species in countries where malaria is not endemic, data originates from small-scale studies and is limited [57, 58]. As of this date, there is no systematic report on the status of *Anopheles* susceptibility to insecticides in the European Region.

5. Concluding remarks

Increasing concern about emerging infectious diseases has rekindled scientific and public interest in malaria. Reminders of widespread malaria endemicity across Europe in the past, the continuing presence of known and emerging vectors and the reality of a substantial population influx—including potential parasite carriers—from endemic areas combined with projections of climate change have raised the question of a possible re-emergence of malaria foci in the continent. Taking geomorphological, climatic and entomological factors into account, the risk of malaria resurgence appears to differ in various parts of Europe. In the northwest, manmade environmental changes in housing and livestock farming has led to continuing loss of breeding sites for *An. atroparvus*, the major vector in the area. In the event of a temperature rise in the region, mosquito survival would increase and *Plasmodium* sporogony would be facilitated, but the scarcity of mosquito vectors and the tendency of relevant species to preferentially feed on animals create an epidemiological setting where there is practically no considerable threat of renewed autochthonous transmission. *An. plumbeus*, with its reported adaptability to urban habitats and increased anthropophily could assume a more epidemiologically significant role as a vector in the future. Even so, however, provided that healthcare retains its current high standards, timely treatment of patients would prevent the buildup of an infectious human reservoir, thus preventing establishment of the parasite in the local mosquito populations. An influx of human gametocyte carriers could result in limited local transmission around untreated patients, which would be spatially and temporally restricted, provided of course that local healthcare services are aware of the risk and effective in early case detection and treatment.

Regarding Southern Europe, there can be no doubt that current climatic conditions are favorable for malaria transmission in selected areas, where competent mosquito vectors like *An. labranchiae* and *An. sacharovi* are also present in epidemiologically significant densities. The recent occurrence of sporadic autochthonous cases and minor outbreaks has demonstrated that previously endemic malaria parasite species, principally *P. vivax,* are still theoretically transmissible in the area. A future temperature rise might expand vector distribution and abundance, increasing the risk for malaria transmission in the long run, but such a change is unlikely to develop overnight. However, two variables that could unpredictably influence vulnerability south of Europe are changing rapidly, that is, population movement and economic hardship. It was only 20 years ago that Turkey and central Asia experienced epidemic malaria resurgence from small residual reservoirs, demonstrating the catalytic impact mass population displacement and socioeconomic upheaval could have on malaria epidemiology in vulnerable areas. Europe is currently witnessing an unprecedented influx of immigrants from malaria endemic areas, many of which are asymptomatic carriers of dormant *Plasmodium* forms. It is believed that the highly organized and efficient European healthcare services can avert malaria re-establishment through prompt diagnosis and treatment, provided that they maintain their current high operational standards. However, nowadays malaria is being imported into Europe through areas severely affected by economic recession, which is putting an increasing strain on available health resources for natives and migrants alike. Therefore, although the resurgence of malaria in Europe is currently unlikely, it is crucially important to improve, maintain and financially support disease awareness, diagnostic expertise, clinical competence, sustained surveillance and vector control to ensure that malaria is not allowed a foothold in the European continent.

Finally, it would be remiss not to mention that malaria history has repeatedly demonstrated the precariousness of malaria control. To quote Bruce-Chwatt and de Zulueta [59] "… *any deterioration of organized services by a major catastrophe or war may bring back to Europe a series of communicable diseases among which malaria would not be the last*." Indeed "…*the simple truth is that there will be no safety from any infectious disease as long as vast reservoirs of pathogens remain in parts of our shrinking world in which the Atlantic and the Pacific Oceans are figuratively demoted to the status of intercontinental rivers."*

Author details

Evangelia-Theophano Piperaki

Address all correspondence to: epiper@med.uoa.gr

Department of Microbiology, Medical School, National and Kapodistrian University of Athens, Athens, Greece

References

[1] WHO. World Malaria Report. 2016. Available from: http://www.who.int/malaria/publications/world-malaria-report-2016/report/en/ [Accessed: 2017-11-29]

[2] Smith DL, Battle KE, Hay SI, Barker CM, Scott TW, et al. Ross, Macdonald, and a theory for the dynamics and control of mosquito-transmitted pathogens. PLoS Pathogens. 2012;**8**(4):e1002588. DOI: 10.1371/journal.ppat.1002588

[3] Macdonald G. The Epidemiology and Control of Malaria. Oxford: Oxford University Press; 1957

[4] Van Thiel PH. On zoophilism and anthropophilism of *Anopheles* biotypes and species. Rivista di Malariologia 1939;**18**:95-124

[5] Kuhn KG, Campbell-Lendrum DH, Davies CR. A continental risk map for malaria mosquito (Diptera: Culicidae) vectors in Europe. Journal of Medical Entomology. 2002 Jul;**39**(4):621-630

[6] Manguin S, Carnevale P, Mouchet J, Coosemans M, Julvez J, Richard-Lenoble D, Sircoulon J. Biodiversity of Malaria in the World. Montrouge, France: John Libbey Eurotext; 2008

[7] Bruce-Chwatt LJ. Essential Malariology. 2nd ed. New York: Wiley; 1985

[8] Cavalli-Sforza LL, Menozzi P, Piazza A. The History and Geography of Human Genes. Princeton, New Jersey: Princeton University Press; 1994

[9] Sallares R. The Ecology of the Ancient Greek World (British History in Perspective). 1st ed. Ithaca, NY: Cornell University Press; 1991

[10] Knottnerus OS. Malaria around the North Sea: A survey. In: Wefer G, Berger WH, Behre KE, Jansen E, editors. Climate Development and History of the North Atlantic Realm. Berlin: Springer; 2002

[11] Majori G. Short history of malaria and its eradication in Italy with short notes on the fight against the infection in the Mediterranean basin. Mediterranean Journal of Hematology and Infectious Diseases. 2012;**4**(1):e2012016. DOI: 10.4084/MJHID.2012.016

[12] Ross R. Malaria in Greece. Journal of Tropical Medicine. 1906;**9**:341-347

[13] Livadas GA, Belios G. Postwar malaria control in Greece and its results on basis of epidemiological data. In: The 4th International Conference on Tropical Medicine and Malaria; Washington, DC, May 1948. Available from http://apps.who.int/iris/bitstream/10665/64067/1/WHO_Mal_27.pdf [Accessed: 2017-11-29]

[14] European Centre for Disease Prevention and Control. Annual Epidemiological Report–Malaria. Stockholm: ECDC; 2016. Available from: https://ecdc.europa.eu/en/publications-data/malaria-annual-epidemiological-report-2016-2014-data [Accessed: 2018-02-08]

[15] European Centre for Disease Prevention and Control. Malaria Annual Epidemiological Report for 2015. Stockholm: ECDC; 2018. Available from: https://ecdc.europa.eu/sites/portal/files/documents/AER_for_2015-malaria.pdf [Accessed: 2018-02-08]

[16] Behrens RH, Neave PE, Jones CO. Imported malaria among people who travel to visit friends and relatives: Is current UK policy effective or does it need a strategic change? Malaria Journal. 2015 Apr 9;**14**:149. DOI: 10.1186/s12936-015-0666-7

[17] Toovey S, Jamieson A. Rolling back malaria: How well is Europe doing? Travel Medicine and Infectious Disease. 2003 Aug;**1**(3):167-175

[18] WHO. Tashkent Declaration: The Move from Malaria Control to Elimination in the WHO European Region. Copenhagen: WHO Regional Office for Europe; 2005. Available from http://www.euro.who.int/__data/assets/pdf_file/0005/98753/E89355.pdf [Accessed: 2017-11-29]

[19] WHO. History of malaria elimination in the European Region. http://www.euro.who.int/__data/assets/pdf_file/0003/307272/Facsheet-malaria-elimination.pdf[Accessed: 2017-11-29]

[20] Santa-Olalla Peralta P, Vazquez-Torres MC, Latorre-Fandos E, Mairal-Claver P, Cortina-Solano P, et al. First autochthonous malaria case due to *Plasmodium vivax* since eradication, Spain, October 2010. Euro Surveillance. 2010 Oct 14;**15**(41):19684

[21] Krüger A, Rech A, Su XZ, Tannich E. Two cases of autochthonous *Plasmodium falciparum* malaria in Germany with evidence for local transmission by indigenous *Anopheles plumbeus*. Tropical Medicine & International Health. 2001;**6**:983-985

[22] Arends JE, Oosterheert JJ, Kraaij-Dirkzwager MM, Kaan JA, Fanoy EB, et al. Two cases of *Plasmodium falciparum* malaria in the Netherlands without recent travel to a malaria-endemic country. The American Journal of Tropical Medicine and Hygiene. 2013;**89**:527-530

[23] Armengaud A, Legros F, D'Ortenzio E, Quatresous I, Barre H, et al. A case of autochthonous *Plasmodium vivax* malaria, Corsica, august 2006. Travel Medicine and Infectious Disease. 2008;**6**:36-40

[24] Baldari M, Tamburro A, Sabatinelli G, Romi R, Severini C, et al. Malaria in Maremma, Italy. Lancet. 1998;**351**(9111):1246-1247

[25] Danis K, Baka A, Lenglet A, Van Bortel W, Terzaki I, et al. Autochthonous *Plasmodium vivax* malaria in Greece, 2011. Euro Surveillance. 2011 Oct 20;**16**(42). pii:19993

[26] European Centre for Disease Prevention and Control. Multiple Reports of Locally-Acquired Malaria Infections in the EU. 20 September 2017. Stockholm: ECDC; 2017. Available from: https://ecdc.europa.eu/en/publications-data/rapid-risk-assessment-multiple-reports-locally-acquired-malaria-infections-eu [Accessed: 2018-02-08]

[27] European Centre for Disease Prevention and Control. Epidemiological update–Indigenous *Plasmodium falciparum* malaria cases in the Apulia region, Italy. 6 Oct 2017. Available from: https://ecdc.europa.eu/en/news-events/epidemiological-update-indigenous-plasmodium-falciparum-malaria-cases-apulia-region [Accessed: 2018-02-08]

[28] Hellenic Center for Disease Control and Prevention. Epidemiological surveillance report malaria in Greece 2017 (up to 15/12/2017). Available from: http://www.keelpno.gr/Portals/0/Files/English%20files/Malaria%20reports/MALARIA_REPORT_15_12_%202017_ENG.pdf [Accessed: 2018-02-08]

[29] WHO Technological Report Series. 1966. p. 324. Available from http://apps.who.int/iris/bitstream/10665/39822/1/WHO_TRS_324.pdf [Accessed: 2017-11-29]

[30] Becker N, Petric D, Zgomba M, Boase C, Madon M, Dahl C, Kaiser A. Mosquitoes and their Control. 2nd ed. Berlin: Springer Verlag; 2010

[31] Hackett LW, Missiroli A. The varieties of *Anopheles maculipennis* and their relation to the distribution of malaria in Europe. Rivista di Malariologia. 1935;**14**:1-67

[32] Sinka ME, Bangs MJ, Manguin S, Coetzee M, Mbogo CM, et al. The dominant *Anopheles* vectors of human malaria in Africa, Europe and the Middle East: Occurrence data, distribution maps and bionomic precis. Parasites & Vectors. 2010 Dec;**3**(3):117. DOI: 10.1186/1756-3305-3-117

[33] Intergovernmental Panel on Climate Change (IPCC). Climate Change 2014 Synthesis Report Fifth Assessment Report. Available from http://ar5-syr.ipcc.ch/ipcc/ipcc/resources/pdf/IPCC_SynthesisReport.pdf [Accessed: 2017-11-29]

[34] Kampen H, Werner D. The recurring necessity of mosquito surveillance and research. Bundesgesundheitsblatt, Gesundheitsforschung, Gesundheitsschutz. 2015 Oct;**58**(10):1101-1109. DOI: 10.1007/s00103-015-2218-2

[35] Ramsdale CD, Coluzzi M. Studies on the infectivity of tropical African strains of *Plasmodium falciparum* to some southern European vectors of malaria. Parassitologia. 1975;**17**:39-48

[36] Daskova NG, Rasnicyn SP. Review of data on susceptibility of mosquitoes in the USSR to imported strains of malaria parasites. Bulletin of the World Health Organization. 1982;**60**(6):893-897

[37] De Zulueta J, Ramsdale CD, Coluzzi M. Receptivity to malaria in Europe. Bulletin of the World Health Organization. 1975;**52**:109-111

[38] Shute PG. Failure to infect English specimens of *Anopheles maculipennis* var. atroparvus with certain strains of *Plasmodium falciparum* of tropical origin. The Journal of Tropical Medicine and Hygiene. 1940;**43**:175-178

[39] Romi R, Boccolini D, Menegon M, Rezza G. Probable autochthonous introduced malaria cases in Italy in 2009-2011 and the risk of local vector-borne transmission. Euro Surveillance. 2012 Nov 29;**17**(48). pii:20325

[40] Eling W, van Gemert G-J, Akinpelu O, Curtis J, Curtis CF. Production of *Plasmodium falciparum* sporozoites by *Anopheles plumbeus*. European Mosquito Bulletin. 2003;**15**:12-13

[41] Blacklock B, Carter HF. The experimental infection, in England, of *Anopheles plumbeus* with *Plasmodium vivax* (sporozoites in salivary glands). Preliminary note. Annals of Tropical Medicine and Parasitology. 1919;**13**:187-188

[42] Schaffner F, Thiéry I, Kaufmann C, Zettor A, Lengeler C, et al. *Anopheles plumbeus* (Diptera: Culicidae) in Europe: A mere nuisance mosquito or potential malaria vector? Malaria Journal. 2012;**11**:393. DOI: 10.1186/1475-2875-11-393

[43] Shute PG. Indigenous *P. vivax* malaria in London believed to have been transmitted by *Anopheles plumbeus*. Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service. 1954;**13**:48-51

[44] UNHCR. Global Trends Forced Displacement in 2014. Geneva, Switzerland: United Nations High Commissioner for Refugees (UNHCR). 2015. Available from: http://ec.europa.eu/eurostat/documents/2995521/7921609/3-16032017-BP-EN.pdf/e5fa98bb-5d9d-4297-9168-d07c67d1c9e1. [Accessed: 2017-11-29]

[45] Roggelin L, Tappe D, Noack B, Addo MM, Tannich E, et al. Sharp increase of imported *Plasmodium vivax* malaria seen in migrants from Eritrea in Hamburg, Germany. Malaria Journal. 2016;**15**:325. DOI: 10.1186/s12936-016-1366-7

[46] Sondén K, Castro E, Trönnberg L, Stenström C, Tegnell A, Färnert A. High incidence of *Plasmodium vivax* malaria in newly arrived Eritrean refugees in Sweden since may 2014 Euro Surveillance. 2014;**19**(35):pii=20890. DOI: 10.2807/1560-7917.ES2014.19.35.20890

[47] Kopel E, Schwartz E, Amitai Z, Volovik I. Relapsing vivax malaria cluster in Eritrean refugees, Israel, June 2010. Euro Surveillance. 2010 Jul 1;**15**(26). pii:19601

[48] Frontex. European Border and Coast Guard Agency. Migratory routes map. Available from http://frontex.europa.eu/trends-and-routes. [Accessed: 2017-11-29]

[49] Kampen H, Proft J, Etti S, Maltezos E, Pagonaki M, et al. Individual cases of autochthonous malaria in Evros Province, northern Greece: Entomological aspects. Parasitology Research. 2003;**89**(4):252-258

[50] Danis K, Lenglet A, Tseroni M, Baka A, Tsiodras S, et al. Malaria in Greece: Historical and current reflections on a re-emerging vector borne disease. Travel Medicine and Infectious Disease. 2013;**11**(1):8-14. DOI: 10.1016/j.tmaid.2013.01.001

[51] Spanakos G, Alifrangis M, Schousboe ML, Patsoula E, Tegos N, et al. Genotyping *Plasmodium vivax* isolates from the 2011 outbreak in Greece. Malaria Journal. 2013;**12**:463. DOI: 10.1186/1475-2875-12-463

[52] Tseroni M, Baka A, Kapizioni C, Snounou G, Tsiodras S, et al. MALWEST Project. Prevention of malaria resurgence in Greece through the Association of Mass Drug Administration (MDA) to immigrants from malaria-endemic regions and standard control measures. PLoS Neglected Tropical Diseases. 2015;**9**(11):e0004215. DOI: 10.1371/journal.pntd.0004215

[53] Centers for Disease Control and Prevention. Overseas Refugee Health Guidelines: MALARIA. Available from https://www.cdc.gov/immigrantrefugeehealth/guidelines/overseas/malaria-guidelines-overseas.html. [Accessed 2017-11-29]

[54] Bouchaud O, Mühlberger N, Parola P, Calleri G, Matteelli A, Peyerl-Hoffmann G, et al. Therapy of uncomplicated falciparum malaria in Europe: MALTHER—A prospective observational multicentre study. Malaria Journal. 2012 Jun 22;**11**:212. DOI: 10.1186/1475-2875-11-212

[55] Lalloo DG, Shingadia D, Bell DJ, Beeching NJ, Whitty CJM, Chiodini PL, PHE Advisory Committee on Malaria Prevention in UK Travellers. UK malaria treatment guidelines 2016. The Journal of Infection. 2016 Jun;**72**(6):635-649. DOI: 10.1016/j.jinf.2016.02.001

[56] WHO. Guidelines for the Treatment of Malaria. 3rd ed. April 2015. Available from: http://apps.who.int/iris/bitstream/10665/162441/1/9789241549127_eng.pdf[Accessed: 2018-02-08]

[57] Fotakis EA, Chaskopoulou A, Grigoraki L, Tsiamantas A, Kounadi S, Georgiou L, Vontas J. Analysis of population structure and insecticide resistance in mosquitoes of the genus *Culex*, *Anopheles* and *Aedes* from different environments of Greece with a history of mosquito borne disease transmission. Acta Tropica. 2017 Oct;**174**:29-37. DOI: 10.1016/j.actatropica.2017.06.005

[58] Zhakhongirov ShM, Saifiev ShT, Abidov ZI. Insecticide resistance in major malaria vectors in Uzbekistan. Meditsinskaia Parazitologiia (Mosk). 2016 Apr-Jun;**2**:31-34

[59] Bruce-Chwatt LJ, de Zulueta J. The Rise and Fall of Malaria in Europe: A Historico-Epidemiological Study. Oxford: Oxford University Press; 1980

Section 4

Approaches and Prospects on Malaria Elimination

Chapter 14

Assessing Malaria Vaccine Efficacy

Laurent Rénia, Yun Shan Goh, Kaitian Peng,
Marjorie Mauduit and Georges Snounou

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.77044

Abstract

After many years of silence, eradication of malaria is, once again, one of the top priorities on the agenda of many international health and development agencies. To meet this idealistic goal, a combination of control tools is needed. From this armentarium, a malaria vaccine is central to prevent infection and/or disease. However, numerous malaria vaccine candidates have shown limited efficacy in Phase II and III studies. One reason for these failures has been that the assessment of efficacy in the context of malaria has been difficult to standardize. In this article, we have reviewed and discussed the different ways to assess the outcome of a malaria vaccination.

Keywords: malaria, vaccines, end-points, immunity, *Plasmodium*

1. Introduction

Malaria remains one of the major infectious diseases with a huge burden, affecting a large fraction of the world population. Although most of the deaths, caused by *Plasmodium falciparum*, *P. vivax* and to a lesser extent *P. malariae* and *P. ovale*, occurred in Africa; significant morbidity is evident in South America and Asia [1]. Different control measures such as insecticide-treated bed nets, powerful drugs (i.e., artemisinin-based combination therapies) and early diagnostics have had a positive impact in reducing malaria mortality worldwide [2]. However, these methods have led to complete eradication of malaria in only a few countries in intertropical zones [3]. This is mainly due to increasing drug resistance of the parasites and the failure of vector control strategies resulting from the change in mosquito behavior and the emergence of insecticide resistance [4, 5]. An antimalarial vaccine is thus a necessity to achieve the goal of complete global malaria eradication [6, 7].

Vaccine development in malaria have employed a composite of rational and empirical approaches and depended on multiple epidemiological and experimental studies. Individuals living in endemic regions acquire immunity over time after repeated exposure to the parasites. Such immunity, also called premonition, is partial, species-specific and biphasic [8, 9]. In the first phase, the hosts still get infected but do not develop clinical symptoms. On the contrary, the second phase, which is the prevention or limitation of parasite multiplication, takes long to develop. This second phase is heavily dependent on parasite exposure - more the exposure the host gets, lesser the time this immunity takes to develop [9, 10]. Hence, the goal of vaccine strategies is to reduce the time needed to acquire protective immunity and to make the immunity long-lasting.

The use of experimental models is critical to vaccine development. Many researchers advocate the use of human parasites in human hosts as it is the optimal experimental model for malaria [11]. However, field studies are inherently limited by the inability to control multiple experimental parameters such as the number of infective mosquito bites, the number of parasite per infective dose, and the genetic background of the host and parasite. In addition, there are numerous ethical considerations, which restrict access to peripheral blood samples for antibody and T cell studies, important for investigating long-term protection. Thus, many researchers have turned to more controllable models, such as monkey or human *Plasmodium* in monkeys [12–14] or rodent *Plasmodium* in mice [15]. Using these models, there have been numerous vaccination studies using genetically-attenuated parasites [16], irradiated parasites [17, 18], chemically-attenuated parasites [19, 20], live parasites under drug prophylaxis [21–23], and defined antigenic formulations [24–30]. These studies have demonstrated that vaccination can reduce parasite development, prevent pathology in infected animals, prevent transmission to mosquitoes, and even induce sterile immunity. Another major advantage of these models is that the outcomes following vaccination is well-defined and easily measurable, such as development of sterile immunity, inhibition of parasite development in the liver or in the blood, and/or prevention of certain pre-defined clinical signs or of pathologies. So far, only whole parasite formulations using irradiated sporozoites [31, 32] or live parasite immunization under chloroquine [33–35], and a limited number of sporozoite antigen formulations, such as RTS,S [36], have been shown to induce sterile immunity in significant proportion of the human volunteers.

2. Vaccine developmental phases

Vaccine clinical testing in humans involves multiple phases. Phase IA involves a small group of naïve volunteers (<100), from non-endemic regions, with no previous experience of malaria, while Phase I involves malaria-exposed individuals from endemic regions. In both phases, vaccine safety and immunogenicity are assessed. Only after the vaccine has shown a good safety profile with encouraging immunogenicity data, phase IIa test study can be initiated with a larger set of volunteers (>100–1000) from non-endemic regions. In Phase IIa, vaccine efficacy is assessed by subjecting the volunteers to a challenge with mosquito bites or intravenous injections of infected red blood cells. Phase IIb involves assessing the vaccine efficacy in a larger set of volunteers from endemic regions. Promising Phase II results qualify moving the

vaccine testing to Phase III, which comprises assessing vaccine safety (including potential side effects) and efficacy over a longer time period in a cohort consisting of thousands of volunteers from endemic regions. If sufficient safety and efficacy has been demonstrated in Phase III (2 to 5 years), the vaccine can then be licensed and marketed for human use, after which mass-deployment for endemic regions can be launched.

3. Testing malaria vaccines in the field

A malaria vaccine could potentially target many different stages of the infection. It could work by: (1) preventing *de novo* infection (either in the liver or the blood), (2) controlling parasite levels in the blood and duration of the blood infection, (3) preventing pathology induced by the infection and thus preventing or reducing morbidity or mortality, and (4) preventing or reducing transmission to mosquitoes. However, not all of these outcomes can be assessed accurately in the field. Only the first and fourth outcomes mentioned can be assessed accurately and experimentally with reproducible results, mainly due to the standard operating procedures that have been implemented over the years.

Outcome assessment in the field is complicated due to the nature of the infection itself. In endemic regions, malaria infections are usually chronic [37]. Low-level parasite persistence may affect immune reactivity by amplifying or down-regulating vaccine-induced immune responses. It may also confuse diagnostics such as fever detection [38]. Occurrence and extent of chronicity may vary according to age, endemicity and host genetics. Thus, in many trials, antimalarial treatments are applied to the tested cohorts to clear prior malarial infections to reduce confounding factors [39].

Presence of co-infections is another factor that makes outcome assessment in the field difficult. Often, endemic cohorts are also infected with other pathogens, such as worms, bacteria or viruses, without being overtly sick [40–44]. Immune responses to these pathogens may either potentiate or inhibit the development of the protective response induced by infection or vaccination [45]. Due to cost constraints, it is rarely possible to make a full analysis for all possible pathogens, but it is advisable to perform retrospective studies to assess their possible influence on the malaria vaccination outcomes.

Another major roadblock for malaria vaccine development is the absence of correlates or surrogate markers of protection. These markers are crucial as they would facilitate the testing of large sets of vaccine formulations and would reduce costs and organization constraints [46]. As an example, vaccine development against Hepatitis B was greatly simplified when it was shown that concentration of Hepatitis B S antigen antibodies over 10 UI/ML level was a surrogate marker of protection (for review Plotkin et al.) [47]. This greatly accelerated the testing of multiple new formulations in a limited number of volunteers and also helped in the development of subsequent improved formulations. There is clearly a gap in our knowledge of the immune correlates of protection against malaria. It is still not clearly known what defense mechanisms are crucial in humans for mediating protection against malaria. This severely handicaps our progress towards effective vaccine development [48].

To assess vaccine efficacy in the field, it is also critical to have epidemiological data concerning the vaccine site. The level of endemicity will have an impact on the surveillance time following the last immunization and also on the size of the cohort. Low endemic conditions will require longer follow-up and a larger cohort to obtain statistically significant results.

4. Vaccines targeting specific parasite stages

The malaria parasite has a complex life cycle, alternating between the human and mosquito host. In the human host, the malaria parasite transits across different body compartments and alternates between intracellular and extracellular locations (**Figure 1**). This developmental complexity of the malaria parasite has a profound impact on the study design of the malaria vaccine and assessment efficacy (**Table 1**).

During the pre-erythrocytic stage, the parasites exist as the extracellular motile sporozoite upon injection by the mosquito during feeding, and the intracellular liver parasites. Vaccines

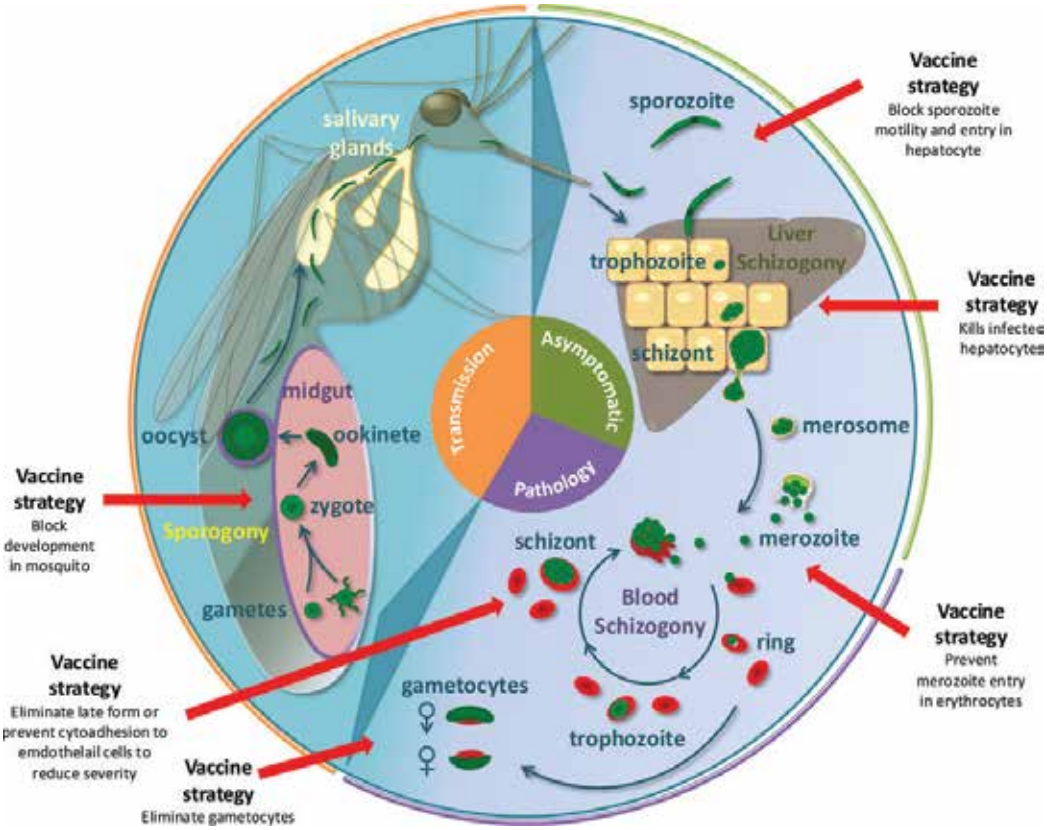


Figure 1. *Plasmodium falciparum* life cycle and vaccine strategies. The cycle in humans includes three stages: the pre-erythrocytic stage, which is asymptomatic; the asexual blood stage, which induces pathology; and the sexual stage, which is transmitted to *Anopheles* mosquitoes. At each of these stages, the parasite expresses various proteins that are targets of vaccine candidates. The different vaccine strategies for each stage are indicated.

Targets	Induced immunity	Mechanisms	Readout
Pre-erythrocytic vaccines			
Sporozoite antigens Liver stage antigens	Inhibition of parasite development and replication/ survival	Antibodies against sporozoites T cells against liver stage	Presence of parasites in the blood
Blood stage vaccines			
Asexual blood stage antigens	Inhibition of erythrocyte invasion and parasite replication/survival	Antibodies Antibody cell dependent inhibition (ADCI) Cellular immunity	Blood parasite load
Parasite derived toxins Parasite adhesion ligands	Inhibition of pathogenesis	Antibodies neutralizing inflammatory factors Antibodies inhibiting parasite/host interactions	Fever Blood parasite load Severe complications*
Transmission-blocking vaccines			
Sexual blood stage antigens Mosquito stage antigens	Inhibition of parasite development in the mosquito	Antibodies blocking gamete mating, ookinete formation or oocyst maturation	Presence of parasites in the mosquito vector (midgut, salivary glands)

Table 1. Targets and mechanisms for anti-malaria vaccines.

developed to target the pre-erythrocytic stage aim at inducing antibodies that target mainly the sporozoites and/or inducing T cells that will eliminate intracellular hepatic forms, thus preventing or controlling the extent of the subsequent blood stage development.

Vaccines targeting the asexual blood stages of the parasites are divided into two categories. The vaccines can be anti-parasite, which aim to control and eliminate parasite development in the blood or anti-disease, which aim to prevent the pathologies induced by the parasite. These vaccines need to induce different types of immune responses targeting different phases of the asexual blood stage. Vaccines targeting the sexual stage parasites aim to prevent transmission of gametocytes to the mosquito and/or gamete mating and ookinete development in the mosquito midgut.

It is worth noting that while the parasite expresses different set of genes at different stages of its life cycle, there are also many antigens that are expressed across the different parasite stages. Vaccination against these shared antigens may have an effect at different phases of the life cycle [22, 49–52], making them just as attractive for vaccine against malaria.

5. Anti-parasite vaccines

For anti-parasite vaccines targeting the pre-erythrocytic stage, the assessment of vaccine efficacy is relatively easy. Complete efficacy for this stage is defined as sterile protection, whereby

no parasite can be detected in blood of immunized individuals after the sporozoite challenge. This is an all or none phenomenon, because a single sporozoite developing in the liver can lead to full-blown blood infections.

Intuitively, one would expect great success of pre-erythrocytic vaccines since the limited numbers of sporozoites (a mean of 5–50) injected by infected mosquitoes [53] would be easily eliminated by the different arms of the immune system induced by the vaccine. However, this has proved to be the contrary. To date, only one vaccine formulation, RTS,S, an hybrid molecule containing a large segment of the circumsporozoite protein and S antigen of the Hepatitis B virus mixed with the AS02 adjuvant, has been shown to induce sterile protection in a substantial proportion of the naïve volunteers [36, 54] but to a much lower extent in field trials [55–58].

One reason that could contribute to the lack of success stories with pre-erythrocytic antiparasite vaccines is the procedures implemented to assess protection. Immunized volunteers were subjected to five mosquito bites, a dose required to ensure that naïve control volunteers would develop patent parasitemia 7–14 days after challenge [59, 60]. Alternative protocols using purified sporozoites injected either intradermal or intravenously have been developed, and so far, have proven to be safe and reproducible [61, 62]. Detection of parasitemia is performed by microscopy on Giemsa-stained blood smears over a 20–25-day period. Once a positive blood film is confirmed, the volunteers were treated with blood schizonticides to eliminate blood parasites and prevent any blood stage parasite-induced pathologies [63]. One limitation of this method of detection is that the time taken to detect parasites in the blood can differ up to 7 days. Hence, a delay of parasitemia does not necessarily translate in reduction of liver load. Moreover, there might be other confounding factors affecting the ability to detect blood parasitemia that are not related to the vaccination. To address this problem, sensitive PCR methods have been developed to detect the first wave of released liver merozoites and to assess the efficacy of the vaccine against pre-erythrocytic parasites. Using elegant regression methods, quantitative PCR techniques [64–67] allow an estimation of the reduction of the parasite liver load and an accurate measure of the effect on the growth rate of blood stage parasites.

Assessing pre-erythrocytic vaccine efficacy in the field is complicated due to factors mentioned earlier, such as the nature of the infection and presence of other co-infections. Evaluation of pre-erythrocytic vaccine in the field had mostly relied on microscopy and long follow-up (usually 6 to 24 months). As mentioned above, this assay may not be the most suitable to accurately assess the efficacy of any formulation targeting the pre-erythrocytic stage. In field conditions, many of the volunteers have been previously infected and, depending on age and exposure, may have developed some immunity against blood stage parasites. Thus, to eliminate possible confounding effects of a synergistic immunity of on-going blood stage infection with immunity induced by vaccination, it is important that volunteers are cleared by drug treatment of low-level parasitemia during immunization and before the surveillance period. It is also necessary that low-level blood infection occurrence be assessed by PCR. When implemented, this approach has reduced the follow-up time period to 1 month, saving costs and allowing the assessment of new formulations [39].

For vaccines against the pre-erythrocytic stage of *P. vivax*, efficacy assessment is further complicated by the fact that this species may produce non-replicating liver form called hypnozoites.

These hypnozoites are responsible for relapse up to 18 months after a sporozoite injection [68], thus complicating analysis and may require longer follow-up to detect relapse. Up to now, few challenges with *P. vivax* sporozoite have been performed [69]. There are no standard protocols and many issues need to be addressed [70]. First, the production of *P. vivax* sporozoites is limited since it requires infected blood from infected patients or monkeys to feed mosquitoes. Second, contrary to *P. falciparum*, no *P. vivax* cloned lines are available. Most of the lines available are derived from infected patients [71] or have been maintained in monkeys [72]. These lines contain multiple clones, which are poorly characterized at the molecular level [73]. This makes it difficult to obtain reproducible infection profile after experimental infection with mosquito bites of naïve volunteers and to characterize hypnozoite relapse profile. Moreover, as with anti-malarial drug studies in the field, the absence of validated genetic or serologic tools to distinguish between reinfection and relapse [74–77] may also prevent detecting strain-specific effect. For anti-parasite vaccines targeting the blood stage, efficacy is assessed after sporozoite or asexual blood stage parasite challenge. Sterile protection occurs when no parasite can be detected in blood of immunized individuals. Detection of parasitemia can be monitored either by microscopy or by PCR, the latter providing more information. Due to its higher sensitivity, it allows the detection of at least 3–5 parasite cycles even before the parasite is detectable by microscopy. PCR [78–81] bar-coding methods [82] can also be applied to genotype blood parasites. This allows assessing multiplicity of infection and determines whether the vaccine efficacy observed is strain-specific [83, 84]. Strain-specific vaccines have little interest since they will select vaccine-resistant parasites.

To assess the vaccine efficacy of anti-parasite vaccines, a challenge is essential. As mentioned earlier, challenge can be performed using sporozoites or blood stage parasites. However, due to the limited availability of insectaries that can provide infected mosquitoes on a regular basis, and the absence of accepted surrogates of protection, there is a necessity for blood stage challenge in healthy volunteers. Contrary to murine or monkey models where direct challenge with blood stage parasites is common, challenge with blood stage parasites in human has only been performed in limited vaccine studies using naïve volunteers [85, 86]. Because of safety reasons, blood parasites used for challenge need to be fully characterized. For a long time, only 3D7, a clone of the NF54 line, has been used. This line is susceptible to a wide range of antimalarials. Other parasite lines have been recently developed [87, 88]. However, since most of blood stage candidates are polymorphic, it is of utmost importance to assess the effect of polymorphism to have an idea of potential vaccine coverage. In addition, blood cells used for blood stage parasite propagation need to be pre-screened for the presence of a wide range of potential pathogens [86].

Despite these limitations, studies have shown that blood stage challenge can be safe and may allow the assessment of anti-asexual blood stage vaccine efficacy [89, 90]. Moreover, as recently reported, blood stage growth *in vivo* could be quantified more accurately after challenge with asexual blood stage parasites than with sporozoites [91], highlighting the advantage of this procedure. However, as it is not possible for safety reasons to let the parasitemia develop to high levels, its application might be limited. Vaccine efficacy may depend on the development of additional immune responses by the host during infection, which requires more time to be active as shown in mouse model [92]. In addition, some immune mechanisms

may need higher parasite challenge dose to be triggered. Antibody-dependent cell immunity or ADCI has been proposed to be effective with parasitemia approaching level detectable by microscopy [93].

In endemic settings, efficacy of blood stage vaccines has been assessed in natural conditions after challenge by mosquito bites. This type of challenge is perfectly suited if the vaccine can induce sterile immunity. However, it might not be the most appropriate when blood parasite multiplication rate must be measured. This rate depends on the numbers of liver merozoites released and timing of their release. As mentioned above, liver merozoite release is not a homogenous phenomenon in terms of quantity and timing. Thus, to obtain parasite growth curve suitable for comparative analysis between individuals and groups, blood sampling must be carefully planned. Indeed, this implies an active and close follow-up of the volunteers to obtain multiple time points. One possibility to obtain more homogenous results would be to perform the challenge with defined number of infected red blood cells at a same time of infection across all groups [86, 94]. However, this requires overcoming a series of hurdles such as the development of standardized inoculums with known number of parasites at the same stage of development and the availability of donor blood, which have to be heavily tested for the presence of any pathogens. In addition, the parasite in the challenge inoculums would also need to be fully characterized and clearly defined in term of parasite clonality.

To accurately assess the efficacy of anti-parasite blood stage vaccine, it is necessary to evaluate any pre-existing immune responses to the antigens in the blood stage vaccine. Individuals leaving in endemic areas acquire immunity over time. The time required to develop this immunity depends on the endemicity level and their genetic background. This immunity may influence growth rate of the parasite. Pre-existing immunity can synergize with the immunity induced by the vaccination. Vaccination may also boost pre-existing antigen-specific immune responses, which would be ideal for any vaccine formulations. On the contrary, pre-existing immune response may inhibit or mask the immune response induced by vaccination. It has been shown that the antibodies to the N-terminal of *P. falciparum* merozoite surface protein 1 can block the inhibitory activity of antibodies recognizing the C-terminal part [95]. Thus, if such an antibody interference mechanism exists for antigen(s) used in vaccine formulation, it would be necessary to evaluate carefully pre-existing immune responses to these antigens.

6. Anti-disease vaccines preventing or reducing morbidity and mortality

Anti-disease vaccines aim to prevent the pathologies induced by the parasite. Hence, to assess the efficacy of these vaccines, it is important to clearly define the symptoms. Symptomatic malaria infections are characterized by recurrent fever and if not treated could develop into more severe complications (i.e. anemia, multi-organ dysfunctions affecting the lungs, kidneys, liver and brain…), and ultimately leading to death. These different clinical occurrences can be considered as end-points when assessing vaccine efficacy. For safety and ethical reasons, these end-points are looked for in experimental clinical trials. However, they are not measured in

many field trials. Active and passive case detections are undertaken to detect clinical malaria episodes and define rate of the first episode or all episodes. Criteria to define a malaria case include presence of fever (≥37.5°C) and detection of malarial parasites in peripheral blood. Careful clinical assessment of the origin of fever is needed to ensure the fever is due to the parasite but not due to concomitant bacterial or viral infection. It should be mandatory to prevent undermining the vaccine efficacy. It is also crucial to clear any asymptomatic infections prior to vaccine testing. Clearing asymptomatic parasitemia allows a better identification of malaria-attributable fever [38]. Assessment of the reduction of severe symptom occurrence and mortality is more difficult to use as end-point. Because of active intervention (drug treatment and patient management), severity and mortality occur only in small fraction of clinical cases. Thus, in order to have sufficient statistical power to assess the vaccine testing, very large cohort is required, resulting in huge cost. Moreover, there have been concerns that decreasing the level of exposure to the parasites might, in return, results in an increase in mortality in the long–term [96]. It has been suggested that reduced exposure prevents the development of naturally-acquired clinical immunity [93], which is thought to result from constant parasite exposure. Thus, for any vaccines entering in Phase III trials, these end-points need to be assessed.

Two types of vaccine strategies aimed at reducing specifically morbidity and mortality are being developed. Anti-sequestration vaccines are based on the assumption that cytoadherence of infected red blood cells leading to parasite sequestration in deep tissues is responsible for most of malaria pathologies. These vaccines are designed to target parasite ligands such as members of the *var.* multigene family encoding the proteins *Plasmodium falciparum* erythrocyte membrane protein-1 (PfEMP-1), which mediates cytoadherence [97, 98]. It has been proposed that parasites sequester to avoid splenic elimination [99]. The more clinically-advanced anti-sequestration vaccine candidate aims at preventing pregnancy-associated malaria [100]. Few *var.* genes, which encode PfEMP-1 binding to chondroitin sulfate A (CSA), have been implicated in placental sequestration, thus making them attractive vaccine candidates [101, 102]. Anti-sequestration vaccines are designed to produce antibodies, which prevent the interactions between infected red blood cells and their cognate host cells (endothelial cells, syncytiotrophoblast…). This will lead to an increase in the circulation of blood parasites at all development stages and hence their elimination by the spleen. Primary end-point measures for such vaccines are both parasitological and clinical. Efficacy of desequestration as measured by the number of mature blood forms can be evaluated simply by microscopical observation of Giemsa stained-blood smears. However, preventing sequestration may lead to rapid increase of parasitemia and possibly a faster development of fever episodes and faster treatment application. Thus, the time-window for monitoring parasite development might be limited. Ultimately, assessment of severity and mortality are the most relevant measures for desequestration vaccines. A large cohort is needed to assess efficacy, like any blood stage vaccine targeting parasite growth.

Another type of anti-disease vaccine is targeting parasite moieties behaving as toxins and inducing immune-mediated pathologies [28, 103]. As these vaccines may have no effect on the parasitemia, the assessment of their efficacies will require very large cohorts to assess clinical outcomes with both active and passive case detection. In terms of safety, a strict clinical follow-up starting as early as the last vaccine dose administration is needed since these may perturb the immune network and induce immunopathology.

7. Transmission blocking vaccines

Transmission blocking vaccines (TBV) are designed to prevent or reduce the development of the sporogonic stage inside the mosquito host. This leads to a reduction in the numbers of infected mosquitoes and hence lesser malaria transmission in the population. As such, experimental clinical trials cannot provide straightforward answers of vaccine efficacy since the effect of such vaccine is at the population level and not at individual level. Assessment of TBV efficacy is done using *in vitro* assays and the membrane-feeding assay. Mosquitoes are fed *in vitro* with *Plasmodium* gametocytes mixed with serums from immunized individuals and the level of sporogonic development is assessed by counting the oocysts in dissected mosquito midguts [104] or, more rarely, the sporozoites in the mosquito salivary glands. Although this assay has been instrumental in identifying target antigens, it remains to be seen whether it might help to define correlate of protections for TBV development.

Currently, none of the TBV has progressed to clinical trials in the field. As the principal outcome of TBV is to reduce the number of infected mosquitoes at the population level, methods for assessing their number in field conditions should be implemented in a timely manner. There are, to date, no standardized methods to estimate the number of infected mosquitoes in the field and estimation would require large sampling size. Moreover, it seems that infected mosquitoes can cluster in discrete locations [105], thus requiring extensive studies on the distribution of infected mosquitos before and after vaccine trials. Since the number of infected mosquitoes depend on the number of circulating gametocytes [106], defining the number of gametocyte carrier prior to vaccine implementation is also a pre-requisite. In addition, defining transmission intensity of the vaccine site is important since it may influence the outcome of the vaccination. This can be defined by seroepidemiology and geographical information system (GIS) applications. For the latter, GPS mapping of mosquitoes and infected humans needs be done. It must be noted that one major limitation of these trials is that they assume that the human and mosquito populations tested are not mobile, which is often not true. An influx of infected individuals can modify the outcome by creating new reservoirs, and an influx of external infected mosquitoes would maintain transmission. It has been suggested that TBV can be tested with accuracy only in enclaved locations such as islands. Ultimately, the main expected outcome is that TBV will reduce transmission and thus reduce morbidity and mortality. The effect of such vaccine is at the population level, a large and costly cohort will be needed to be assessed over a long period of time. However, recent advances in modeling might facilitate TBV assessment by identifying end-point measures, which may serve as correlates of protection [107, 108]. Different end-point measures have been developed to assess TBV efficacy. For TBV that targets gametocytes, numbers of gametocytes and duration of gametocytes are important measures since it is expected that reduction in the number of gametocyte-carriers (reservoir) will decrease transmission. Microscopic determination of gametocytes on Giemsa-stained smears has long been used but they must be complemented with PCR methods since they have shown to underestimate gametocyte load [109]. Gametocyte infectivity to mosquitoes can be measured with the membrane-feeding assay mentioned earlier or with feeding of mosquitoes directly on the skin of gametocyte carriers, which reproduces the natural situation. An honest correlation

between the two assays has been described in few studies [110] but the membrane-feeding assay still awaits definitive validation [111]. In summary, it would be relevant for future TBV trials to perform feeding directly on gametocyte carriers using local mosquitoes. These mosquitoes would have to be raised in local insectaries and tested for the absence of any other human pathogens. Measures of TBV efficacy should not be limited to development of oocysts but also to salivary glands sporozoites since the latter are the infectious forms to humans. Hence, future studies should aim at measuring salivary gland sporozoite loads and sporozoite infectivity.

8. Concluding remarks

Here we discussed the different types of malaria vaccines and the different ways to access the vaccine efficacy. We also highlighted the limitations involved and the difficulties encountered by researchers aiming to develop an efficacious vaccine against a complex parasite such as *Plasmodium*. Despite decades of research efforts in vaccine development, no efficient malaria vaccine (i.e. with an efficacy >50%) has been developed. The most clinically-advanced RTS,S, which has been tested in Phase III, conferred at best 30–40% protection against clinical malaria [112]. Modeling studies have been proposed that, together with other malaria interventions, RTS,S vaccination may reduce the incidence of clinical malaria and deaths in many sub-Saharan African countries [113, 114]. Thus, this has led to the approval of licensure for the RTS,S vaccines by the European Union. However, the World Health Organization has not recommended its use in the extended program of immunization for children due to its discouraging vaccine efficacy data. Thus, the future of this vaccine for mass deployment remains uncertain. One of the major reasons of the limited efficacy of RTS,S vaccines and the discontinuation of various other vaccine development efforts is certainly due to antigen polymorphism [83]. In addition to antigen polymorphism, the malaria parasite utilizes many other immune escape mechanisms [115], which have severely hampered the development of malaria vaccines. With the renewed interest in malaria eradication, the development of an effective malaria vaccine is high on the agenda. Diverse strategies are being proposed to develop better vaccines: identification of new vaccine candidate [116], combinations of different antigens targeting the same stage or different stages [117]; new delivery systems and prime-boost strategies using different modalities [118]; and new adjuvants to induce stronger and longer lasting efficient immune responses [119–122]. However, for all vaccine types described, the absence of validated surrogates of protection to help select and prioritize different vaccine formulations is a major roadblock, which should be given priority to accelerate vaccine testing.

Acknowledgements

This work was supported by the Agency for Science Technology and Research (A*STAR), Singapore.

Author details

Laurent Rénia[1]*, Yun Shan Goh[1], Kaitian Peng[1], Marjorie Mauduit[1] and Georges Snounou[2]

*Address all correspondence to: laurentrenia@gmail.com

1 Singapore Immunology Network, Agency for Science, Technology and Research (A*STAR), Biopolis, Singapore

2 Sorbonne Universités, UPMC Univ Paris 06, Inserm (Institut National de la Santé et de la Recherche Médicale), Centre d'Immunologie et des Maladies Infectieuses (CIMI-Paris), UMR 1135, ERL CNRS 8255 (Centre National de la Recherche Scientifique), Paris, France

References

[1] Gething PW, Hay SI, Lim SS. *Plasmodium falciparum* mortality in Africa between 1990 and 2015. New England Journal of Medicine. 2017;**376**:2494. DOI: 10.1056/NEJMc1701144

[2] Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, Battle KE, Moyes CL, Henry A, Eckhoff PA, Wenger EA, Briet O, Penny MA, Smith TA, Bennett A, Yukich J, Eisele TP, Griffin JT, Fergus CA, Lynch M, Lindgren F, Cohen JM, Murray CL, Smith DL, Hay SI, Cibulskis RE, Gething PW. The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. Nature. 2015;**526**:207-211. DOI: 10.1038/nature15535

[3] Rabinovich RN, Drakeley CJ, Djimde AA, Hall BF, Hay SI, Hemingway J, Kaslow DC, Noor A, Okumu F, Steketee R, Tanner M, Wells TNC, Whittaker MA, Winzeler EA, Wirth DF, Whitfield K, Alonso PL. MalERA: An updated research agenda for malaria elimination and eradication. PLoS Medicine. 2017;**14**:e1002456. DOI: 10.1371/journal.pmed.1002456

[4] Wiebe A, Longbottom J, Gleave K, Shearer FM, Sinka ME, Massey NC, Cameron E, Bhatt S, Gething PW, Hemingway J, Smith DL, Coleman M, Moyes CL. Geographical distributions of African malaria vector sibling species and evidence for insecticide resistance. Malaria Journal. 2017;**16**:85. DOI: 10.1186/s12936-017-1734-y

[5] Hemingway J, Ranson H, Magill A, Kolaczinski J, Fornadel C, Gimnig J, Coetzee M, Simard F, Roch DK, Hinzoumbe CK, Pickett J, Schellenberg D, Gething PW, Hoppe M, Hamon N. Averting a malaria disaster: will insecticide resistance derail malaria control? Lancet. 2016;**387**:1785-1788. DOI: 10.1016/S0140-6736(15)00417-1

[6] Malaria vaccine. WHO position paper – January 2016. Weekly Epidemiology Record. 2016; **4**:35-52

[7] Healer J, Cowman AF, Kaslow DC, Birkett AJ. Vaccines to accelerate malaria elimination and eventual eradication. Cold Spring Harbour Perspectives in Medicine. 2017;**7**:a025627. DOI: 10.1101/cshperspect.a025627

[8] Sergent E, Parrot L. L'Immunité, la prémunition et la resistance. Annales de l'institut Pasteur d'Algers. 1935;**13**:279-315

[9] McGregor IA. Malarial immunity: Current trends and prospects. Annals of Tropical Medicine and Parasitology. 1987;**81**:647-656

[10] Sinton JA. A summary of our present knowledge of the mechanism of immunity in malaria. Journal of Malaria Institute of India. 1939;**2**:71-83

[11] Druilhe P, Hagan P, Rook G. The importance of models of infection in the study of disease resistance. Trends in Microbiology. 2002;**10**:S38-S46

[12] Collins WE, Chin W, Skinner JC. *Plasmodium fragile* and *Macaca mulatta* monkeys as a model system for the study of malaria vaccines. American Journal of Tropical Medicine and Hygiene. 1979;**28**:948-954

[13] Gysin J. Relevance of the squirrel monkey as a model for experimental human malaria. Research in Immunology. 1991;**142**:649-654

[14] Heppner DG Jr, Cummings JF, Ockenhouse CF, Kester KE, Lyon JA, Gordon DM. New world monkey efficacy trials for malaria vaccine development: Critical path or detour? Trends in Parasitology. 2001;**17**:419-425

[15] Vanderberg JP. Reflections on early malaria vaccine studies, the first successful human malaria vaccination, and beyond. Vaccine. 2009;**27**:2-9. DOI: 10.1016/j.vaccine.2008.10.028

[16] Mueller AK, Labaied M, Kappe SH, Matuschewski K. Genetically modified *Plasmodium* parasites as a protective experimental malaria vaccine. Nature. 2005;**433**:164-167

[17] Richards WHG. Active immunization of chicks against *Plasmodium gallinaceum* by inactivated homologous sporozoites and erythrocytic parasites. Nature. 1966;**212**:1492-1494

[18] Nussenzweig RS, Vanderberg JP, Most H, Orton CG. Protective immunity produced by injection of X-irradiated sporozoites of *Plasmodium berghei*. Nature. 1967;**216**:160-162

[19] Purcell LA, Yanow SK, Lee M, Spithill TW, Rodriguez A. Chemical attenuation of *Plasmodium berghe*i sporozoites induces sterile immunity in mice. Infection and Immunity. 2008;**76**:1193-1887. DOI: 10.1128/IAI.01399-07

[20] Yanow SK, Purcell LA, Spithill TW. The a/T-specific DNA alkylating agent adozelesin inhibits *Plasmodium falciparum* growth in vitro and protects mice against *Plasmodium chabaudi adami* infection. Molecular Biochemical and Parasitology. 2006;**148**:52-59. DOI: 10.1128/IAI.01399-07

[21] Orjih AU, Cochrane AH, Nussenzweig RS. Comparative studies on the immunogenicity of infective and attenuated sporozoites of *Plasmodium berghei*. Transactions of the Royal Society of Tropical Medecine and Hygiene. 1982;**76**:57-61

[22] Belnoue E, Costa FTM, Frankenberg T, Vigario AM, Voza T, Leroy N, Rodrigues MM, Landau I, Snounou G, Renia L. Protective T cell immunity against malaria liver stage after vaccination with live sporozoites under chloroquine treatment. Journal of Immunology. 2004;**172**:2487-2495

[23] Friesen J, Silvie O, Putrianti ED, Hafalla JCR, Matuschewski K, Borrmann S. Natural immunization against malaria: Causal prophylaxis with antibiotics. Science Translational Medicine. 2010;**2**:40ra49. DOI: 10.1126/scitranslmed.3001058

[24] Zavala F, Tam JP, Barr PJ, Romero P, Ley V, Nussenzweig RS, Nussenzweig V. Synthetic peptide vaccine confers protection against murine malaria. Journal of Experimental Medicine. 1987;**166**:1591-1596

[25] Ling IT, Ogun SA, Holder AA. Immunization against malaria with a recombinant protein. Parasite Immunology. 1994;**16**:63-67

[26] Marussig M, Renia L, Motard A, Miltgen F, Pétour P, Chauhan VS, Corradin G, Mazier D. Linear and multiple antigen peptides containing defined T and B epitopes of the *Plasmodium yoelii* circumsporozoite protein: Antibody-mediated protection and boosting by sporozoite infection. International Immunology. 1997;**9**:1817-1824

[27] Miyahira Y, Garcia-Sastre A, Rodriguez D, Rodriguez JR, Murata K, Tsuji M, Palese P, Esteban M, Zavala F, Nussenzweig RS. Recombinant viruses expressing a human malaria antigen can elicit potentially protective immune CD8+ responses in mice. Proceedings of the National Academy of Sciences USA. 1998;**95**:3954-3959

[28] Schofield L, Hewitt MC, Evans K, Siomos MA, Seeberger PH. Synthetic GPI as a candidate anti-toxic vaccine in a model of malaria. Nature. 2002;**418**:785-789

[29] Draper SJ, Moore AC, Goodman AL, Long CA, Holder AA, Gilbert SC, Hill F, AVS H. Effective induction of high-titer antibodies by viral vector vaccines. Nature Medicine. 2008;**14**:819-821. DOI: 10.1038/nm.1850

[30] Li Y, Leneghan DB, Miura K, Nikolaeva D, Brian IJ, Dicks MD, Fyfe AJ, Zakutansky SE, de CS, Long CA, Draper SJ, AVS H, Hill F, Biswas S. Enhancing immunogenicity and transmission-blocking activity of malaria vaccines by fusing Pfs25 to IMX313 multimerization technology. Science Reports. 2016;**6**:18848. DOI: 10.1038/srep18848

[31] Hoffman SL, Goh LM, Luke TC, Schneider I, Le TP, Doolan DL, Sacci Jr JB, De la Vega P, Dowler M, Paul C, Gordon DM, Stoute JA, Church LW, Sedegah M, Heppner DG Jr, Ballou WR, Richie TL. Protection of humans against malaria by immunization with radiation-attenuated *Plasmodium falciparum* sporozoites. Journal of Infectious Diseases. 2002;**185**:1155-1164

[32] Seder RA, Chang LJ, Enama ME, Zephir KL, Sarwar UN, Gordon IJ, Holman LA, James ER, Billingsley PF, Gunasekera A, Richman A, Chakravarty S, Manoj A, Velmurugan S, Li M, Ruben AJ, Li T, Eappen AG, Stafford RE, Plummer SH, Hendel CS, Novik L, Costner PJ, Mendoza FH, Saunders JG, Nason MC, Richardson JH, Murphy J, Davidson SA, Richie TL, Sedegah M, Sutamihardja A, Fahle GA, Lyke KE, Laurens MB, Roederer MW, Tewari K, Epstein JE, Sim BK, Ledgerwood JE, Graham BS, Hoffman SL. Protection against malaria by intravenous immunization with a nonreplicating Sporozoite vaccine. Science. 2013;**341**:1359-1365. DOI: 10.1126/science.1241800

[33] Roestenberg M, McCall M, Hopman J, Wiersma J, Luty AJF, van Gemert GJ, van Der Vegte-Boklmer M, van Schaijk BCL, Teelen K, Arens T, Spaarman L, de Mast Q, Roeffen WFG, Snounou G, Renia L, van Der Ven AJAM, Hermsen CC, Sauerwein RW. Protection against a malaria challenge by sporozoite inoculation. New England Journal of Medicine. 2009;**361**:468-477

[34] Bijker EM, Schats R, Obiero JM, Behet MC, van Gemert GJ, Vegte-Bolmer MG, Graumans W, van Lieshout L, Bastiaens GJ, Teelen K, Hermsen CC, Scholzen A, Visser LG, Sauerwein RW. Sporozoite immunization of human volunteers under mefloquine prophylaxis is safe, immunogenic and protective: A double-blind randomized controlled clinical trial. PLoS One. 2014;**9**:e112910. DOI: 10.1371/journal.pone.0112910

[35] Mordmueller BG, Surat G, Lagler H, Chakravarty S, Ishizuka AS, Lalremruata A, Gmeiner M, Campo JJ, Esen M, Ruben AJ, Held J, Calle CL, Mengue JB, Gebru T, Ibanez J, Sulyok M, James ER, Billingsley PF, Natasha KC, Manoj A, Murshedkar T, Gunasekera A, Eappen AG, Li T, Stafford RE, Li M, Felgner PL, Seder RA, Richie TL, Sim BK, Hoffman SL, Kremsner PG. Sterile protection against human malaria by chemoattenuated PfSPZ vaccine. Nature. 2017;**542**:445-449. DOI: 10.1038/nature21060

[36] Kester KE, McKinney DA, Tornieporth N, Ockenhouse CF, Heppner DG Jr, Hall T, Krzych U, Delchambre M, Voss G, Dowler MG, Palensky J, Wittes J, Cohen J, Ballou WR. Efficacy of recombinant circumsporozoite protein vaccine regimens against experimental *Plasmodium falciparum* malaria. Journal of Infectious Diseases. 2001;**183**:640-647. DOI: 10.1086/318534

[37] Garnham PCC. Comments on biology of human malaria. Military Medicine. 1966;**131** (Suppl 2):131-132

[38] Bejon PA, Mwangi T, Lowe B, Peshu N, Hill AVS, Marsh K. Clearing asymptomatic parasitaemia increases the specificity of the definition of mild febrile malaria. Vaccine. 2007;**25**:8198-8202. DOI: 10.1016/j.vaccine.2007.07.057

[39] Imoukhuede EB, Andrews L, Milligan P, Berthoud T, Bojang KA, Nwakanma D, Ismaili J, Buckee CO, Njie F, Keita S, Sowe M, Lang T, Gilbert SC, Greenwood BM, Hill AVS. Low-level malaria infections detected by a sensitive polymerase chain reaction assay and use of this technique in the evaluation of malaria vaccines in an endemic area. American Journal of Tropical Medicine and Hygiene. 2007;**76**:486-493

[40] Menezes RAO, Gomes MDSM, Mendes AM, Couto AARA, Nacher M, Pimenta TS, Sousa ACP, Baptista ARS, Jesus MI, Enk MJ, Cunha MG, RLD M. Enteroparasite and vivax malaria co-infection on the Brazil-French Guiana border: Epidemiological, haematological and immunological aspects. PLoS One. 2018;**13**:e0189958. DOI: 10.1371/journal.pone.0190808

[41] Diallo MA, Kane BS, Ndiaye M, Dieng M, Diongue K, Badiane AS, Seck MC, Ndiaye D. *Plasmodium falciparum* malaria co-infection with tick-borne relapsing fever in Dakar. Malaria Journal. 2017;**16**:24. DOI: 10.1186/s12936-017-1682-6

[42] Shah PD, Mehta TK. Evaluation of concurrent malaria and dengue infections among febrile patients. Indian Journal of Medical Microbiology. 2017;**35**:402-405. DOI: 10.4103/ijmm.IJMM_15_455

[43] Waxman M, Aluisio AR, Rege S, Levine AC. Characteristics and survival of patients with Ebola virus infection, malaria, or both in Sierra Leone: A retrospective cohort study. Lancet Infectious Diseases. 2017;**17**:30112-30113. DOI: 10.1016/S1473-3099(17)30112-3

[44] Ayorinde AF, Oyeyiga AM, Nosegbe NO, Folarin OA. A survey of malaria and some arboviral infections among suspected febrile patients visiting a health Centre in Simawa, Ogun state, Nigeria. Journal of Infection and Public Health. 2016;**9**:52-59. DOI: 10.1016/j.jiph.2015.06.009

[45] Druilhe P, Tall A, Sokhna CS. Worms can worsen malaria: Towards a new means to roll back malaria? Trends in Parasitology. 2005;**21**:359-362. DOI: 10.1016/j.pt.2005.06.011

[46] Plotkin SA. Complex correlates of protection after vaccination. Clinical of Infectious Diseases. 2013;**56**:1458-1465

[47] Plotkin SA. Immunologic correlates of protection induced by vaccination. Pediatric Infectious Diseases Journal. 2001;**20**:63-75

[48] Dobano C, Campo JJ. Understanding protective immune mechanisms induced by malaria vaccines in the context of clinical trials. Human Vaccines. 2009;**5**:562-565

[49] Belnoue E, Voza T, Costa FTM, Gruner AC, Mauduit M, Rosa DS, Depinay N, Kayibanda M, Vigario AM, Mazier D, Snounou G, Sinnis P, Renia L. Vaccination with live *Plasmodium yoelii* blood stage parasites under chloroquine cover induces cross-stage immunity against malaria liver stage. Journal of Immunology. 2008;**181**:8552-8558

[50] Mazier D, Goma J, Pied S, Renia L, Nussler A, Miltgen F, Mattei DM, Grau GE. Hepatic phase of malaria: A crucial role as "go-between" with other stages. Bulletin of the World Health Organisation. 1990;**68**:126-131

[51] Mazier D, Renia L, Nussler A, Pied S, Marussig M, Goma J, Grillot D, Miltgen F, Drapier JC, Corradin G. Hepatic phase of malaria is the target of cellular mechanisms induced by the previous and the subsequent stages. A crucial role for liver nonparenchymal cells. Immunology Letters. 1990;**25**:65-70

[52] Motard A, Marussig M, Renia L, Baccam D, Landau I, Mattei DM, Targett G, Mazier D. Immunization with the malaria heat shock like protein hsp70-1 enhances transmission to the mosquito. International Immunology. 1995;**7**:147-150

[53] Beier JC, Davis JR, Vaughan JA, Noden BH, Beier MS. Quantitation of *Plasmodium falciparum* sporozoites transmitted *in vitro* by experimentally infected *Anopheles gambiae* and *Anopheles stephensi*. American Journal of Tropical Medicine and Hygiene. 1991;**44**:564-570

[54] Stoute JA, Slaoui M, Heppner DG Jr, Momin P, Kester KE, Desmons P, Wellde BT, Garcon N, Krzych U, Marchand M. A preliminary evaluation of a recombinant circumsporozoite protein vaccine against *Plasmodium falciparum* malaria. RTS,S Malaria Vaccine Evaluation Group. New England Journal of Medicine. 1997;**336**:86-91. DOI: 10.1056/NEJM199701093360202

[55] Bojang KA, Milligan PJM, Pinder M, Vigneron L, Alloueche A, Kester KE, Ballou WR, Conway DJ, Reece WH, Gothard PK, Yamuah LK, Delchambre M, Voss G, Greenwood BM, Hill AVS, McAdam KPWJ, Tornieporth N, Cohen JD, Doherty TM. Efficacy of RTS,S/AS02 malaria vaccine against *Plasmodium falciparum* infection in semi-immune

adult men in the Gambia: A randomised trial. Lancet. 2001;**358**:1927-1934. DOI: 10.1016/S0140-6736(01)06957-4

[56] Alonso PL, Sacarlal J, Aponte JJ, Leach A, Macete E, Milman J, Mandomando I, Spiessens B, Guinovart C, Espasa M, Bassat Q, Aide P, Ofori-Anyinam O, Navia MM, Corachan S, Ceuppens M, Dubois MC, Demoitie MA, Dubovsky F, Menendez C, Tornieporth N, Ballou WR, Thompson R, Cohen JD. Efficacy of the RTS,S/AS02A vaccine against *Plasmodium falciparum* infection and disease in young African children: Randomised controlled trial. Lancet. 2004;**364**:1411-1420

[57] Bejon PA, Lusingu J, Olotu AI, Leach A, Lievens M, Vekemans J, Mshamu S, Lang T, Gould J, Dubois MC, Demoitie MA, Stallaert JF, Vansadia P, Carter T, Njuguna P, Awuondo KO, Malabeja A, Abdul O, Gesase S, Mturi N, Drakeley CJ, Savarese B, Villafana T, Ballou WR, Cohen JD, Riley EM, Lemnge MM, Marsh K, von Seidlein LV: Efficacy of RTS,S/AS01E vaccine against malaria in children 5 to 17 months of age. New England Journal of Medicine. 2008;**359**:2521-2532. DOI: 10.1016/S0140-6736(04)17223-1

[58] RTS,S Clinical Trials Partnership, Agnandji ST, Lell B, Soulanoudjingar SS, Fernandes JF, Abossolo BP, Conzelmann C, Methogo BG, Doucka Y, Flamen A, Mordmueller BG, Issifou S, Kremsner PG, Sacarlal J, Aide P, Lanaspa M, Aponte JJ, Nhamuave A, Quelhas D, Bassat Q, Mandjate S, Macete E, Alonso P, Abdulla S, Salim N, Juma O, Shomari M, Shubis K, Machera F, Hamad AS, Minja R, Mtoro A, Sykes A, Ahmed S, Urassa AM, Ali AM, Mwangoka G, Tanner M, Tinto H, D'Alessandro U, Sorgho H, Valea I, Tahita MC, Kabore W, Ouedraogo S, Sandrine Y, Guiguemde RT, Ouedraogo JB, Hamel MJ, Kariuki S, Odero C, Oneko M, Otieno K, Awino N, Omoto J, Williamson J, Muturi-Kioi V, Laserson KF, Slutsker L, Otieno W, Otieno L, Nekoye O, Gondi S, Otieno A, Ogutu B, Wasuna R, Owira V, Jones D, Onyango AA, Njuguna P, Chilengi R, Akoo P, Kerubo C, Gitaka J, Maingi C, Lang T, Olotu A, Tsofa B, Bejon PA, Peshu N, Marsh K, Owusu-Agyei S, Asante KP, Osei-Kwakye K, Boahen O, Ayamba S, Kayan K, Owusu-Ofori R, Dosoo D, Asante I, Adjei G, Adjei G, Chandramohan D, Greenwood BM, Lusingu J, Gesase S, Malabeja A, Abdul O, Kilavo H, Mahende C, Liheluka E, Lemnge M, Theander TG, Drakeley CJ, Ansong D, Agbenyega T, Adjei S, Boateng HO, Rettig T, Bawa J, Sylverken J, Sambian D, Agyekum A, Owusu L, Martinson F, Hoffman I, Mvalo T, Kamthunzi P, Nkomo R, Msika A, Jumbe A, Chome N, Nyakuipa D, Chintedza J, Ballou WR, Bruls M, Cohen J, Guerra Y, Jongert E, Lapierre D, Leach A, Lievens M, Ofori-Anyinam O, Vekemans J, Carter T, Leboulleux D, Loucq C, Radford A, Savarese B, Schellenberg D, Sillman M, Vansadia P. First results of phase 3 trial of RTS,S/AS01 malaria vaccine in African children. New England Journal of Medicine. 2011;**365**:1863-1875. DOI: 10.1056/NEJMoa1102287

[59] Roestenberg M, Mordmueller BG, Ockenhouse CF, Mo A, Yazdanbakhsh M, Kremsner PG. The frontline of controlled human malaria infections: A report from the controlled human infection models workshop in Leiden University Medical Centre 5 May 2016. Vaccine. 2017;**35**:7065-7069. DOI: 10.1016/j.vaccine.2017.10.092

[60] Roestenberg M, O'Hara GA, Duncan CJ, Epstein JE, Edwards NJ, Scholzen A, van Der Ven AJAM, Hermsen CC, Hill AVS, Sauerwein RW. Comparison of clinical and parasitological

data from controlled human malaria infection trials. PLoS One. 2012;**7**:e38434. DOI: 10.1371/journal.pone.0038434

[61] Roestenberg M, Bijker EM, Sim BK, Billingsley PF, James ER, Bastiaens GJ, Teirlinck AC, Scholzen A, Teelen K, Arens T, van der Ven AJ, Gunasekera A, Chakravarty S, Velmurugan S, Hermsen CC, Sauerwein RW, Hoffman SL. Controlled human malaria infections by intradermal injection of cryopreserved *Plasmodium falciparum* sporozoites. American Journal of Tropical Medicine and Hygiene. 2013;**88**:5-13. DOI: 10.4269/ajtmh.2012.12-0613

[62] Sissoko MS, Healy SA, Katile A, Omaswa F, Zaidi I, Gabriel EE, Kamate B, Samake Y, Guindo MA, Dolo A, Niangaly A, Niare K, Zeguime A, Sissoko K, Diallo H, Thera I, Ding K, Fay MP, O'Connell EM, Nutman TB, Wong-Madden S, Murshedkar T, Ruben AJ, Li M, Abebe Y, Manoj A, Gunasekera A, Chakravarty S, Sim BK, Billingsley PF, James ER, Walther M, Richie TL, Hoffman SL, Doumbo O, Duffy PE. Safety and efficacy of PfSPZ vaccine against *Plasmodium falciparum* via direct venous inoculation in healthy malaria-exposed adults in Mali: A randomised, double-blind phase 1 trial. Lancet Infectious Diseases. 2017;**17**:498-509. DOI: 10.1016/S1473-3099(17)30104-4

[63] Sauerwein RW, Roestenberg M, Moorthy VS. Experimental human challenge infections can accelerate clinical malaria vaccine development. Nature Reviews. Immunology. 2011;**11**:57-64. DOI: 10.1038/nri2902

[64] Hermsen CC, De Vlas SJ, van Gemert GJ, Telgt DS, Verhage DF, Sauerwein RW. Testing vaccines in human experimental malaria: Statistical analysis of parasitemia measured by a quantitative real-time polymerase chain reaction. American Journal of Tropical Medicine and Hygiene. 2004;**71**:196-201

[65] Andrews L, Andersen RF, Webster D, Dunachie S, Walther RM, Bejon PA, Hunt-Cooke A, Bergson G, Sanderson F, Hill AVS, Gilbert SC. Quantitative real-time polymerase chain reaction for malaria diagnosis and its use in malaria vaccine clinical trials. American Journal of Tropical Medicine and Hygiene. 2005;**73**:191-198

[66] Bejon PA, Andrews L, Andersen RF, Dunachie S, Webster D, Walther M, Gilbert SC, Peto TEA, Hill AVS. Calculation of liver-to-blood Inocula, parasite growth rates, and Preerythrocytic vaccine efficacy, from serial quantitative polymerase chain reaction studies of volunteers challenged with malaria Sporozoites. Journal of Infectious Diseases. 2005;**191**:619-626. DOI: 10.1086/427243

[67] Roestenberg M, De Vlas SJ, Nieman AE, Sauerwein RW, Hermsen CC: Efficacy of Preerythrocytic and blood-stage malaria vaccines can be assessed in small sporozoite challenge trials in human volunteers. Journal of Infectious Diseases. 2012;**206**:319-323. DOI: 10.1093/infdis/jis355

[68] Coatney GR, Cooper WC, Young MD. Studies on human malaria. XXX. A summary of 204 sporozoite-induced infections with the Chesson strain of *Plasmodium vivax*. Journal of the National Malaria Society. 1951;**10**:381-396

[69] Arevalo-Herrera M, Forero-Pena DA, Rubiano K, Gomez-Hincapie J, Martinez NL, Lopez-Perez M, Castellanos A, Cespedes N, Palacios R, Onate JM, Herrera S. *Plasmodium vivax* sporozoite challenge in malaria-naive and semi-immune colombian volunteers. PLoS One. 2014;**9**:e99754. DOI: 10.1371/journal.pone.0099754

[70] Mueller I, Moorthy VS, Brown GV, Smith PG, Alonso PL, Genton B. Guidance on the evaluation of *Plasmodium vivax* vaccines in populations exposed to natural infection. Vaccine. 2009;**27**:5633-5643. DOI: 10.1016/j.vaccine.2009.07.018

[71] Payne RO, Griffin PM, McCarthy JS, Draper SJ. *Plasmodium vivax* controlled human malaria infection - progress and prospects. Trends in Parasitology. 2017;**33**:141-150. DOI: 10.1016/j.pt.2016.11.001

[72] Collins WE, Sullivan JS, Strobert E, Galland GG, Williams A, Nace D, Williams T, Barnwell JW. Studies on the Salvador I strain of *Plasmodium vivax* in non-human primates and anopheline mosquitoes. American Journal of Tropical Medicine and Hygiene. 2009, 2009;**80**:228-235

[73] Ntumngia FB, McHenry AM, Barnwell JW, Cole-Tobian JL, King CL, Adams JH. Genetic variation among *Plasmodium vivax* isolates adapted to non-human primates and the implication for vaccine development. American Journal of Tropical Medicine and Hygiene. 2009;**80**:218-227

[74] Imwong M, Snounou G, Pukrittayakamee S, Tanomsing N, Kim JR, Nandy A, Guthmann JP, Nosten F, Carlton JMR, Looareesuwan S, Nair S, Sudimack D, Day NP, Anderson TJC, White NJ. Relapses of *Plasmodium vivax* infection usually result from activation of heterologous Hypnozoites. Journal of Infectious Diseases. 2007;**195**:927-933. DOI: 10.1086/512241

[75] Imwong M, Boel ME, Pagornrat W, Pimanpanarak M, McGready RM, Day NP, Nosten F, White NJ. The first *Plasmodium vivax* relapses of life are usually genetically homologous. Journal of Infectious Diseases. 2012;**205**:860-863. DOI: 10.1093/infdis/jir806

[76] Lin JT, Patel JC, Kharabora O, Sattabongkot J, Muth S, Ubalee R, Schuster AL, Rogers WO, Wongsrichanalai C, Juliano JJ. *Plasmodium vivax* isolates from Cambodia and Thailand show high genetic complexity and distinct patterns of *P. vivax* multidrug resistance gene 1 (pvmdr1) polymorphisms. American Journal of Tropical Medicine and Hygiene. 2013;**88**:1116-1123. DOI: 10.4269/ajtmh.12-0701

[77] Chuquiyauri R, Molina DM, Moss EL, Wang R, Gardner MJ, Brouwer KC, Torres S, Gilman RH, Llanos-Cuentas A, Neafsey DE, Felgner PL, Liang X, Vinetz JM. Genome-scale protein microarray comparison of human antibody responses in *Plasmodium vivax* relapse and reinfection. American Journal of Tropical Medicine and Hygiene. 2015;**93**:801-809. DOI: 10.4269/ajtmh.15-0232

[78] Snounou G. Genotyping of *Plasmodium* spp. Nested PCR. Malaria Methods and Protocols. 2002:103-116. DOI: 10.1385/1-59259-271-6:189

[79] Perandin F, Manca N, Calderaro A, Piccolo G, Galati L, Ricci L, Medici MC, Arcangeletti MC, Snounou G, Dettori G, Chezzi C. Development of a real-time PCR assay for detection of *Plasmodium falciparum*, *Plasmodium vivax,* and *Plasmodium ovale* for routine clinical diagnosis. Journal of Clinical Microbiology. 2004;**42**:1214-1219

[80] Mugittu K, Adjuik M, Snounou G, Ntoumi F, Taylor W, Mshinda H, Olliaro P, Beck HP. Molecular genotyping to distinguish between recrudescents and new infections in treatment trials of *Plasmodium falciparum* malaria conducted in sub-Saharan Africa: Adjustment of parasitological outcomes and assessment of genotyping effectiveness. Tropical Medicine and Intrnational Health. 2006;**11**:1350-1359. DOI: 10.1111/j.1365-3156.2006.01688.x

[81] Imwong M, Hanchana S, Malleret B, Renia L, Day NP, Dondorp AM, Nosten F, Snounou G, White NJ. High throughput ultra-sensitive molecular techniques to quantify low density malaria parasitaemias. Journal of Clinical Microbiology. 2014;**52**:3303-3309. DOI: 10.1128/JCM.01057-14

[82] Preston MD, Campino SG, Assefa SA, Echeverry DF, Ocholla H, Amambua-Ngwa A, Stewart LB, Conway DJ, Borrmann S, Michon P, Zongo I, Ouedraogo JB, Djimde AA, Doumbo OK, Nosten F, Pain A, Bousema JT, Drakeley CJ, Fairhurst RM, Sutherland CJ, Roper C, Clark TG. A barcode of organellar genome polymorphisms identifies the geographic origin of *Plasmodium falciparum* strains. Nature Communications. 2014;**5**:4052. DOI: 10.1038/ncomms5052

[83] Neafsey DE, Juraska M, Bedford T, Benkeser D, Valim C, Griggs A, Lievens M, Abdulla S, Adjei S, Agbenyega T, Agnandji ST, Aide P, Anderson S, Ansong D, Aponte JJ, Asante KP, Bejon PA, Birkett AJ, Bruls M, Connolly KM, D'Alessandro U, Dobano C, Gesase S, Greenwood BM, Grimsby J, Tinto H, Hamel MJ, Hoffman I, Kamthunzi P, Kariuki S, Kremsner PG, Leach A, Lell B, Lennon NJ, Lusingu J, Marsh K, Martinson F, Molel JT, Moss EL, Njuguna P, Ockenhouse CF, Ragama OB, Otieno W, Otieno L, Otieno K, Owusu-Agyei S, Park DJ, Pelle K, Robbins D, Russ C, Ryan EM, Sacarlal J, Sogoloff B, Sorgho H, Tanner M, Theander TG, Valea I, Volkman SK, Yu Q, Lapierre D, Birren BW, Gilbert PB, Wirth DF. Genetic diversity and protective efficacy of the RTS,S/AS01 malaria vaccine. New England Journal of Medicine. 2015;**373**:2025-2037. DOI: 10.1056/NEJMoa1505819

[84] Thera MA, Doumbo OK, Coulibaly D, Laurens MB, Ouattara A, Kone AK, Guindo AB, Traore K, Traore I, Kouriba B, Diallo DA, Diarra I, Daou M, Dolo A, Tolo Y, Sissoko MS, Niangaly A, Sissoko M, Takala-Harrison SL, Lyke KE, Wu Y, Blackwelder WC, Godeaux O, Vekemans J, Dubois MC, Ballou WR, Cohen JD, Thompson D, Dube T, Soisson L, Diggs CL, House B, Lanar DE, Dutta S, Heppner Jr DG, Plowe CV. A field trial to assess a blood-stage malaria vaccine. New England Journal of Medicine. 2011;**365**:1004-1013. DOI: 10.1056/NEJMoa1008115

[85] Duncan CJA, Draper SJ. Controlled human blood stage malaria infection: Current status and potential applications. American Journal of Tropical Medicine and Hygiene. 2012;**86**:561-565. DOI: 10.1056/NEJMoa1008115

[86] Stanisic DI, McCarthy JS, Good MF. Controlled human malaria infection: Applications, advances, and challenges. Infection and Immunity. 2018;**86**:e00479017. DOI: 10.1128/IAI.00479-17

[87] Teirlinck AC, Roestenberg M, van Der Ven AJAM, Scholzen A, Heinrichs MJ, Siebelink-Stoter R, Graumans W, van Gemert GJ, Teelen K, Vos MW, Nganou-Makamdop K, Borrmann S, Rozier YP, Erkens MA, Luty AJF, Hermsen CC, Sim BK, van Lieshout AW, Hoffman SL, Visser LG, Sauerwein RW. NF135.C10: A new *Plasmodium falciparum* clone for controlled human malaria infections. Journal of Infectious Diseases. 2013;**207**:656-666. DOI: 10.1093/infdis/jis725

[88] Lyke KE, Ishizuka AS, Berry AA, Chakravarty S, DeZure A, Enama ME, James ER, Billingsley PF, Gunasekera A, Manoj A, Li M, Ruben AJ, Li T, Eappen AG, Stafford RE, Kc N, Murshedkar T, Mendoza FH, Gordon IJ, Zephir KL, Holman LA, Plummer SH, Hendel CS, Novik L, Costner PJ, Saunders JG, Berkowitz NM, Flynn BJ, Nason MC, Garver LS, Laurens MB, Plowe CV, Richie TL, Graham BS, Roederer M, Sim BK, Ledgerwood JE, Hoffman SL, Seder RA. Attenuated PfSPZ vaccine induces strain-transcending T cells and durable protection against heterologous controlled human malaria infection. Proceedings of the National Academy of Sciences of USA. 2017;**114**:2711-2716. DOI: 10.1073/pnas.1615324114

[89] Lawrence GW, Cheng Q, Reed C, Taylor D, Stowers AW, Cloonan N, Rzepczyk CM, Smillie A, Anderson K, Pombo DJ, Allworth A, Eisen D, Anders RF, Saul AJ. Effect of vaccination with 3 recombinant asexual-stage malaria antigens on initial growth rates of *Plasmodium falciparum* in non-immune volunteers. Vaccine. 2000;**18**:1925-1931

[90] Pombo DJ, Lawrence G, Hirunpetcharat C, Rzepczyk CM, Bryden M, Cloonan N, Anderson K, Mahakunkijcharoen Y, Martin LB, Wilson D, Elliot S, Eisen DP, Weinberg JB, Saul AJ, Good MF. Immunity to malaria after administration of ultra-low doses of red cells infected with *Plasmodium falciparum*. Lancet. 2002;**360**:610-617. DOI: 10.1016/S0140-6736(02)09784-2

[91] Sanderson F, Andrews L, Douglas AD, Hunt-Cooke A, Bejon PA, Hill AVS. Blood-stage challenge for malaria vaccine efficacy trials: A pilot study with discussion of safety and potential value. American Journal of Tropical Medicine and Hygiene. 2008;**78**:878-883

[92] Hirunpetcharat C, Vukovic P, Liu XQ, Kaslow DC, Miller LH, Good MF. Absolute requirement for an active immune response involving B cells and Th cells in immunity to *Plasmodium yoelii* passively acquired with antibodies to the 19-kDa carboxyl-terminal fragment of merozoite surface protein-1. Journal of Immunology. 1999;**162**:7309-7314

[93] Druilhe P, Perignon JL. A hypothesis about the chronicity of malaria infection. Parasitology Today. 1997;**13**:353-357

[94] Griffin P, Pasay C, Elliott S, Sekuloski S, Sikulu M, Hugo L, Khoury D, Cromer D, Davenport M, Sattabongkot J, Ivinson K, Ockenhouse CF, McCarthy J. Safety and reproducibility of a clinical trial system using induced blood stage *Plasmodium vivax* infection and its potential as a model to evaluate malaria transmission. PLoS Neglected Tropical Diseases. 2016;**10**:e0005139. DOI: 10.1371/journal.pntd.00051390

[95] Guevara Patino JA, Holder AA, McBride JS, Blackman MJ. Antibodies that inhibit malaria merozoite surface protein-1 processing and erythrocyte invasion are blocked by naturally acquired human antibodies. Journal of Experimental Medicine. 1997;**186**:**1**689-1699

[96] Ghani AC, Sutherland CJ, Riley EM, Drakeley CJ, Griffin JT, Gosling RD, Filipe JA. Loss of population levels of immunity to malaria as a result of exposure-reducing interventions: Consequences for interpretation of disease trends. PLoS One. 2009;**4**:e4383. DOI: 10.1371/journal.pone.0004383

[97] Miller LH, Baruch DI, Marsh K, Doumbo OK. The pathogenic basis of malaria. Nature. 2009;**415**:673-679. DOI: 10.1038/415673a

[98] Sutherland CJ. The flip-side of cytoadherence immune selection, antigenic variation and the var genes of *Plasmodium falciparum*. Parasitology Today. 1998;**14**:329-332

[99] David PH, Hommel M, Miller LH, Udeinya IJ, Oligino LD. Parasite sequestration in *Plasmodium falciparum* malaria: Spleen and antibody modulation of cytoadherence of infected erythrocytes. Proceedings of the National Academy of Sciences USA. 1983;**80**:5075-5079

[100] Gamain B, Smith JD, Viebig NK, Gysin J, Scherf A. Pregnancy-associated malaria: Parasite binding, natural immunity and vaccine development. International Journal of Parasitology. 2007;**37**:273-283. DOI: 10.1016/j.ijpara.2006.11.011

[101] Fried M, Duffy PE. Adherence of *Plasmodium falciparum* to chondroitin sulfate a in the human placenta. Science. 1996;**272**:1502-1504

[102] Gangnard S, Lewit-Bentley A, Dechavanne S, Srivastava A, Amirat F, Bentley GA, Gamain B. Structure of the DBL3X-DBL4epsilon region of the VAR2CSA placental malaria vaccine candidate: Insight into DBL domain interactions. Science Reports. 2015;**5**:14868. DOI: 10.1038/srep14868

[103] Schofield L. Antidisease vaccines. Chemistry and Immunolology. 2002;**80**:322-342

[104] Lensen AHW, van Druten JA, Bolmer MG, van Gemert GJ, Eling WMC, Sauerwein RW. Measurement by membrane feeding of reduction in *Plasmodium falciparum* transmission induced by endemic sera. Transactions of the Royal Society in Tropical Medicine and Hygiene. 1996;**90**:20-22

[105] Gaudart J, Poudiougou B, Dicko A, Ranque S, Toure O, Sagara I, Diallo M, Diawara S, Ouattara A, Diakite M, Doumbo OK. Space-time clustering of childhood malaria at the household level: A dynamic cohort in a Mali village. BMC Public Health. 2006;**6**:286. DOI: 10.1186/1471-2458-6-286

[106] Pett H, Goncalves BP, Dicko A, Nebie I, Tiono AB, Lanke K, Bradley J, Chen I, Diawara H, Mahamar A, Soumare HM, Traore SF, Baber I, Sirima SB, Sauerwein RW, Brown J, Gosling R, Felger I, Drakeley CJ, Bousema T. Comparison of molecular quantification of *Plasmodium falciparum* gametocytes by Pfs25 qRT-PCR and QT-NASBA in relation to mosquito infectivity. Malaria Journal. 2016;**15**:539. DOI: 10.1186/s12936-016-1584-z

[107] Saul AJ. Efficacy model for mosquito stage transmission blocking vaccines for malaria. Parasitology. 2008;**135**:1497-1506. DOI: 10.1017/S0031182008000280

[108] Okell LC, Paintain LS, Webster J, Hanson K, Lines J. From intervention to impact: Modelling the potential mortality impact achievable by different long-lasting, insecticide-treated net delivery strategies. Malaria Journal. 2012;**11**:327. DOI: 10.1186/1475-2875-11-327

[109] Schneider P, Bousema T, Omar S, Gouagna L, Sawa P, Schallig H, Sauerwein RW. (sub) microscopic *Plasmodium falciparum* gametocytaemia in Kenyan children after treatment with sulphadoxine-pyrimethamine monotherapy or in combination with artesunate. International Journal of Parasitology. 2006;**36**:403-408. DOI: 10.1016/j.ijpara.2006.01.002

[110] Bonnet S, Gouagna LC, Paul RE, Safeukui I, Meunier JY, Boudin C. Estimation of malaria transmission from humans to mosquitoes in two neighbouring villages in South Cameroon: Evaluation and comparison of several indices. Transactions of the Royal Society in Tropical Medicine and Hygiene. 2003;**97**:53-59

[111] Nikolaeva D, Draper SJ, Biswas S. Toward the development of effective transmission-blocking vaccines for malaria. Expert Reviews in Vaccines. 2015;**14**:653-680. DOI: 10.1586/14760584.2015.993383

[112] Greenwood B, Dicko A, Sagara I, Zongo I, Tinto H, Cairns M, Kuepfer I, Milligan P, Ouedraogo JB, Doumbo O, Chandramohan D. Seasonal vaccination against malaria: A potential use for an imperfect malaria vaccine. Malaria Journal. 2017;**16**:182. DOI: 10.1186/s12936-017-1841-9

[113] Sauboin CJ, Van Bellinghen LA, Van de Velde N, Van Vlaenderen I. Potential public health impact of RTS. S malaria candidate vaccine in sub-Saharan Africa: a modelling study. Malaria Journal. 2015;**14**:524. DOI: 10.1186/s12936-015-1046-z

[114] Penny MA, Pemberton-Ross, P, Smith TA: The time-course of protection of the RTS,S vaccine against malaria infections and clinical disease. Malaria Journal. 2015;**14**:437. DOI: 10.1186/s12936-015-0969-8

[115] Renia L, Goh YS. Malaria parasites: The great escape. Frontiers in Microbiology. 2016;**7**: 463. DOI: 10.3389/fimmu.2016.00463

[116] Osier FH, Mackinnon MJ, Crosnier C, Fegan G, Kamuyu G, Wanaguru M, Ogada E, McDade B, Rayner JC, Wright GJ, Marsh K. New antigens for a multicomponent blood-stage malaria vaccine. Science Translational Medicine. 2014;**247**:247ra102. DOI: 10.1126/scitranslmed.3008705

[117] Peng K, Siau A, Franetich JF, Goh YS, Chia WC, Ong ASM, Malleret B, Wu YY, Snounou G, Hermsen CC, Adams JH, Mazier D, Preiser PR, Sauerwein RW, Grüner AC, Rénia L. Breadth of humoral response and sporozoite-neutralizing antibodies are associated with sterile protection induced by controlled human malaria infection. Cellular Microbiology. 2016;**18**:1739-1750. DOI: 10.1111/cmi.12608

[118] Bustamante LY, Powell GT, Lin YC, Macklin MD, Cross N, Kemp A, Cawkill P, Sanderson T, Crosnier C, Muller-Sienerth N, Doumbo OK, Traore B, Crompton PD,

Cicuta P, Tran TM, Wright GJ, Rayner JC. Synergistic malaria vaccine combinations identified by systematic antigen screening. Proceedings of the National Academy of Science USA. 2017;**114**:12045-12050. DOI: 10.1073/pnas.1702944114

[119] Cabrera-Mora M, Fonseca JA, Singh B, Zhao C, Makarova N, Dmitriev I, Curiel DT, Blackwell J, Moreno A. A recombinant chimeric Ad5/3 vector expressing a multistage *Plasmodium* antigen induces protective immunity in mice using heterologous prime-boost immunization regimens. Journal of Immunology. 2016;**197**:2748-2761. DOI: 10.4049/jimmunol.1501926

[120] Camargo TM, de Freitas EO, Gimenez AM, Lima LC, Almeida d, Caramico K, Francoso KS, Bruna-Romero O, Andolina C, Nosten F, Renia L, HCJ E, Nussenzweig RS, Nussenzweig V, Rodrigues MM, Reyes-Sandoval A, Soares IS. Prime-boost vaccination with recombinant protein and adenovirus-vector expressing *Plasmodium vivax* circumsporozoite protein (CSP) partially protects mice against Pb/Pv sporozoite challenge. Science Reports. 2018;**8**:1118. DOI: 10.1038/s41598-017-19063-6

[121] Ewer KJ, Lambe T, Rollier CS, Spencer AJ, Hill AVS, Dorrell L. Viral vectors as vaccine platforms: From immunogenicity to impact. Current Opinion in Immunology. 2016;**41**:43-54. DOI: 10.1016/j.coi.2016.05.014

[122] Garcon N. Di Pasquale a: From discovery to licensure, the adjuvant system story. Human Vaccines and Immunotherapy. 2017;**13**:19-33. DOI: 10.1080/21645515.2016.1225635

Chapter 15

Malaria Transmission-Blocking Vaccines: Present Status and Future Perspectives

Ogobara K. Doumbo, Karamoko Niaré,
Sara A. Healy, Issaka Sagara and Patrick E. Duffy

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.77241

Abstract

Transmission-blocking vaccines (TBVs) utilize *Plasmodium* sexual stage proteins to induce antibodies that prevent parasites from infecting blood-fed mosquitoes. This type of vaccine, which can be considered a "vaccine of solidarity," reduces *Plasmodium* infections within communities without conferring direct protective immunity to the vaccine recipients. The leading TBV candidates have advanced to field clinical trials, where vaccine-induced antibody function has been demonstrated in mosquito-feeding assays. However, the duration of functional antibody responses has been short-lived; hence current development has focused on improved adjuvant and vaccine delivery systems to generate long-lasting immune responses. For the future implementation of TBVs, community perceptions and understandings should be considered, and education should be provided on the concept and its value. Implementation will need to be undertaken in harmony with current malaria control policies.

Keywords: transmission-blocking vaccine, malaria, mosquito-feeding assays, elimination, eradication

1. Introduction

Malaria elimination and eradication have received renewed attention as the best long-term solution to this ancient scourge. However, existing tools that have been programmatically implemented have been insufficient to achieve elimination in areas of Africa, as well as at a global scale. Thus, new products are needed to pursue elimination, and efficacious vaccines are generally conceded to be ideal population-based interventions to support disease eradication. In this chapter, we describe the rationale and status of transmission-blocking vaccines

(TBV), which will contribute to malaria elimination and eradication. We place TBV in the context of overall malaria vaccine development and highlight the role and challenges of TBV field trials, which are needed to confirm activity and guide implementation.

2. Current malaria burden and need for elimination/eradication

Malaria is the most important parasitic disease and is endemic across the globe, most importantly sub-Saharan Africa, South and Southeast Asia, Papua New Guinea, and South America. Its burden remains unacceptably high, especially in sub-Saharan Africa, despite the significant gains with the use of current tools including vector control, diagnostics, chemoprevention and treatment. In 2016, 216 million malaria cases and 445,000 deaths were recorded worldwide mostly caused by *Plasmodium falciparum,* 90% of which occurred in sub-Saharan Africa [1]. Since artemisinins constitute the core component of the current malaria treatments, the recent emergence of artemisinin resistance in Southeast Asia [2–4] has become a serious obstacle for the malaria elimination agenda. Although the current phenotypes of artemisinin resistance are limited to slow parasite clearance and parasite recrudescence, their impact in malaria-endemic areas could result in a considerable increase of malaria cases, deaths, and economic costs according to predictive models [5, 6]. Indeed, the probable spread of artemisinin resistance to sub-Saharan Africa, where the burden of malaria is the highest, could jeopardize the lives of millions of children. Furthermore, the spread of insecticide and mosquito behavioral resistance compromises malaria control via the failure of vector control interventions such as indoor residual spraying (IRS) and insecticide-treated nets (ITN). Given that current strategies will eventually fail, new tools are urgently needed for malaria control and treatment. To overcome these constraints, transmission-blocking vaccines (TBVs) offer a new approach by targeting developing parasites in the mosquito host (a bottleneck in the malaria parasite lifecycle) and thereby contributing to malaria elimination and potentially eradication.

3. Need for malaria vaccine strategy

Vaccines are powerful tools that could accelerate malaria elimination efforts. Historically, vaccine-based strategies have contributed to the successful eradication of infectious diseases in humans and animals, including smallpox and rinderpest [7, 8]. Poliomyelitis is now close to eradication through routine Expanded Programme on Immunization (EPI) and massive immunization campaigns in some areas. Vaccination is a safe and cost-effective strategy that is easily implemented in large populations to reduce or even eliminate disease morbidity and mortality. Vaccine-induced immune responses protect individuals against infection or disease and can also stop transmission of the causative agent. With high coverage, vaccines protect not only recipients but also non-immunized individuals within the population through the effect of herd immunity. Malaria vaccines, even those with modest efficacy, such as the RTS,S product (see below in "Current status of malaria vaccine research"), are expected to avert millions of clinical malaria cases and thousands of severe malaria cases, hospitalizations, and

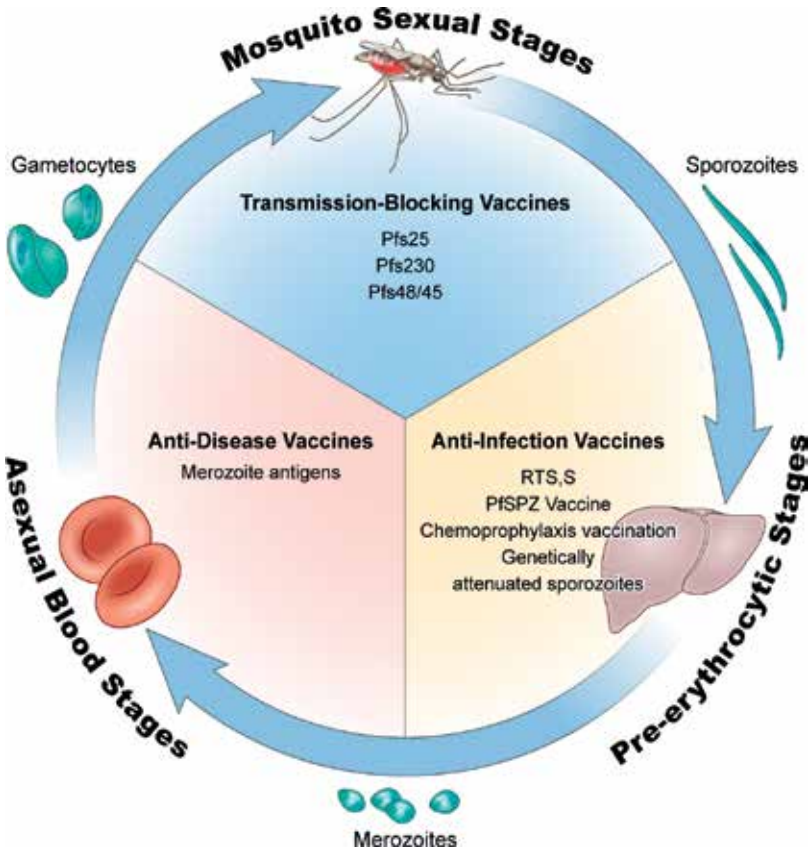


Figure 1. Malaria parasite life cycle and vaccine targets. Transmission-blocking vaccines are directed against the sexual stages of malaria parasite development in the mosquito, while other stages of the parasite life cycle can be targeted with different vaccine approaches. The vaccine concepts, candidate vaccines, and candidate antigens discussed in this chapter are presented according to their targeted stage of the parasite life cycle, as well as their anticipated biological effects: transmission-blocking, anti-infection, and anti-disease effects. Illustration by Alan Hoofring, Medical Arts Design Section, NIH.

deaths, according to prevalence-based predictive models [9–11]. The complex malaria parasite lifecycle (**Figure 1**) offers several stages that can be targeted by various vaccine strategies, which in combination may interrupt transmission.

4. Benefits of malaria transmission-blocking vaccines

Current malaria vaccine approaches target various parasite lifecycle stages including liver and blood stages in the individual and sexual stages in the mosquito (**Figure 1**). Liver stage vaccines, best typified by whole sporozoite (SPZ) vaccines that induce sterile protection [12], presumably act through T cell responses [13] and possibly antibodies and prevent progression of liver stage infections to blood stage parasitemia. Blood stage vaccines on the other hand

confer protection that reduces malaria episodes, disease severity, and/or parasitemia. Additionally, immunity against VAR2CSA, a member of the *P. falciparum* erythrocyte membrane protein 1 family that binds to chondroitin sulfate A, may prevent placental malaria [14].

Vaccines that target the sexual stages, known as TBVs, are the focus of this chapter. TBVs do not directly protect immunized individuals but specifically block onward transmission by preventing mosquito infection. TBVs utilize antigens expressed during mosquito parasite stages (gametocytes, gametes, zygotes and ookinetes) to induce functional antibodies that attack the parasite in the mosquito and impair its viability, inhibit its development, or impede its interaction with the mosquito midgut. The effector antibody responses involved in these types of vaccines include neutralization and complement-mediated lysis. A broader concept coined as a Vaccine to Interrupt Malaria Transmission (VIMT) by the Malaria Eradication Research Agenda (MalERA) includes not only TBVs but also pre-erythrocytic and blood stage vaccines, as well as mosquito molecules involved in parasite development [15] such as *Anopheles gambiae* aminopeptidase 1 (AnAPN1), carboxypeptidase, and saglin.

Ideally, TBVs will elicit effective antibodies that prevent malaria parasite development in mosquitoes after uptake of blood meals. This will reduce the number of circulating infectious mosquitoes below a threshold that sustains transmission. TBVs are among the tools being encouraged for use during pre-elimination and elimination phases of malaria eradication according to malERA [15] and could be an effective alternative or adjunct to vector control. Compared to vector control interventions, TBVs are ecologically safer, cost-effective, and readily enable high coverage of populations.

Most TBV antigens are genetically conserved, which may be due to limited immune pressure. The effect of immune pressure exerted by TBV against the parasite remains unknown and will need to be monitored in future. Notably, sexual stages are critical for the generation of parasite genetic diversity and regulation of parasite virulence, hence the effects of TBVs on these phenomena also warrant monitoring. In addition, malaria parasites experience a considerable population bottleneck in the mosquito for only a handful of parasite zygotes progressing to oocysts on the mosquito midgut. Altogether, while these observations make the mosquito phase an attractive target for vaccine development, much remains to be done to achieve implementable and effective TBVs.

5. Current status of malaria vaccine research

The development of effective vaccines against eukaryotic organisms is far from easy and has been particularly difficult for *P. falciparum*, a protozoan parasite characterized by three genomes (nuclear, mitochondrial, and apicoplastid), an adenine-thymine rich (~80%) nuclear genome [16] encoding >5000 genes, and a complex lifecycle involving several developmental stages between vertebrate and invertebrate hosts. Malaria vaccine development has been hampered by several factors during a century of effort, including the genetic diversity of *P. falciparum*, complexity of its biology, and difficulty obtaining long-lasting effective immunity. Interestingly, adults living in hyperendemic settings are continuously exposed to infective

mosquito bites and naturally acquire immunity that controls parasitemia and reduces clinical episodes of malaria over time. Responses against some parasite proteins have been associated with this natural protection, which makes them promising vaccine targets [17].

Today, the most advanced malaria vaccine is RTS,S, a pre-erythrocytic stage vaccine consisting of a virus-like particle (VLP) that displays hepatitis B surface antigen alone (S) and fused with a *P. falciparum* circumsporozoite protein fragment containing its central repeats and T cell epitopes (RTS). RTS,S has completed Phase III clinical trial (vaccine given to thousands of people and tested for efficacy and safety) and showed an efficacy of 51.3% (95%CI, 47.5–54.9) against clinical malaria in 5- to 17-month children over 12 months after three doses of the vaccine. A fourth dose was required to sustain protection over longer periods [18]. RTS,S is currently in pilot implementation studies involving 360,000 young children, expected to be given the vaccine in Ghana, Kenya, and Malawi. Although this represents important progress given the absence of any other human vaccine against a eukaryotic pathogen, more research is needed to develop vaccines that meet the Malaria Vaccine Technology Roadmap goals of 50% efficacy against severe malaria for more than one year and ≥75% long lasting efficacy against clinical malaria. For example, alternative dosages, timing and number of doses, are being evaluated as strategies to improve RTS,S efficacy [19, 20].

Attenuated whole SPZ vaccines have shown high-level sterile protection (>90%) against homologous challenge in early clinical trials [21] and thus have been heralded as a promising malaria vaccine approach. The concept of immunization using the whole SPZ was first attempted in 1910 by the French scientist Sergent using an avian model of malaria [22]. Several decades later, protective immunity was induced in mice following inoculation of X-irradiated SPZ of *P. berghei* [12]. In 1973, this approach was shown to be protective in humans, using X-irradiated SPZ of *P. falciparum* to vaccinate, followed by challenge with the non-irradiated homologous strain delivered by mosquito bites [23]. More recently, inoculation of non-attenuated fully infectious SPZ from chemo-sensitive strains along with administration of effective antimalarial drugs, known as chemoprophylaxis vaccination, was shown to induce sterilizing immunity [24]. Immunity induced by chemoprophylaxis vaccination is dose-dependent and requires substantially smaller SPZ inocula compared to irradiated SPZ [25].

Finally, genetic attenuation of parasites through the deletion of liver developmental stage-specific genes by homologous recombination is also being pursued to generate whole SPZ vaccines [26]. Numerous technologies may generate genetically attenuated parasite vaccines, including flippase (Flp)/Flp recognition target, Cre/loxP recombination, zing-finger nucleases, and the clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (CRISPR/Cas9) system [27–30]. Genetic attenuation enables generation of parasites that arrest at late liver stages, exposing a broader liver stage-specific antigen repertoire to the immune system over a longer duration. However, genetic attenuation can be incompletely effective for preventing breakthrough to blood stage parasitemia, and this needs to be monitored carefully in clinical studies. Further, the requirement for mosquitoes to deliver SPZ vaccines had been considered as an insuperable obstacle to development of a whole SPZ vaccine for mass immunization. This obstacle has been partially overcome by the production of purified, aseptic, and cryopreserved SPZ for syringe injection by Sanaria Inc. [31].

The blood stage is another important focus for malaria vaccine research, as this stage is responsible for the clinical manifestations of malaria. People living in endemic areas are repeatedly exposed to blood stage parasites and acquire protective antibodies over years that control parasitemia and prevent disease; consequently, a blood stage vaccine can be composed of antigens targeted by naturally acquired immunity that prevent clinical episodes of malaria [17, 32]. For example, immune responses to combinations of merozoite antigens were associated with 100% protection against clinical episodes of malaria in Kenyan observational cohorts [17]. Unfortunately, the efficacy of merozoite antigen vaccines in interventional trials has been poor, limited in part by antigenic diversity, which must be overcome for effective strain-transcending vaccines [33, 34].

6. Transmission-blocking vaccine (TBV) development

The first demonstration of antibodies that prevented mosquito infection was reported in 1958 using the avian species *Plasmodium gallinaceum* [35]. However, it remained until 1976 for studies to show that such antibodies might recognize gamete proteins and therefore act against the parasite in the invertebrate rather than vertebrate host [36, 37]. These gamete proteins were subsequently characterized to be P230 and P48/45, and later the zygote/ookinete surface proteins P28 and P25 were shown to be TBV targets; "P" refers to *Plasmodium* (the antigens have homologs in all *Plasmodium* species to date), and the number refers to their molecular weights on SDS-PAGE [38, 39]. P28 and P25 are paralogs, most abundant on the surface of zygotes and ookinetes, glycosylphosphatidylinositol (GPI)-anchored, and involved in ookinete formation. Today, these four parasite proteins represent the leading TBV candidates. Ookinete-secreted proteins have also been identified as targets for TBVs, including chitinase 1, von Willebrand factor-A domain-related protein, thrombospondin-related anonymous protein-related protein, membrane-attack ookinete protein, secreted ookinete adhesive protein (SOAP), and cell-traversal protein for ookinetes and sporozoites (CelTOS) [40, 41].

TBVs that have reached human clinical trials include only Pfs25 and its *Plasmodium vivax* ortholog Pvs25, and Pfs230. Early clinical trials of Pfs25 and Pvs25 yielded poor results due to either poor production of antibodies with transmission-blocking activity or to significant reactogenicity attributed to adjuvant formulations [42–44]. These challenges have been addressed by advances in vaccine expression systems, delivery platforms, and adjuvant formulations. Production of recombinant TBV antigen has been assessed in numerous systems, including *Escherichia coli*, *Saccharomyces cerevisiae*, *Pichia pastoris*, and baculovirus/insect cells, to yield better-folded proteins that are stable in solution and recreate conformational epitopes. Pfs230 and Pfs45/48 vaccines in particular are hampered by difficulty in expressing them in their appropriate conformations. To overcome this, research has focused on the expression of immunogenic fragments rather than full-length proteins [45, 46].

Several approaches to vaccine particle preparation have also been pursued to increase immunogenicity. These include conjugation to carriers (such as *Pseudomonas aeruginosa* exoprotein A (EPA) [47] and bacterial outer membrane protein complex (OMPC) [48]) or fusion to partners that complex to generate particles (such as C4 bp oligomerization domain (IMX313) expressed

in *E. coli* [49, 50] or modified lichenase carrier (LiKM) produced in *Nicotiana benthamiana* [50]). Viral vector vaccines, such as Chad63/Modified Vaccinia Ankara, are also being assessed to improve immunogenicity [49].

Adjuvants, such as Alhydrogel® and Montanide®, have been used for clinical trials of TBVs with reactogenicity issues observed with both; however, recent trials of Alhydrogel®-formulated TBV have demonstrated good safety and reactogenicity profiles. Recently, GSK®'s liposomal adjuvant AS01 has been considered for TBVs. AS01 incorporates the TLR4 ligand MPL and the saponin derivative QS-21, and because AS01 is used for formulating the pre-erythrocytic vaccine RTS,S, this would simplify future efforts to combine products.

TBV candidates that are in clinical and preclinical developments are summarized in **Table 1**. Human studies of Pfs25 and Pvs25 showed that priming doses of the vaccines do not induce detectable antibody levels [42, 51]. Antibody production is measurable after the first boost and then rapidly declines; additional boosts are required to retain antibody titers. However, the raised antibodies have been proven to be functional, i.e. capable of reducing oocyst formation in mosquito-feeding assays, and this activity strongly correlates to antibody titer [42, 51]. In addition to Pfs25/Pvs25 candidates, Pfs230 conjugated to EPA and adjuvanted in Alhydrogel® or AS01 has advanced to clinical trials for evaluation either alone or in coadministration with Pfs25 (https://clinicaltrials.gov/, trials NCT02334462 and NCT02942277).

Vaccine candidate	Type	Stage of development	Clinical trial identifier or reference number
Pfs25M-EPA/AS01 and/or Pfs230D1M-EPA/AS01	Subunit vaccine	Phase 1	NCT02942277
Pfs230D1M-EPA/Alhydrogel® and/or Pfs25 EPA/Alhydrogel®	Subunit vaccine	Phase 1	NCT02334462
Pfs25-EPA/Alhydrogel®	Subunit vaccine	Phase 1	NCT01867463, 51
Pfs25 VLP-FhCMB	VLP vaccine	Phase 1	NCT02013687
ChAd63 Pfs25-IMX313+/-MVA Pfs25-IMX313	Viral vector & nanoparticle vaccines	Phase 1	NCT02532049
Pfs25 & Pvs25/Montanide ISA 51	Subunit vaccine	Phase 1	[43]
Pvs25H/Alhydrogel®	Subunit vaccine	Phase 1	[42]
Pfs25-Pfs25	Conjugate vaccine	Phase 1	NCT00977899
Plant-Produced Pfs230 LiKM	Subunit vaccine	Preclinical	[57]
Pfs48/45	Subunit vaccine	Preclinical	[58, 59]
Pvs48/45	DNA vaccine	Preclinical	[60]
Pvs47	DNA vaccine	Preclinical	[60]
Pfs28	Subunit vaccine	Preclinical	[61, 62]
PfHAP2	Subunit or viral vector vaccine	Preclinical	[55, 63]
AnAPN1	Subunit vaccine	Preclinical	[59, 64]

Table 1. Transmission-blocking vaccine (TBV) candidates in clinical and preclinical developments.

7. Field studies of TBVs and Malian experiences

Field clinical trials are a major component of TBV development. TBV candidates are generally discovered in laboratories in the North with extensive infrastructure for modern biological sciences to conduct discovery research. After passing preclinical evaluations, TBV candidates must be tested in humans to qualify as viable vaccines. Safety and tolerability of the products are assessed first, generally in malaria-naïve individuals in non-endemic countries during a first-in-human phase 1 study. If the product meets acceptable safety and tolerability criteria, then it advances to Phases I, II, and III field clinical trials, which often means evaluation in malaria-endemic settings. Field studies are essential for assessing interruption of malaria transmission in the communities living in malaria-endemic areas.

A field clinical trial is not just a simple study but rather a multifaceted activity that builds on strong partnerships between research institutions and affected communities. Various capabilities are required for successful clinical trials, including confidence-based collaborative research teams, facilities, equipment, written procedures, training programs, community engagement, collaboration with ethics review committees, and collaborations with health and political authorities. The partnerships include vaccine inventors, developers, sponsors/funders, and institutions that have appropriate capacities and experience in conducting field clinical trials of malaria vaccines.

The main components of a TBV field clinical trial comprise immunization of study volunteers with prime and boost doses, intensive safety follow-up and reporting, mosquito-feeding assays, and the measurement of antibody responses using enzyme-linked immunosorbent assay (ELISA) for titers and standard membrane-feeding assay (SMFA) for activity. The immunization of volunteers is a major event that involves professionals with sundry expertise. The professionals include clinicians who assess volunteers for inclusion/exclusion criteria and monitor their health after receiving the vaccination, pharmacists who manage the randomization list as well as vaccine preparation, physicians who administer the vaccines, and intensivists who provide care for any post-immunization emergency. Medical biologists ensure proper biological sample collection, processing, transport, and storage as well as immediate measurement of biological parameters. Medical entomologists perform mosquito-feeding assays and associated dissections for endpoint analysis, and data managers enter and ensure quality of data according to established procedures.

8. Measuring vaccine activity

Unlike other vaccines where controlled human malaria infection (CHMI) studies are useful for assessing efficacy, field assessment of TBV efficacy currently requires mosquito-feeding assays on individuals living in malaria-endemic areas. Several mosquito-feeding assays can be utilized to assess the capacity of vaccine-induced antibodies to interfere with mosquito infectivity including direct skin-feeding (DSF) assay, direct membrane-feeding assay (DMFA), and standard membrane-feeding assay (SMFA). The DMFA entails feeding of laboratory-reared uninfected mosquitoes on venous blood immediately after collection from study participants

(**Figure 2A**). Feeding occurs through various types of membranes, such as pig intestine or Parafilm®, to access infected blood housed in a heated chamber that attracts mosquitoes. This method takes the diversity of infection in the population into account. The standard membrane-feeding assay (SMFA) is the gold-standard technique for functional evaluation of antibodies in TBV studies, given its use of a well-characterized laboratory parasite isolate and mosquito line that lend themselves to standardization. Mosquitoes feed on cultured gametocytes together with either volunteer serum or purified immunoglobulin (**Figure 2B**). SMFA is similar to DMFA in the machinery and process for feeding but fails to capture parasite diversity effects on vaccine activity.

In DSF assays, cups of field-adapted, laboratory-reared mosquitoes are fed on the skin of human volunteers to assess the ability of vaccine antibody responses to block malaria transmission in near-natural conditions (**Figure 3**). In a recent advance, the Malaria Research and Training Center (MRTC) in Bamako (Mali) and the Laboratory of Malaria Immunology and Vaccinology (LMIV) at NIAID/NIH, in Rockville, MD (USA) have established the infrastructure, logistics, and safety database to support scale up of DSF assays on a community-wide basis (**Figure 3**). These DSF assays use a line of locally caught *Anopheles coluzzii* recently adapted for breeding in a contained insectary. In these studies [52], uninfected mosquitoes (generally 30–60 per assay) are fed on study volunteers on a regular basis during the malaria transmission season, with the expectation that an effective vaccine will reduce the number of infected mosquitoes compared to controls. We believe DSFs, in which insectary-raised clean mosquitoes are directly fed on infected individuals, may be more likely to be predictive of an intervention's impact on transmission than membrane feeds.

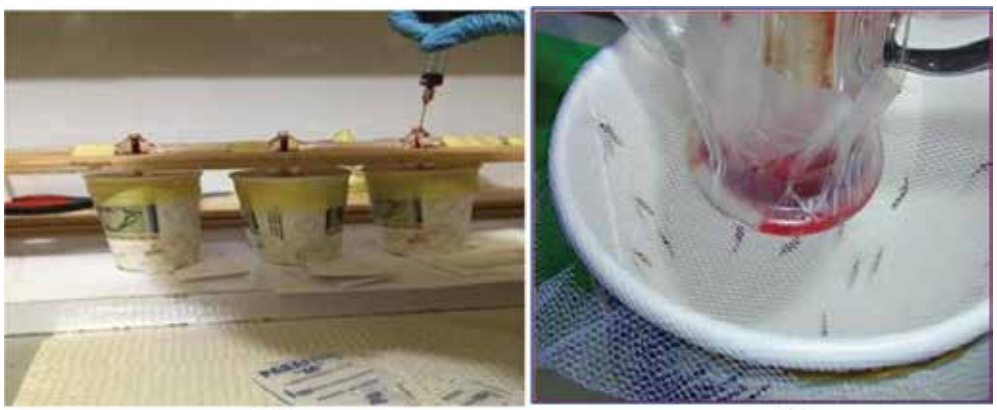


Figure 2. Membrane-feeding assays. (A) Overall setup of a direct membrane-feeding assay (DMFA). DMFA was performed at the Malaria Research and Training Center (MRTC), Mali with mosquitoes feeding through a membrane on whole venous blood taken in citrate-phosphate-dextrose or heparin from infected donors. The mosquitoes used were F1 or F2 progeny of wild-caught mosquitoes, or MRTC colony-bred mosquitoes (*Anopheles coluzzii*) maintained for many generations after local capture. The gametocyte source was fresh venous blood collected from infected study volunteers in Mali. (B) Standard membrane-feeding assay (SMFA) showing an individual feeding chamber. SMFA performed at the Laboratory of Malaria Immunology and Vaccinology (LMIV), National Institute of Allergy and Infectious Diseases (NIAID)/National Institutes of Health (NIH), Rockville, MD, USA with mosquitoes feeding through a membrane on laboratory-cultured parasites (gametocytes) suspended in media with immune or nonimmune serum/plasma or IgG. The mosquitoes used were an established laboratory strain (commonly *Anopheles stephensi*).

Figure 3. Direct skin feeds (DSF). DSF was performed at the Malaria Research and Training Center (MRTC), Mali at the study sites. MRTC colony-bred mosquitoes (*Anopheles coluzzii*) raised in containment fed directly on skin of consenting study participants. The gametocyte source was infected study volunteers in Mali. Ethical approvals were obtained from both the NIAID/NIH IRB (National Institute of Allergy and Infectious Diseases/National Institutes of Health, Rockville, MD, USA Institutional Review Board) and the Mali FMPOS (Faculty of Medicine, Pharmacy and Odonto-Stomatology) Ethics Committee.

For all these methods (SMFA, DMFA, DSF), mosquitoes are dissected about a week after feeding for oocyst detection and counting by microscopy. The results of the mosquito-feeding assay allow the calculation of transmission-blocking activity and transmission reducing

activity, which are respectively the ratio of the proportions of infected mosquitoes and the mean counts of oocysts, between test and control mosquitoes.

The measurement of antibody titers during field clinical trials of TBVs (generally by ELISA) is required to assess the immunogenicity of vaccine candidates. Samples are assayed to detect any pre-existing immunity against vaccine candidates, to determine vaccine immunogenicity after prime and boost doses, and to monitor the decay of antibody titers during the follow-up. In addition, antibody titer data can be linked with mosquito-feeding assay data to determine the correlation between antibody production and functionality. Many further investigations can be performed with samples collected from the study volunteers for other exploratory objectives.

9. The Malaria Research and Training Center (MRTC) field trials experience

The achievement of all field activities requires an institution endowed with strong capacities and trained staff. Malaria Research and Training Center (MRTC), founded in 1992 at the Department of Parasitic Diseases Epidemiology based at the University of Sciences, Techniques and Technologies of Bamako (USTTB) in Mali, is a leading institution in conducting field clinical trials of TBVs in Africa. The center is the result of a partnership between the Malian government and US National Institutes of Health (NIH) to build capacity in malaria research and training in Mali. The USTTB collaboration has been designated by NIH as an International Center for Excellence in Research (ICER).

MRTC comprises six equipped and autonomous clinical trial sites located in malaria-endemic Malian villages that fulfill International Conference on Harmonization (ICH) requirements and adhere to Good Clinical Practices (GCP) and a central laboratory in Bamako (the capital city of Mali) where several teams are based. The MRTC infrastructure includes a clinical laboratory certified by the College of American Pathologists and an equipped insectary, among others. In addition, the center has 15 other field sites that host epidemiological studies and other collaborations with external partners.

From 2003 to 2016, MRTC completed 13 asexual stage vaccine clinical trials and one TBV field clinical trial evaluating Pfs25H-EPA/Alhydrogel® (Bancoumana, Mali, from 2013 to 2016, NCT01867463) in collaboration with LMIV at NIH. Field clinical trials of six more candidate TBVs are ongoing: Pfs25M-EPA/Alhydrogel® and Pfs230D1M-EPA/Alhydrogel®, as well as the combination of Pfs25M-EPA/Alhydrogel® + Pfs230D1M-EPA/Alhydrogel® (Bethesda, USA and Bancoumana, Mali; started in 2015, NCT02334462), and Pfs25M-EPA/AS01, Pfs230D1M-EPA/AS01, and the combination of Pfs25M-EPA/AS01 + Pfs230D1M-EPA/AS01 (Bamako, Doneguebougou and Bancoumana, Mali; started in 2016, NCT02942277). The success of these studies depends on MRTC's technical capacities and strength in community engagement. Dynamic MRTC teams execute intense programs of volunteer immunization and thorough follow-up (**Figure 4**), as well as high-throughput processing of samples in laboratories, rearing of mosquitoes, mosquito-feeding assays, dissection of mosquitoes, real-time data entry and cleaning, and constant transportation of persons, samples, and materials between central laboratory and study sites.

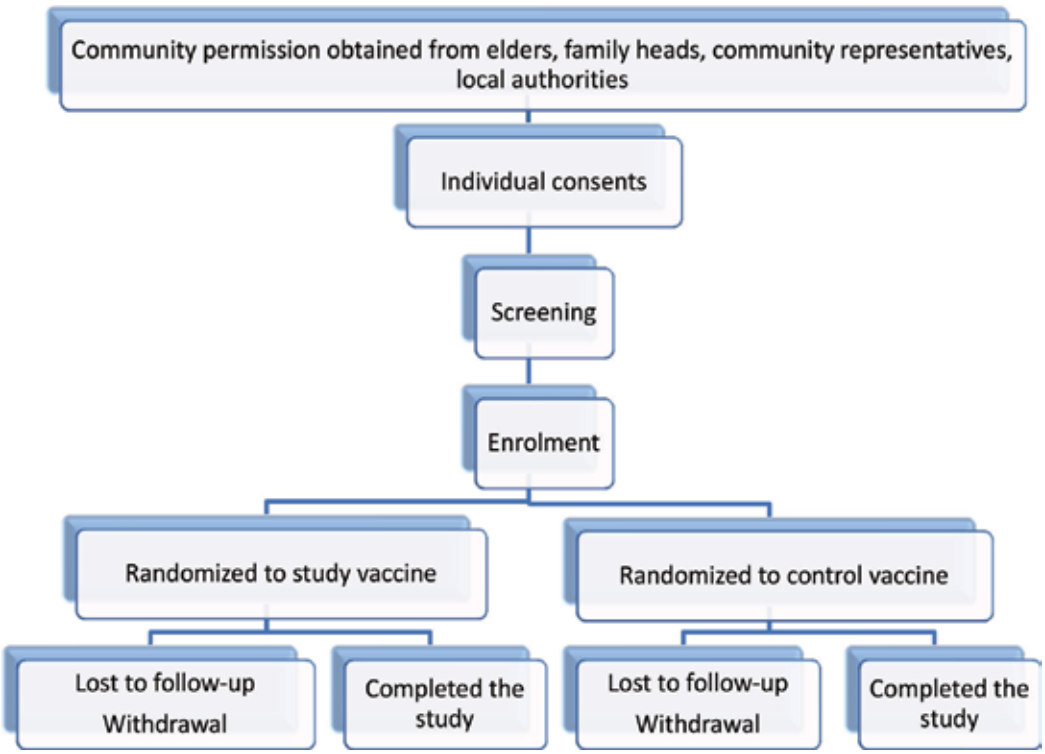


Figure 4. General profile of a malaria transmission-blocking vaccine trial in Mali. This is general study process (flow-chart) followed at the Malaria Research and Training Center (MRTC), Mali in carrying out a malaria vaccine study.

For community engagement, MRTC has built participative, durable, and confidence-based partnerships with communities at and around the study sites. Initially, community leaders comprising village heads, elderly, family heads, women's and youth's representatives, local health providers, and school teachers are consulted for permission to build research facilities and conduct clinical trials in the communities [53] (**Figure 4**). Subsequently, individual consents are obtained from interested volunteers according to international guidelines.

Local residents are fully involved and meaningfully impacted by the research activities. The presence of research teams and facilities, along with successful execution of clinical trials, has contributed to significant improvement of the local healthcare system and even the economy of the host villages. This positive impact has reinforced community confidence towards clinical trials and subsequently strengthened community engagement, positioning MRTC to successfully conduct TBV field clinical trials in Mali.

10. Bottlenecks and perspectives for TBVs

The future development and potential implementation of current TBV candidates will need to address important regulatory issues. A major issue involves the conceptual framework for a

vaccine that does not directly benefit a recipient, but instead benefits a community only when the proportion of the population that receives the vaccine achieves the threshold needed to reduce malaria prevalence and incidence. This threshold may vary between communities depending on the baseline intensity and ecology of malaria transmission. A TBV can be considered a "vaccine of solidarity," whereby the individual accepts vaccination to contribute to protection of his/her community and thereby ultimately to his/her own benefit. Before any decision to participate, individuals must balance the risks of immunization and the benefits for the community and ultimately themselves. To address this issue during clinical trials, candidate study participants must pass a test of comprehension that indicates the participant's understanding that the TBV has no potential to directly prevent their own infections. At present, there are few data in the literature on individual and community perceptions and understanding of TBV. Education on the mechanisms of action and potential community/individual benefits of TBV will be required before implementation.

Additionally, this community-based approach and vaccine coverage goal sets a high bar to achieve, even for a malaria vaccine. The vaccine needs to be efficacious not only in children but also in the majority of individuals in the community that contribute to transmission. The impact of factors such as malaria exposure, immunodeficiency, coinfection, pregnancy, and age on antibody responses will require careful study after qualification of promising TBV candidates. Thus, further research is needed to understand transmission dynamics within a community, durability and boosting of TBVs, and immunogenicity throughout all individuals within a community.

Furthermore, the DSF assay that experimentally exposes humans to mosquito bites raises ethical concerns and logistical burdens that may impact scientific objectives and study designs, and in some communities, recruitment and retention of volunteers. Mosquitoes used for DSF are reared in controlled and contained conditions to ensure the safety of the volunteers. While DSF has approval by ethical review committees, community perceptions should be considered to reassure participants and promote participant engagement in the process and rationale to prevent loss to follow-up that can compromise trial objectives. Our experience in Mali shows that strong community relationships and individual education in study design and goals have resulted in high participation and retention rates for studies that incorporate DSF assays.

The leading TBV candidates are either not exposed (Pfs25) to the immune system naturally or induce only modest responses naturally, and thus natural boosting may do little or nothing to extend antibody responses. Human antibodies should act quickly before degradation in the mosquito and before parasite traversal of midgut epithelium (~24 h) that limits its accessibility to antibody. The need for TBV to maintain high levels of potent antibody that preclude mosquito infection is the major hurdle for developing TBV. Today, several strategies are being pursued to overcome this hurdle, including the improved adjuvants and delivery systems discussed above. Additional antigens are also under exploration such as Pfs48/45 [54] and PfHAP2 [55] that could expand the portfolio of TBV in future.

As there is no existing human-based intervention that uniquely aims at blocking parasite transmission, such a tool could shift interest toward the interruption of transmission in comparison to the urgent need for interventions that protect and treat children under five and pregnant women. Once TBV tolerability and impact in the field are confirmed in advanced

clinical trials, consideration must be given for how this vaccine should be implemented alongside other malaria control tools. Ultimately, TBVs are envisioned as one tool in the toolbox of interventions, including antimalarial drugs, vector control measures, and other vaccines, that will be required for malaria elimination.

11. Conclusions

Malaria control has improved with the redoubling of efforts and financial resources in recent years, but existing tools will lose their effectiveness over time. The best long-term strategy to address the malaria scourge is its elimination and ultimately eradication, but existing tools are insufficient for this purpose. This gap has been recognized by the WHO Roadmap for Malaria Vaccines that specifically calls for vaccines that can be used for elimination [56]. TBVs prevent human-to-mosquito transfer of parasites and hence are well-suited for use in elimination and eradication programs. Development of TBV has been hindered by the nature of the target antigens, which have been difficult to express in proper conformation and are poorly immunogenic, as well as the dearth of resources dedicated to their development. However, increasing attention to elimination by policy makers and funding agencies has reignited interest in this research area, and improved platforms for vaccine expression and delivery have yielded promising new TBV candidates that have in some cases advanced to field trials. Collaborative multidisciplinary teams are now rising to the task of testing TBVs in the field, which also require specialized facilities to measure transmission-blocking activity of vaccines. Regulatory issues will need to be addressed as TBVs are developed and implemented, particularly because these products are designed to benefit the community and not the individual directly. However, vaccines have been essential for eliminating or eradicating other infectious agents, and TBVs could be a vital component of a multipronged effort to eradicate malaria from the face of the earth.

Acknowledgements

Karamoko Niaré, Issaka Sagara and Ogobara Doumbo are supported by the Mali malaria vaccine programmes of Malaria Research and Training Center (MRTC)-International Center for Excellence in Research (ICER); University of Sciences, Techniques and Technologies of Bamako (USTTB); and National Institute of Allergy and Infectious Diseases (NIAID)/National Institutes of Health (NIH).

Sara Healy and Patrick Duffy are supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases. J. Patrick Gorres assisted in proof reading and editing the chapter.

Acronyms

AnaPN1	*Anopheles gambiae* aminopeptidase 1
AS01	GSK's adjuvant system containing liposome, 3-O-desacyl-4′-monophosphoryl lipid A (MPL) and *Quillaja saponaria* Molina saponin fraction 21 (QS21)
CELTOS	Cell-traversal protein for ookinetes and sporozoites
CHMI	Controlled human malaria infection
CRISPR/Cas9	Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9)
DMSFA	Standard membrane-feeding assay
DSF	Direct skin-feeding assay
ELISA	Enzyme-linked immunosorbent assay
EPA	*Pseudomonas aeruginosa* exoprotein A.
EPI	Expanded Programme on Immunization
Flp	Flippase
FMPOS	Faculty of Medicine, Pharmacy and Odonto-Stomatology
GCP	Good Clinical Practices
GPI	Glycosylphosphatidylinositol
GSK	GlaxoSmithKline
ICER	International Center for Excellence in Research
ICH	International Conference on Harmonization
IMX313	C4 bp oligomerization domain
IRB	Institutional Review Board
IRS	Indoor residual spraying
ITN	Insecticide-treated nets
LikM	Modified lichenase carrier
LMIV	Laboratory of Malaria Immunology and Vaccinology
MalERA	Malaria Eradication Research Agenda
MPL	3-O-desacyl-4′-monophosphoryl lipid A

MRTC	Malaria Research and Training Center
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OMPC	Outer membrane protein complex
QS21	GSK's *Quillaja saponaria* Molina saponin fraction 21
RTS,S	Hepatitis B surface antigen alone (S) and fused with *Plasmodium falciparum* circumsporozoite protein fragment containing its central repeats and T cell epitopes (RTS)
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SMFA	Standard membrane-feeding assay
SOAP	Secreted ookinete adhesive protein
SPZ	Sporozoite
TBV	Transmission-blocking vaccine
TLR4	Toll-like receptor 4
USTTB	University of Sciences, Techniques and Technologies of Bamako
VAR2CSA	Member of *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) family binding to chondroitin sulfate A (CSA)
VIMT	Vaccine to Interrupt Malaria Transmission
VLP	Virus-like particle
WHO	World Health Organization

Author details

Ogobara K. Doumbo[1]*, Karamoko Niaré[1], Sara A. Healy[2], Issaka Sagara[1] and Patrick E. Duffy[2]

*Address all correspondence to: okd@icermali.org

1 Malaria Research and Training Center-International Center for Excellence in Research, University of Sciences, Techniques and Technologies of Bamako, Mali

2 Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland, USA

References

[1] WHO. World Malaria Report 2017. Geneva: World Health Organization; 2017. 114 p

[2] Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, Sreng S, Anderson JM, Mao S, Sam B, Sopha C, Chuor CM, Nguon C, Sovannaroth S, Pukrittayakamee S, Jittamala P, Chotivanich K, Chutasmit K, Suchatsoonthorn C, Runcharoen R, Hien TT, Thuy-Nhien NT, Thanh NV, Phu NH, Htut Y, Han K-T, Aye KH, Mokuolu OA, Olaosebikan RR, Folaranmi OO, Mayxay M, Khanthavong M, Hongvanthong B, Newton PN, Onyamboko MA, Fanello CI, Tshefu AK, Mishra N, Valecha N, Phyo AP, Nosten F, Yi P, Tripura R, Borrmann S, Bashraheil M, Peshu J, Faiz MA, Ghose A, Hossain MA, Samad R, Rahman MR, Hasan MM, Islam A, Miotto O, Amato R, MacInnis B, Stalker J, Kwiatkowski DP, Bozdech Z, Jeeyapant A, Cheah PY, Sakulthaew T, Chalk J, Intharabut B, Silamut K, Lee SJ, Vihokhern B, Kunasol C, Imwong M, Tarning J, Taylor WJ, Yeung S, Woodrow CJ, Flegg JA, Das D, Smith J, Venkatesan M, Plowe CV, Stepniewska K, Guerin PJ, Dondorp AM, Day NP, White NJ. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. New England Journal of Medicine. 2014;**371**:411-423. DOI: 10.1056/NEJMoa1314981

[3] Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, Lwin KM, Ariey F, Hanpithakpong W, Lee SJ, Ringwald P, Silamut K, Imwong M, Chotivanich K, Lim P, Herdman T, An SS, Yeung S, Singhasivanon P, Day NPJ, Lindegardh N, Socheat D, White NJ. Artemisinin resistance in *Plasmodium falciparum* malaria. The New England Journal of Medicine. 2009;**361**:455-467. DOI: 10.1056/NEJMoa0808859

[4] Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, Khim N, Kim S, Duru V, Bouchier C, Ma L, Lim P, Leang R, Duong S, Sreng S, Suon S, Chuor CM, Bout DM, Ménard S, Rogers WO, Genton B, Fandeur T, Miotto O, Ringwald P, Le Bras J, Berry A, Barale JCMD. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. Nature. 2014;**505**:50-55. DOI: 10.1038/nature12876

[5] Slater HC, Griffin JT, Ghani AC, Okell LC. Assessing the potential impact of artemisinin and partner drug resistance in sub-Saharan Africa. Malaria Journal. 2016;**15**:10. DOI: 10.1186/s12936-015-1075-7

[6] Lubell Y, Dondorp A, Guérin PJ, Drake T, Meek S, Ashley E, Day NP, White NJ, White LJ. Artemisinin resistance—Modelling the potential human and economic costs. Malaria Journal. 2014;**13**:452. DOI: 10.1186/1475-2875-13-452

[7] Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. Smallpox and Its Eradication. Geneva: World Health Organization; 1988. 1500p

[8] Normile D. Rinderpest: Driven to extinction. Science. 2008;**319**:1606-1609. DOI: 10.1126/science.319.5870.1606

[9] Sauboin CJ, Van Bellinghen L-A, Van De Velde N, Van Vlaenderen I. Potential public health impact of RTS,S malaria candidate vaccine in sub-Saharan Africa: A modelling study. Malaria Journal. 2015;**14**:524. DOI: 10.1186/s12936-015-1046-z

[10] Penny MA, Verity R, Bever CA, Sauboin C, Galactionova K, Flasche S, White MT, Wenger EA, Van de Velde N, Pemberton-Ross P, Griffin JT, Smith TA, Eckhoff PA, Muhib F, Jit M, Ghani AC. Public health impact and cost-effectiveness of the RTS,S/AS01 malaria vaccine: A systematic comparison of predictions from four mathematical models. Lancet. 2016;**387**:367-375. DOI: 10.1016/S0140-6736(15)00725-4

[11] Nunes JK, Cárdenas V, Loucq C, Maire N, Smith T, Shaffer C, Måseide K, Brooks A. Modeling the public health impact of malaria vaccines for developers and policymakers. BMC Infectious Diseases. 2013;**13**:295. DOI: 10.1186/1471-2334-13-295

[12] Nussenzweig RS, Vanderberg J, Most H, Orton C. Protective immunity produced by the injection of x-irradiated sporozoites of *Plasmodium berghei*. Nature. 1967;**216**:160-162

[13] Reyes-Sandoval A, Wyllie D, Bauza K, Milicic A, Forbes E, Rollier C, Hill A. CD8 + T effector memory cells protect against liver-stage malaria. Journal of Immunology. 2011; **187**:1347-1357. DOI: 10.4049/jimmunol.1100302

[14] Fried M, Duffy PE. Designing a VAR2CSA-based vaccine to prevent placental malaria. Vaccine. 2015;**33**:7483-7488. DOI: 10.1016/j.vaccine.2015.10.011

[15] Alonso PL, Brown G, Arevalo-Herrera M, Binka F, Chitnis C. A research agenda to underpin malaria eradication. PLoS Medicine. 2011;**8**:e1000406. DOI: 10.1371/journal.pmed.1000406

[16] Gardner MJ, Hall N, Fung E, White O, Berriman M, Hyman RW, Carlton JM, Pain A, Nelson KE, Bowman S, Paulsen IT, James K, Eisen JA, Rutherford K, Salzberg SL, Craig A, Kyes S, Chan M-S, Nene V, Shallom SJ, Suh B, Peterson J, Angiuoli S, Pertea M, Allen J, Selengut J, Haft D, Mather MW, Vaidya AB, Martin DMA, Fairlamb AH, Fraunholz MJ, Roos DS, Ralph SA, McFadden GI, Cummings LM, Subramanian GM, Mungall C, Venter JC, Carucci DJ, Hoffman SL, Newbold C, Davis RW, Fraser CM, Barrell B. Genome sequence of the human malaria parasite *Plasmodium falciparum*. Nature. 2002;**419**:498-511. DOI: 10.1038/nature01097

[17] Osier FH, Mackinnon MJ, Crosnier C, Fegan G, Kamuyu G, Wanaguru M, Ogada E, McDade B, Rayner JC, Wright GJ, Marsh K. New antigens for a multicomponent blood-stage malaria vaccine. Science Translational Medicine. 2014;**6**:247ra102. DOI: 10.1126/scitranslmed.3008705

[18] Vandoolaeghe P, Schuerman L. The RTS,S/AS01 malaria vaccine in children 5 to 17 months of age at first vaccination. Expert Review of Vaccines. 2016;**15**:1481-1493. DOI: 10.1080/14760584.2016.1236689

[19] Regules JA, Cicatelli SB, Bennett JW, Paolino KM, Twomey PS, Moon JE, Kathcart AK, Hauns KD, Komisar JL, Qabar AN, Davidson SA, Dutta S, Griffith ME, Magee CD, Wojnarski M, Livezey JR, Kress AT, Waterman PE, Jongert E, Wille-Reece U, Volkmuth W, Emerling D, Robinson WH, Lievens M, Morelle D, Lee CK, Yassin-Rajkumar B, Weltzin R, Cohen J, Paris RM, Waters NC, Birkett AJ, Kaslow DC, Ballou WR, Ockenhouse CF, Vekemans J. Fractional third and fourth dose of RTS,S/AS01 malaria candidate vaccine: A phase 2a controlled human malaria parasite infection and immunogenicity study. Journal of Infectious Diseases. 2016;**5**:762-771. DOI: 10.1093/infdis/jiw237

[20] Chaudhury S, Regules JA, Darko CA, Dutta S, Wallqvist A, Waters NC, Jongert E, Lemiale F, Bergmann-Leitner ES. Delayed fractional dose regimen of the RTS,S/AS01 malaria vaccine candidate enhances an IgG4 response that inhibits serum opsonophagocytosis. Scientific Reports. 2017;**1**:7998. DOI: 10.1038/s41598-017-08526-5

[21] Hoffman SL, Goh LML, Luke TC, Schneider I, Le TP, Doolan DL, Sacci J, de la Vega P, Dowler M, Paul C, Gordon DM, Stoute JA, Church LWP, Sedegah M, Heppner DG, Ballou WR, Richie TL. Protection of humans against malaria by immunization with radiation-attenuated *Plasmodium falciparum* sporozoites. The Journal of Infectious Diseases. 2002; **185**:1155-1164. DOI: 10.1086/339409

[22] Sergent E. Sur l'immunité dans le paludisme des oiseaux. Conservation in vitro des sporozoites de *Plasmodium relictum*. Immunité relative obtenue par inoculation de ces sporozoites. Comptes rendus de l'Académie des Sciences. 1910;**151**:407-409

[23] Clyde DF, Most H, McCarthy VC, Vanderberg JP. Immunization of man against sporozoite-induced *falciparum* malaria. The American Journal of the Medical Sciences. 1973;**266**:169-177

[24] Roestenberg M, McCall M, Hopman J, Wiersma J, Luty AJF, van Gemert GJ, van de Vegte-Bolmer M, van Schaijk B, Teelen K, Arens T, Spaarman L, de Mast Q, Roeffen W, Snounou G, Rénia L, van der Ven A, Hermsen CC, Sauerwein R. Protection against a malaria challenge by sporozoite inoculation. The New England Journal of Medicine. 2009;**361**: 468-477. DOI: 10.1056/NEJMoa0805832

[25] Bijker EM, Teirlinck AC, Schats R, van Gemert G-J, van de Vegte-Bolmer M, van Lieshout L, IntHout J, Hermsen CC, Scholzen A, Visser LG, Sauerwein RW. Cytotoxic markers associate with protection against malaria in human volunteers immunized with *Plasmodium falciparum* sporozoites. The Journal of Infectious Diseases. 2014;**210**:1605-1615. DOI: 10.1093/infdis/jiu293

[26] Mueller A-K, Labaied M, Kappe SHI, Matuschewski K. Genetically modified *Plasmodium* parasites as a protective experimental malaria vaccine. Nature. 2005;**433**:164-167. DOI: 10.1038/nature03188

[27] Combe A, Giovannini D, Carvalho TG, Spath S, Boisson B, Loussert C, Thiberge S, Lacroix C, Gueirard P, Ménard R. Clonal conditional mutagenesis in malaria parasites. Cell Host & Microbe. 2009;**5**:386-396. DOI: 10.1016/j.chom.2009.03.008

[28] Singer M, Marshall J, Heiss K, Mair GR, Grimm D, Mueller A-K, Frischknecht F. Zinc finger nuclease-based double-strand breaks attenuate malaria parasites and reveal rare microhomology-mediated end joining. Genome Biology. 2015;**16**:249. DOI: 10.1186/s13059-015-0811-1

[29] Ghorbal M, Gorman M, Macpherson CR, Martins RM, Scherf A, Lopez-Rubio J-J. Genome editing in the human malaria parasite *Plasmodium falciparum* using the CRISPR-Cas9 system. Nature Biotechnology. 2014;**32**:819-821. DOI: 10.1038/nbt.2925

[30] Singer M, Frischknecht F. Time for genome editing: Next-generation attenuated malaria parasites. Trends in Parasitology. 2017;**33**:202-213. DOI: 10.1016/j.pt.2016.09.012

[31] Hoffman SL, Billingsley PF, James E, Richman A, Loyevsky M, Li T, Chakravarty S, Gunasekera A, Chattopadhyay R, Li M, Stafford R, Ahumada A, Epstein JE, Sedegah M, Reyes S, Richie TL, Lyke KE, Edelman R, Laurens MB, Plowe CV, Sim BKL. Development

of a metabolically active, non-replicating sporozoite vaccine to prevent *Plasmodium falciparum* malaria. Human Vaccines. 2010;**6**:97-106. DOI: 10.4161/hv.6.1.10396

[32] Richards JS, Arumugam TU, Reiling L, Healer J, Hodder AN, Fowkes FJI, Cross N, Langer C, Takeo S, Uboldi AD, Thompson JK, Gilson PR, Coppel RL, Siba PM, King CL, Torii M, Chitnis CE, Narum DL, Mueller I, Crabb BS, Cowman AF, Tsuboi T, Beeson JG. Identification and prioritization of merozoite antigens as targets of protective human immunity to *Plasmodium falciparum* malaria for vaccine and biomarker development. Journal of Immunology. 2013;**191**:795-809. DOI: 10.4049/jimmunol.1300778

[33] Thera MA, Doumbo OK, Coulibaly D, Laurens MB, Ouattara A, Kone AK, Guindo AB, Traore K, Traore I, Kouriba B, Diallo DA, Diarra I, Daou M, Dolo A, Tolo Y, Sissoko MS, Niangaly A, Sissoko M, Takala-Harrison S, Lyke KE, Wu Y, Blackwelder WC, Godeaux O, Vekemans J, Dubois M-C, Ballou WR, Cohen J, Thompson D, Dube T, Soisson L, Diggs CL, House B, Lanar DE, Dutta S, Heppner DG, Plowe CV. A field trial to assess a blood-stage malaria vaccine. The New England Journal of Medicine. 2011;**365**:1004-1013. DOI: 10.1056/NEJMoa1008115

[34] Genton B, Betuela I, Felger I, Al-Yaman F, Anders RF, Saul A, Rare L, Baisor M, Lorry K, Brown GV, Pye D, Irving DO, Smith TA, Beck H, Alpers MP. A recombinant blood-stage malaria vaccine reduces *Plasmodium falciparum* density and exerts selective pressure on parasite populations in a phase 1–2b trial in Papua New Guinea. The Journal of Infectious Diseases. 2002;**185**:820-827. DOI: 10.1086/339342

[35] Huff CG, Marchbank DF, Shiroishi T. Changes in infectiousness of malarial gametocytes. II. Analysis of the possible causative factors. Experimental Parasitology. 1958;**7**:399-417

[36] Carter R, Chen DH. Malaria transmission blocked by immunization with gametes of the malaria parasite. Nature. 1976;**263**:57-60. DOI: 10.1038/263057a0

[37] Gwadz RW. Successful immunization against the sexual stages of *Plasmodium gallinaceum*. Science. 1976;**193**:1150-1151. DOI: 10.1126/science.959832

[38] Kaushal DC, Carter R, Rener J, Grotendorst CA, Miller LH, Howard RJ. Monoclonal antibodies against surface determinants on gametes of *Plasmodium gallinaceum* block transmission of malaria parasites to mosquitoes. Journal of Immunology. 1983;**131**:2557-2562

[39] Rener J, Graves PM, Carter R, Williams JL, Burkot TR. Target antigens of transmission-blocking immunity on gametes of *Plasmodium falciparum*. The Journal of Experimental Medicine. 1983;**158**:976-981

[40] Dessens JT, Sidén-Kiamos I, Mendoza J, Mahairaki V, Khater E, Vlachou D, Xu X-J, Kafatos FC, Louis C, Dimopoulos G, Sinden RE. SOAP, a novel malaria ookinete protein involved in mosquito midgut invasion and oocyst development. Molecular Microbiology. 2003;**49**: 319-329. DOI: 10.1046/j.1365-2958.2003.03566.x

[41] Kariu T, Ishino T, Yano K, Chinzei Y, Yuda M. CelTOS, a novel malarial protein that mediates transmission to mosquito and vertebrate hosts. Molecular Microbiology. 2006; **59**:1369-1379. DOI: 10.1111/j.1365-2958.2005.05024.x

[42] Malkin EM, Durbin AP, Diemert DJ, Sattabongkot J, Wu Y, Miura K, Long CA, Lambert L, Miles AP, Wang J, Stowers A, Miller LH, Saul A. Phase 1 vaccine trial of Pvs25H: A transmission blocking vaccine for *Plasmodium vivax* malaria. Vaccine. 2005;**23**:3131-3138. DOI: 10.1016/j.vaccine.2004.12.019

[43] Wu Y, Ellis RD, Shaffer D, Fontes E, Malkin EM, Mahanty S, Fay MP, Narum D, Rausch K, Miles AP, Aebig J, Orcutt A, Muratova O, Song G, Lambert L, Zhu D, Miura K, Long C, Saul A, Miller LH, Durbin AP. Phase 1 trial of malaria transmission blocking vaccine candidates Pfs25 and Pvs25 formulated with Montanide ISA 51. Ratner AJ, ed. PLoS One. 2008;**3**(7):e2636. DOI: 10.1371/journal.pone.0002636

[44] Ockenhouse CF, Sun PF, Lanar DE, Wellde BT, Hall BT, Kester K, Stoute JA, Magill A, Krzych U, Farley L, Wirtz RA, Sadoff JC, Kaslow DC, Kumar S, Church LW, Crutcher JM, Wizel B, Hoffman S, Lalvani A, Hill AV, Tine JA, Guito KP, de Taisne C, Anders R, Ballou WR. Phase I/IIa safety, immunogenicity, and efficacy trial of NYVAC-Pf7, a pox-vectored, multiantigen, multistage vaccine candidate for *Plasmodium falciparum* malaria. The Journal of Infectious Diseases. 1998;**177**:1664-1673

[45] Outchkourov NS, Roeffen W, Kaan A, Jansen J, Luty A, Schuiffel D, van Gemert GJ, van de Vegte-Bolmer M, Sauerwein RW, Stunnenberg HG. Correctly folded Pfs48/45 protein of *Plasmodium falciparum* elicits malaria transmission-blocking immunity in mice. Proceedings of the National Academy of Sciences 2008;**105**:4301-4305. DOI:10.1073/pnas.0800459105

[46] Williamson KC, Keister DB, Muratova O, Kaslow DC. Recombinant Pfs230, a *Plasmodium falciparum* gametocyte protein, induces antisera that reduce the infectivity of *Plasmodium falciparum* to mosquitoes. Molecular and Biochemical Parasitology. 1995;**75**:33-42. DOI: 10.1016/0166-6851(95)02507-3

[47] Qian F, Wu Y, Muratova O, Zhou H, Dobrescu G, Duggan P, Lynn L, Song G, Zhang Y, Reiter K, MacDonald N, Narum DL, Long CA, Miller LH, Saul A, Mullen GED. Conjugating recombinant proteins to *Pseudomonas aeruginosa* ExoProtein A: A strategy for enhancing immunogenicity of malaria vaccine candidates. Vaccine. 2007;**25**:3923-3933. DOI: 10.1016/j.vaccine.2007.02.073

[48] Wu Y, Przysiecki C, Flanagan E, Bello-Irizarry SN, Ionescu R, Muratova O, Dobrescu G, Lambert L, Keister D, Rippeon Y, Long CA, Shi L, Caulfield M, Shaw A, Saul A, Shiver J, Miller LH. Sustained high-titer antibody responses induced by conjugating a malarial vaccine candidate to outer-membrane protein complex. Proceedings of the National Academy of Sciences. 2006;**103**:18243-18248. DOI: 10.1073/pnas.0608545103

[49] Li Y, Leneghan DB, Miura K, Nikolaeva D, Brian IJ, Dicks MDJ, Fyfe AJ, Zakutansky SE, de Cassan S, Long CA, Draper SJ, Hill AVS, Hill F, Biswas S. Enhancing immunogenicity and transmission-blocking activity of malaria vaccines by fusing Pfs25 to IMX313 multimerization technology. Scientific Reports. 2016;**6**:18848. DOI: 10.1038/srep18848

[50] Ogun SA, Dumon-Seignovert L, Marchand J-B, Holder AA, Hill F. The oligomerization domain of C4-binding protein (C4bp) acts as an adjuvant, and the fusion protein comprised of the 19-kilodalton merozoite surface protein 1 fused with the murine C4bp

domain protects mice against malaria. Infection and Immunity. 2008;**76**:3817-3823. DOI: 10.1128/IAI.01369-07

[51] Talaat KR, Ellis RD, Hurd J, Hentrich A, Gabriel E, Hynes NA, Rausch KM, Zhu D, Muratova O, Herrera R, Anderson C, Jones D, Aebig J, Brockley S, MacDonald NJ, Wang X, Fay MP, Healy SA, Durbin AP, Narum DL, Wu Y, Duffy PE. Safety and immunogenicity of Pfs25-EPA/Alhydrogel®, a transmission blocking vaccine against *Plasmodium falciparum*: An open label study in malaria naïve adults. Borrow R, ed. PLoS One. 2016; **11**:e0163144. DOI: 10.1371/journal.pone.0163144

[52] Coulibaly MB, Gabriel EE, Sinaba Y, Sylla D, Sacko A, Sylla L, Coulibaly B, Hume JCC, Baber I, Assadou MH, Sagara I, Wu Y, Healy SA, Doumbo O, Traore SF, Duffy P. Optimizing direct membrane and direct skin feeding assays for *Plasmodium falciparum* transmission-blocking vaccine trials in Bancoumana, Mali. The American Journal of Tropical Medicine and Hygiene. 2017;**97**:719-725. DOI: 10.4269/ajtmh.16-1000

[53] Diallo DA, Doumbo OK, Plowe CV, Wellems TE, Emanuel EJ, Hurst SA. Community permission for medical research in developing countries. Clinical Infectious Diseases. 2005;**41**:255-259. DOI: 10.1086/430707

[54] Theisen M, Jore MM, Sauerwein R. Towards clinical development of a *Pf* s48/45-based transmission blocking malaria vaccine. Expert Review of Vaccines. 2017;**16**:329-336. DOI: 10.1080/14760584.2017.1276833

[55] Miura K, Takashima E, Deng B, Tullo G, Diouf A, Moretz SE, Nikolaeva D, Diakite M, Fairhurst RM, Fay MP, Long CA, Tsuboi T. Functional comparison of *Plasmodium falciparum* transmission-blocking vaccine candidates by the standard membrane-feeding assay. Adams JH, ed. Infection and Immunity. 2013;**81**:4377-4382. DOI: 10.1128/IAI.01056-13

[56] Malaria Vaccine Funders Group. Malaria Vaccine Technology Roadmap 2013. Available from: http://www.who.int/immunization/sage/meetings/2013/april/6_Draft_roadmap_update_v_5_March.pdf [Accessed: 2018-03-28]

[57] Farrance CE, Chichester JA, Musiychuk K, Shamloul M, Rhee A, Manceva SD, Jones RM, Mamedov T, Sharma S, Mett V, Streatfield SJ, Roeffen W, van de Vegte-Bolmer M, Sauerwein RW, Wu Y, Muratova O, Miller L, Duffy P, Sinden R, Yusibov V. Antibodies to plant-produced *Plasmodium falciparum* sexual stage protein Pfs25 exhibit transmission blocking activity. Human Vaccines. 2011;**7**:191-198. DOI: 10.4161/hv.7.0.14588

[58] Merino KM, Bansal GP, Kumar N. Reduced immunogenicity of *Plasmodium falciparum* gamete surface antigen (Pfs48/45) in mice after disruption of disulphide bonds-evaluating effect of interferon-γ-inducible lysosomal thiol reductase. Immunology. 2016;**148**:433-447. DOI: 10.1111/imm.12621

[59] Pritsch M, Ben-Khaled N, Chaloupka M, Kobold S, Berens-Riha N, Peter A, Liegl G, Schubert S, Hoelscher M, Löscher T, Wieser A. Comparison of intranasal outer membrane vesicles with cholera toxin and injected MF59C.1 as adjuvants for malaria transmission

blocking antigens AnAPN1 and Pfs48/45. Journal of Immunology Research. 2016;**2016**: 3576028. DOI: 10.1155/2016/3576028

[60] Tachibana M, Suwanabun N, Kaneko O, Iriko H, Otsuki H, Sattabongkot J, Kaneko A, Herrera S, Torii M, Tsuboi T. *Plasmodium vivax* gametocyte proteins, Pvs48/45 and Pvs47, induce transmission-reducing antibodies by DNA immunization. Vaccine. 2015;**33**:1901-1908. DOI: 10.1016/j.vaccine.2015.03.008

[61] Duffy PE, Kaslow DC. A novel malaria protein, Pfs28, and Pfs25 are genetically linked and synergistic as *falciparum* malaria transmission-blocking vaccines. Infection and Immunity. 1997;**65**:1109-1113

[62] Qian F, Aebig JA, Reiter K, Barnafo E, Zhang Y, Shimp RL Jr, Rausch KM, Jones DS, Zhu D, Lambert L, Mullen GED, Narum DL, Miller LH, Wu Y. Enhanced antibody responses to *Plasmodium falciparum* Pfs28 induced in mice by conjugation to ExoProtein A of *Pseudomonas aeruginosa* with an improved procedure. Microbes and Infection. 2009;**11**:408-412. DOI: 10.1016/j.micinf.2008.12.009

[63] Kapulu MC, Biswas S, Blagborough A, Gilbert SC, Sinden RE, Hill AV. Viral vectored transmission blocking vaccines against *Plasmodium falciparum*. Malaria Journal. 2010;**9**: O22. DOI: 10.1186/1475-2875-9-S2-O22

[64] Atkinson SC, Armistead JS, Mathias DK, Sandeu MM, Tao D, Borhani-Dizaji N, Tarimo BB, Morlais I, Dinglasan RR, Borg NA. The *Anopheles*-midgut APN1 structure reveals a new malaria transmission–blocking vaccine epitope. Nature Structural & Molecular Biology. 2015;**22**:532-539. DOI: 10.1038/nsmb.3048

Chapter 16

Newer Approaches for Malaria Vector Control and Challenges of Outdoor Transmission

John C. Beier, André B.B. Wilke and Giovanni Benelli

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75513

Abstract

The effective and reliable management of malaria vectors is still a global challenge. Recently, it has been noted that the first vaccine against *Plasmodium falciparum* malaria, RTS,S/AS01 showed only transient protection, particularly in infants, and rapid resistance has been developing to artemisinin-based drugs. Therefore, the control of malaria mosquito vectors according to strategies of integrated vector management (IVM) is receiving emphasis. A rather wide number of novel mosquito control tools have been tested, including attractive toxic sugar baits, eave tubes, nano-synthesized pesticides loaded with microbial- and plant-borne compounds, biocontrol agents with little non-target effects, new adult repellents, oviposition deterrents, and even acoustic larvicides. However, their real-world applications remain limited. Most National Malaria Control Programs in Africa still rely on indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) to reduce malaria incidence but generally have insufficient impact on malaria prevalence. Here, we focus on facts, trends, and current challenges in the employment of the above-mentioned vector control tools in the fight against malaria. We emphasize the needs for better vector control tools used in IVM to overcome the challenges posed by outdoor transmission and growing levels of insecticide resistance, which are threatening the efficacy of LLINs and IRS.

Keywords: *Anopheles*, attractive toxic sugar baits, eave tubes, long-lasting insecticidal nets, mosquito insecticide resistance, *Plasmodium falciparum*, *Plasmodium vivax*

1. Introduction

Malaria is a major challenge to public health; it is caused by *Plasmodium* parasites, obligatorily transmitted to humans through the bites of infected female mosquitoes of the genus

Anopheles (Diptera: Culicidae). There are five known species of *Plasmodium* that cause malaria in humans, *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi* [1–5]. Currently, 91 countries are endemic for malaria [6]. However, the African region is the most affected with 90% of the cases and 92% of deaths [7–9]. Added to that, malaria has a major impact on the economic development of these countries accounting for both direct and indirect medical costs, such as long-term disabilities and decrease in tourism [10–13].

In the past decade, two significant developments for malaria prevention and treatment were achieved. The first was the discovery of artemisinin, a very effective drug against *Plasmodium falciparum*; this molecule has been studied by the Chinese scientist Y. Tu [14–16]. The second was the development of the vaccine against *P. falciparum* (RTS,S/AS01), by GlaxoSmithKline Biologicals, the PATH Malaria Vaccine Initiative, supported by the Bill & Melinda Gates Foundation, and carried out at several African research centers [17, 18]. However, the vaccine only protected transiently the subjects against malaria [19].

Importantly, new drugs and vaccines are needed to achieve further substantial decrease in the prevalence and incidence of malaria globally and address the increasingly resistance of *Plasmodium* to the drugs currently available such as chloroquine and artemisinin [20–22]. More importantly, effective and scientific-driven control strategies for reducing *Anopheles* vector densities remain the gold standard to prevent malaria transmission [23–25]. However, controlling mosquito populations is a difficult task and is unlikely to be achieved by employing only one tool, such as the use of insecticides commonly employed in the past [26, 27]. Now it is clear that local malaria elimination across different endemic environments will not be achieved with current vector control tools, but will require using several approaches together in the form of integrated vector management (IVM) [28].

2. New tools to fight malaria vectors in an IVM perspective

To decrease the risk of vector-borne disease transmission and increase the effectiveness and sustainability of IVM in reducing mosquito populations, local features should be considered [29]. Therefore, guidelines were developed by the global vector control response (GVCR) including: (1) strengthening inter- and intra-sectoral action and collaboration; (2) enhance vector control surveillance and evaluation of interventions; (3) scale up and integrate tools and approaches; and (4) engage and mobilize communities. The goals of this initiative included increasing the effectiveness of reducing mosquito vectors for both capacity and capability as well as encouraging applied research and innovation [13].

The use of IVM aiming for optimum mosquito control contrasts with strategies used in the past that heavily relied on insecticide spraying. Current mosquito control strategies make use of every available tool. For that reason, regular assessments of local disease transmission dynamics and scientific-driven decision-making criteria are important for achieving effective vector-borne disease transmission reduction [27].

Several tools have been proposed to control vector mosquitoes, especially for the *Anopheles* genus [23, 30]. However, current malaria management programs widely rely on indoor

residual spraying (IRS), and long-lasting insecticidal nets (LLINs) [5], contrasting with contemporary IVM guidelines. Moreover, residual transmission of malaria has been commonly found using both IRS and LLINs mosquito control strategies [31]. The presence of the insecticide can be translated as a powerful selective pressure, selecting mosquitoes that are able to avoid contact with it. Key shifts in mosquito behavior such as seeking for human hosts outdoors, avoiding contact with LLINs, and finding resting places outside houses decrease the effectiveness of long-lasting insecticidal strategies [32, 33]. The efficacy of LLINs and IRS can be increased if used together with new tools and guidelines available for controlling mosquito populations, as recommended by the Vector Control Advisory Group (VCAG). Some environments are also suited for using *Bacillus thuringiensis* serovar. *israelensis* (Bti) to manage breeding sites [34–38]. Moreover, promising new tools for mosquito control are being developed, the most notable being "eave tubes" and attractive toxic sugar baits (ATSB).

Rural houses in African countries often are constructed with a gap between the walls and the roof to improve ventilation. *Anopheles* mosquitoes usually enter the houses exploiting this architectural structure exposing the residents to infective bites [39]. The "eave tubes" technology comprises the use of plastic tubes with adulticide-coated mesh under the roofline and the installation of a screen to close the remaining gap (**Figure 1**). When mosquitoes try to enter the house through the eaves, they come in contact with the insecticide and die. This technique is based on the attractive power that the human residents represent for the *Anopheles* mosquitoes comprising an "attract and kill" strategy (**Figure 2**) [40, 41]. The ATSB method is also found under the same strategy of "lure and kill"; it exploits the instinct of mosquitoes, both males and females to seek and feed on sugar sources [42, 43]. The ATBS can be deployed in bait stations or sprayed on plants and are co-formulated with low-risk toxic substances, such as boric acid [44–50]. Even though more studies and epidemiological field trials are required, "eaves tubes" and ATSB methods are leading new technologies for vector control that are highly effective, target-specific, and with minimal nontarget effects and contamination of the environment.

Several other modern strategies exploiting different approaches are being developed, including the use of cytoplasmic incompatibility caused by *Wolbachia* endosymbiotic bacteria. This

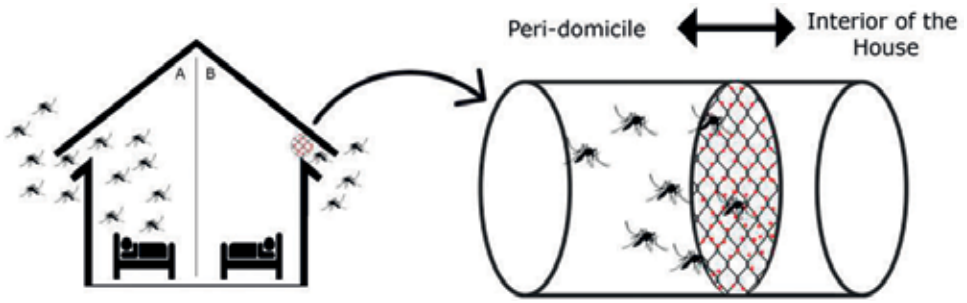


Figure 1. The "eave tubes" technology comprises the use of plastic tubes with adulticide-coated mesh under the roofline and the installation of a screen to close the remaining gap. (A) Graphic representation of a house without "eaves tubes" and (B) with "eaves tubes".

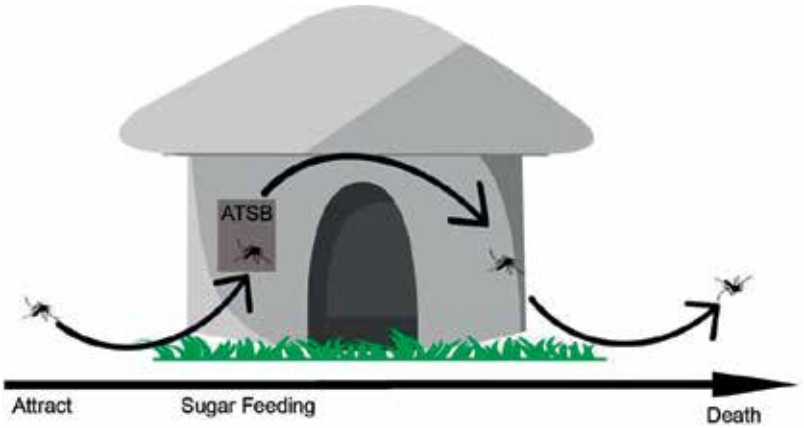


Figure 2. Attractive toxic sugar baits (ATSB) employing an "attract and kill" strategy. This technique consists of using natural attractants such as fruit or flower scent to lure mosquitoes to sugar feeding in a solution containing toxic substances that will lead to its death.

technique has been used to control *Aedes aegypti* and has achieved promising results [51]. Currently, it is undergoing field testing in Brazil and Colombia; however, further studies are needed to transfer this technology to other mosquito species since there are inherent risks for the release of mosquitoes infected with *Wolbachia*, and the result should be monitored for undesirable effects such as increased levels of West Nile virus infection observed in *Culex tarsalis* mosquitoes [52–54]. Other species of bacteria such as *Enterobacter* Esp_Z and *Chromobacterium Csp_P* have been used to inhibit the development of *Plasmodium* in mosquitoes such as *Anopheles stephensi* [55], by increasing the mosquito immune response to *Plasmodium* parasites [31, 56].

The release of irradiated sterile male mosquitoes that will seek and mate with wild females impairing the production of offspring (SIT) is once more being considered as a promising tool for controlling mosquitoes. However, its effectiveness is likely to be decreased by the presence of cryptic species and the presence of multiple *Anopheles* vectors. The same issue should be considered with the use of genetically modified mosquitoes carrying a lethal gene (RIDL), since this technique is species specific and may not be indicated to control outdoor malaria transmission. Genetically modified mosquito techniques based on impairing the *Plasmodium* life cycle inside the mosquito is still in preliminary phases of development and is not likely to be available in the near future [30, 57–61].

The above strategies can be used in the IVM context along with well-established control tools, such as selective microbial and plant-borne pesticides effective against immature mosquitoes, oviposition deterrents, insecticide-coated clothes and other surfaces for personal protection, spatial repellents reducing human-vector contact such as microencapsuled insecticide paint formulation, as well as synthetic and plant-borne repellents [23, 62–69].

The development of plant-based larvicides is of particular interest, and several plant species were successfully used for the synthesis of nano-mosquitocides; nonetheless, plant-based

ovicidal and ovideterrent products are still scarce. This technology can provide rapid synthesis of toxic substances and mosquito repellents useful to manage mosquito populations, with minimal toxicity to humans. Even though mosquito control strategies relying on plant-based larvicides are a fast-growing research area, it is still in the preliminary phase of development and several steps should be taken into account, that is, (1) development, characterization, and optimization of potential botanical components suitable for nano-biosynthesis; (2) identification of potential toxic nanoparticles; (3) feasibility of utilization of plant-based industrial by-products as nano-mosquitocides; (4) field evaluation of the effectiveness of plant-based nanoparticles to control mosquito populations; and (5) effect of plant-based nanoparticles on non-target species and environment [70, 71].

Natural predators also have been used to control immature mosquitoes including cyclopoid copepods, *Toxorhynchites* mosquitoes, water bugs, backswimmers, tadpoles, and fishes [72–74]. The efficacy of mosquito predators may vary accordingly to different environmental settings and their impact on non-target aquatic species and difficulty in using multiple or artificial breeding containers should be considered for their use in control strategies [71, 75]. Another approach for controlling mosquitoes is based on endectocide ivermectin, a molecule that has been used for more than 30 years to control lymphatic filariasis. This molecule remains in the human bloodstream following a standard oral dose and can kill *Anopheles* mosquitoes that feed on the blood of medicated persons [76–79]. Controlling vector mosquito populations is a difficult task and so the addition of new technologies to be considered for IVM will help improve the effectiveness of vector-borne disease transmission [80–83].

Current strategies for malaria vector control used in most African countries still rely on LLINs and IRS, which generally are not sufficient to achieve successful malaria control and local elimination [13, 25, 84]. Even though LLINs and IRS are very effective for in-house reduction of malaria transmission, in endemic areas, it has been showed that insecticide-treated bed nets reduce malaria prevalence only by 13% [85–91]. Furthermore, due to the high abundance of mosquitoes, even low levels of *Plasmodium* transmission undermine efforts to reduce the prevalence of malaria, since human hosts are bitten multiple times increasing the chance of coming in contact with the parasite. The prevalence of *P. falciparum* is strongly related to the number of infective bites per person per year or annual entomological inoculation rates (EIRs), ranging from <1 to >500. Malaria prevalence is positively associated with high EIRs; however, even low annual EIRs (<5) can be associated with malaria prevalence levels of 40–60%. For a significant reduction in the prevalence of malaria, EIRs must be lower than 1 [92]. Vector control strategies implemented in Africa have so far been unable to achieve such low levels of malaria transmission [93].

Besides, with the increase in the control efforts focused into indoor mosquitoes, the dynamics of malaria transmission is shifting from the highly endophilic to more exophilic outdoor-adapted species within the *Anopheles gambiae* complex [94–99]. In Asia, the main malaria vectors of the *Anopheles dirus* complex are exophagic and difficult to target with conventional control strategies [31]. Moreover, increasing resistance to insecticides renders LLINs and IRS less effective for controlling *Anopheles* populations. As well, even though larvicides are effective against immature mosquitoes, they are not recommended for application in rural areas [100–105].

3. Conclusions and issues to watch for

The importance of basic knowledge on mosquito vector behavior and ecology for the development of tailor-made vector control strategies is considered key in the recent WHO Health and Environment Linkages Initiative (HELI), highlighting its importance for sustainable long-term mosquito control actions [106–111].

Recently, an updated research agenda for malaria elimination and eradication (malERA) was published [26, 112–114]. It comprises a multidisciplinary approach to the most important challenges of controlling malaria. Several factors significantly impact the dynamics of malaria transmission. Specifically, shifts in mosquito ecology and behavior caused by anthropogenic alterations in the environment have a major impact on the effectiveness of control strategies. These alterations include, but not limited to, urbanization, human movement, availability of breeding containers and water bodies, hosts for blood feeding and availability of sugar sources and resting places. Moreover, mosquito insecticide resistance, behavioral avoidance, high vector biodiversity, competitive and food web interactions, mosquito population dynamics and dispersion also play a major role in the complex scenario comprising the dynamics of malaria transmission [17, 115, 116].

The development of reliable and effective mosquito control strategies is no easy task, and several challenges must be overcome to achieve a long-term sustainable reduction of mosquito populations. Most of the new strategies and tools developed for controlling vector mosquito populations are not rigorously tested, and most of the time, their real epidemiological impact is not properly assessed rendering the deployment of ineffective mosquito control strategies with limited result on the prevalence of vector-borne diseases [117]. These challenges can be classified as systemic, structural, informational, environmental, human movement, political and financial ones [13]. Key core issues have to be addressed in order to decrease the prevalence of malaria, such as (1) vector surveillance is often neglected or insufficient in most countries at risk of mosquito-borne diseases, rendering control efforts ineffective; (2) malaria endemic countries are often endemic for more than one major mosquito-borne disease depleting the availability of resources; (3) there is a lack of scientific evidence to guide the efforts for mosquito control; (4) anthropogenic alterations in the environment and global warming are responsible for driving the abundance of vector mosquitoes, directly affecting the effectiveness of control strategies; (5) the increase in the human population and movement of people is associated with the dispersion of vector mosquitoes, exposing non-immune populations to new diseases; and (6) funds for vector surveillance are negligible and even though financial support has been made available for LLINs and IRS for controlling *Anopheles* mosquitoes, other vector-borne diseases are largely neglected [13, 17, 118, 119].

Priorities in vector control should be defined by the national vector-borne disease control program and studies designed and performed in consultation with national and international experts in the relevant field. The plan should consider a list of strategic key areas necessary to implement vector control in a given country, followed by research guidance from academic institutes and companies [27]. The most important topics to be considered that are also in agreement with the WHO criteria, comprise: (1) assessment of the health system limitations to improve processes and methods aiming for the improvement in efficacy of vector control; (2) implementation

http://dx.doi.org/10.5772/intechopen.75513

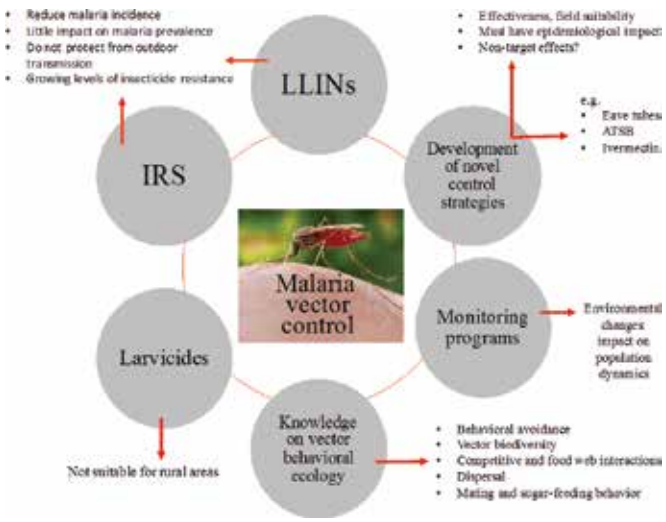


Figure 3. Main challenges and trends in current malaria vector control research (adapted and modified from [120]).

of mosquito surveillance for the development of guidelines and models of the risk of disease transmission (**Figure 3**); (3) development of effective and environmentally friendly strategies to reduce malaria and other vector-borne disease transmission, following the recommendations by VCAG and considering the increase of insecticide resistance [100, 102]. To our understanding, traditional insecticide-based control efforts, such as IRS and LLIN, should be used in combination with novel eco-friendly tools, such as "eave tubes technology," ATSB methods, and even the employment of the ectendocide ivermectin [40, 44, 76]. These new mosquito control tools should be accompanied by (4) an evaluation of their effectiveness, assessment of their usefulness and impact through randomized controlled trials with entomological and epidemiological outcomes (**Figure 3**), this has been done for traditional control strategies such as LLINs and IRS; (5) the monitoring of man-made alteration in the environment and its impact in the dynamics of malaria vectors; and (6) the establishment of a multi-disciplinary team with different areas of expertise (**Figure 3**) [100, 102, 120]. Indeed, the transdisciplinary cooperation among professionals is important for ensuring adequate evaluation of the epidemiological impact triggered by novel mosquito vector control strategies.

Here we illustrate the complex scenario comprising the epidemiology of malaria and how anthropogenic selective pressures are modulating the ecology and behavior of vector mosquitoes. To our understanding, there is no other choice rather to use rigorous, science-driven strategies for controlling vector mosquito populations.

Acknowledgements

This study was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under Award Number R01AI100968. The content is solely the responsibility of the authors and does not necessarily represent the official views of the

National Institutes of Health. This research was also supported by CDC (https://www.cdc.gov/) grant 1U01CK000510-01: Southeastern Regional Center of Excellence in Vector-Borne Diseases: The Gateway Program.

Author details

John C. Beier[1]*, André B.B. Wilke[1] and Giovanni Benelli[2,3]

*Address all correspondence to: jbeier@med.miami.edu

1 Department of Public Health Sciences, University of Miami Miller School of Medicine, Miami, FL, United States

2 Department of Agriculture, Food and Environment, University of Pisa, Pisa, Italy

3 The BioRobotics Institute, Sant'Anna School of Advanced Studies, Pisa, Italy

References

[1] Aly ASI, Vaughan AM, Kappe SHI. Malaria parasite development in the mosquito and infection of the mammalian host. Annual Review of Microbiology. 2009;**63**:195-221

[2] Beier JC. Malaria parasite development in mosquitoes. Annual Review of Entomology. 1998;**43**:519-543

[3] Capanna E. Grassi versus Ross: Who solved the riddle of malaria? International Microbiology. 2006;**9**:69-74

[4] Hempelmann E, Krafts K. Bad air, amulets and mosquitoes: 2,000 years of changing perspectives on malaria. Malaria Journal. 2013;**12**:232

[5] WHO. Malaria Fact Sheet. 2017. (Updated April 2017)

[6] WHO. World Malaria Report 2017. Available at: http://www.who.int/malaria/publications/world-malaria-report-2015/report/en/Malaria report 2017

[7] White NJ. Declining malaria transmission and pregnancy outcomes in southern Mozambique. The New England Journal of Medicine. 2015;**373**:1670-1671

[8] Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, et al. The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. Nature. 2015; **526**:207-211

[9] White MT, Conteh L, Cibulskis R, Ghani AC. Costs and cost-effectiveness of malaria control interventions - A systematic review. Malaria Journal. 2011;**10**:337

[10] Sachs J, Malaney P. The economic and social burden of malaria. Nature. 2002;**415**:680-685

[11] Gallup JL, Sachs JD. The economic burden of malaria. The American Journal of Tropical Medicine and Hygiene. 2001;**64**:85-96

[12] Malaney P, Spielman A, Sachs J. The malaria gap. The American Journal of Tropical Medicine and Hygiene. 2004;**71**:141-146

[13] WHO. Global Vector Control Response 2017-2030; 2017

[14] Tu Y. The discovery of artemisinin (qinghaosu) and gifts from Chinese medicine. Nature Medicine. 2011;**17**:1217-1220

[15] Callaway E, Cyranoski D. Anti-parasite drugs sweep Nobel prize in medicine 2015. Nature. 2015;**526**:174-175

[16] Su X-Z, Miller LH. The discovery of artemisinin and the Nobel prize in physiology or medicine. Science China. Life Sciences. 2015;**58**:1175-1179

[17] Benelli G, Mehlhorn H. Declining malaria, rising of dengue and Zika virus: Insights for mosquito vector control. Parasitology Research. 2016;**115**:1747-1754

[18] WHO. Background Brief: Malaria Vaccine RTS,S/AS01; 2015. (Updated 23 October 2015)

[19] Gosling R, von Seidlein L. The future of the RTS,S/AS01 malaria vaccine: An alternative development plan. PLoS Medicine. 2016;**13**:1-6

[20] Jensen M, Mehlhorn H. Seventy-five years of Resochin® in the fight against malaria. Parasitology Research. 2009;**105**:609-627

[21] Burrows JN, Duparc S, Gutteridge WE, van Huijsduijnen RH, Kaszubska W, Macintyre F, et al. New developments in anti-malarial target candidate and product profiles. Malaria Journal. BioMed Central. 2017;**16**:151

[22] WHO. Updates on Artemisinin Resistance; 2016. (Updated 6 October 2016)

[23] Benelli G. Research in mosquito control: Current challenges for a brighter future. Parasitology Research. 2015;**114**:2801-2805

[24] Chanda E, Ameneshewa B, Bagayoko M, Govere JM, Macdonald MB. Harnessing integrated vector management for enhanced disease prevention. Trends in Parasitology. 2017;**33**:30-41

[25] Hemingway J, Shretta R, Wells TNC, Bell D, Djimdé AA, Achee N, et al. Tools and strategies for malaria control and elimination: What do we need to achieve a grand convergence in malaria? PLoS Biology. 2016;**14**:1-14

[26] Nájera JA, González-Silva M, Alonso PL. Some lessons for the future from the global malaria eradication programme (1955-1969). PLoS Medicine. 2011;**8**:e1000412

[27] WHO. Health and Environment Linkages Initiative − HELI priority environment and health risks; 2017. (www.who.int/heli/risks/vectors/malariacontrol/en/index3. html)

[28] Lizzi KM, Qualls WA, Brown SC, Beier JC. Expanding integrated vector management to promote healthy environments. Trends in Parasitology. 2014;**30**:394-400

[29] WHO. Handbook for integrated vector management. Outlooks on Pest Management. 2013;**24**:142-143

[30] Bourtzis K, Lees RS, Hendrichs J, Vreysen MJB. More than one rabbit out of the hat: Radiation, transgenic and symbiont-based approaches for sustainable management of mosquito and tsetse fly populations. Acta Tropica. 2016;**157**:115-130

[31] Durnez L, Coosemans M. Residual transmission of malaria: An old issue for new approaches. In: Manguin S, editor. *Anopheles* Mosquitoes–New Insights into Malaria Vectors. Rijeka: InTech Open Access; 2013. DOI: 10.5772/55925

[32] Killeen GF, Chitnis N. Potential causes and consequences of behavioural resilience and resistance in malaria vector populations: A mathematical modelling analysis. Malaria Journal. 2014;**13**:97

[33] Killeen GF, Govella NJ, Lwetoijera DW, Okumu FO. Most outdoor malaria transmission by behaviourally-resistant *Anopheles arabiensis* is mediated by mosquitoes that have previously been inside houses. Malaria Journal. BioMed Central. 2016;**15**:225

[34] Bravo A, Gill SS, Soberón M. Mode of action of *Bacillus thuringiensis* cry and Cyt toxins and their potential for insect control. Toxicon. 2007;**49**:423-435

[35] Boyce R, Lenhart A, Kroeger A, Velayudhan R, Roberts B, Horstick O. *Bacillus thuringiensis israelensis* (*Bti*) for the control of dengue vectors: Systematic literature review. Tropical Medicine & International Health. 2013;**18**:564-577

[36] Pavela R. Essential oils for the development of eco-friendly mosquito larvicides: A review. Industrial Crops and Products. 2015;**76**:174-187

[37] Lacey LA. *Bacillus thuringiensis* serovariety *israelensis* and *Bacillus sphaericus* for mosquito control. Journal of the American Mosquito Control Association. 2007;**23**:133-163

[38] WHO. Vector Control Advisory Group (VCAG) on New Paradigms; 2017. Available at: http://www.who.int/neglected_diseases/vector_ecology/VCAG/en/

[39] Knols BGJ, Farenhorst M, Andriessen R, Snetselaar J, Suer RA, Osinga AJ, et al. Eave tubes for malaria control in Africa: An introduction. Malaria Journal. 2016;**15**:404

[40] Sternberg ED, Ng'habi KR, Lyimo IN, Kessy ST, Farenhorst M, Thomas MB, et al. Eave tubes for malaria control in Africa: Initial development and semi-field evaluations in Tanzania. Malaria Journal. 2016;**15**:447

[41] Waite JL, Lynch PA, Thomas MB. Eave tubes for malaria control in Africa: A modelling assessment of potential impact on transmission. Malaria Journal. 2016;**15**:449

[42] Beier JC, Muller GC, Gu W, Arheart KL, Schlein Y. Attractive toxic sugar bait (ATSB) methods decimate populations of *Anopheles* malaria vectors in arid environments regardless of the local availability of favoured sugar-source blossoms. Malaria Journal. 2012;**11**:31

[43] Allan SA. Susceptibility of adult mosquitoes to insecticides in aqueous sucrose baits. Journal of Vector Ecology. 2011;**36**:59-67

[44] Müller GC, Beier JC, Traore SF, Toure B, Traore MM, Bah S, et al. Successful field trial of attractive toxic sugar bait (ATSB) plant-spraying methods against malaria vectors in the *Anopheles gambiae* complex in Mali, West Africa. Malaria Journal. 2010;**9**:210

[45] Naranjo DP, Qualls WA, Müller GC, Samson DM, Roque D, Alimi T, et al. Evaluation of boric acid sugar baits against *Aedes albopictus* (Diptera: Culicidae) in tropical environments. Parasitology Research. 2013;**112**:1583-1587

[46] Stewart ZP, Oxborough RM, Tungu PK, Kirby MJ, Rowland MW, Irish SR. Indoor application of attractive toxic sugar bait (ATSB) in combination with mosquito nets for control of pyrethroid-resistant mosquitoes. PLoS One. 2013;**8**:6-12

[47] Xue R-D, Mueller GC, Kline DL, Barnard DR, Müller GC, Kline DL, et al. Effect of application rate and persistence of boric acid sugar baits applied to plants for control of *Aedes albopictus*. Journal of the American Mosquito Control Association. 2011;**27**:56-60

[48] Xue R-D, Ali A, Kline DL, Barnard DR. Field evaluation of boric acid- and fipronil-based bait stations against adult mosquitoes. Journal of the American Mosquito Control Association. 2008;**24**:415-418

[49] Xue R-D, Kline DL, Ali A, Barnard DR. Application of boric acid baits to plant foliage for adult mosquito control. Journal of the American Mosquito Control Association. 2006;**22**:497-500

[50] Müller GC, Kravchenko VD, Schlein Y. Decline of *Anopheles sergentii* and *Aedes caspius* populations following presentation of attractive toxic (spinosad) sugar bait stations in an oasis. Journal of the American Mosquito Control Association. 2008;**24**:147-149

[51] Walker T, Johnson PH, Moreira LA, Iturbe-Ormaetxe I, Frentiu FD, McMeniman CJ. et al, The *wMel Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. Nature. 2011;**476**:450-453

[52] Aliota MT, Walker EC, Uribe Yepes A, Dario Velez I, Christensen BM, Osorio JE. The *wMel* strain of *Wolbachia* reduces transmission of chikungunya virus in *Aedes aegypti*. PLoS Neglected Tropical Diseases. 2016;**10**:e0004677

[53] Callaway E. Rio fights Zika with biggest release yet of bacteria-infected mosquitoes. Nature. 2016;**539**:17-18

[54] Dodson BL, Hughes GL, Paul O, Matacchiero AC, Kramer LD, Rasgon JL. *Wolbachia* enhances West Nile virus (WNV) infection in the mosquito *Culex tarsalis*. PLoS Neglected Tropical Diseases. 2014;**8**:e2965

[55] Eappen AG, Smith RC, Jacobs-Lorena M. Enterobacter-activated mosquito immune responses to *Plasmodium* involve activation of SRPN6 in *Anopheles stephensi*. PLoS One. 2013;**8**:e62937

[56] Ramirez JL, Short SM, Bahia AC, Saraiva RG, Dong Y, Kang S, et al. *Chromobacterium* Csp_P reduces malaria and dengue infection in vector mosquitoes and has entomopathogenic and in vitro anti-pathogen activities. PLoS Pathogens. 2014;**10**:e1004398

[57] Ito J, Ghosh A, Moreira L, Wimmer E, Jacobs-Lorena M. Transgenic anopheline mosquitoes impaired in transmission of a malaria parasite. Nature. 2002;**417**:452-455

[58] Wiwatanaratanabutr I, Allan S, Linthicum K, Kittayapong P. Strain-specific differences in mating, oviposition, and host-seeking behavior between *Wolbachia*-infected and uninfected *Aedes albopictus*. Journal of the American Mosquito Control Association. 2010;**26**:265-273

[59] Oliva CF, Jacquet M, Gilles J, Lemperiere G, Maquart PO, Quilici S, et al. The sterile insect technique for controlling populations of *Aedes albopictus* (Diptera: Culicidae) on Reunion Island: Mating vigour of sterilized males. PLoS One. 2012;**7**:1-8

[60] Oliva CF, Damiens D, Vreysen MJB, Lemperière G, Gilles J. Reproductive strategies of *Aedes albopictus* (Diptera: Culicidae) and implications for the sterile insect technique. PLoS One. 2013;**8**:e78884

[61] Bouyer J, Lefrançois T. Boosting the sterile insect technique to control mosquitoes. Trends in Parasitology. 2014;**30**:271-273

[62] Pavela R, Benelli G. Essential oils as ecofriendly biopesticides? Challenges and constraints. Trends in Plant Science. 2016;**21**:1000-1007

[63] Pavela R. Encapsulation–A convenient way to extend the persistence of the effect of ecofriendly mosquito larvicides. Current Organic Chemistry. 2016;**20**:2674-2680

[64] Xue R-D, Barnard DR, Ali A. Laboratory and field evaluation of insect repellents as oviposition deterrents against the mosquito *Aedes albopictus*. Medical and Veterinary Entomology. 2001;**15**:126-131

[65] Benelli G. Green synthesized nanoparticles in the fight against mosquito-borne diseases and cancer—A brief review. Enzyme and Microbial Technology. 2016;**95**:58-68

[66] Rajaganesh R, Murugan K, Panneerselvam C, Jayashanthini S, Aziz AT, Roni M, et al. Fern-synthesized silver nanocrystals: Towards a new class of mosquito oviposition deterrents? Research in Veterinary Science. 2016;**109**:40-51

[67] Banks SD, Murray N, Wilder-Smith A, Logan JG. Insecticide-treated clothes for the control of vector-borne diseases: A review on effectiveness and safety. Medical and Veterinary Entomology. 2014;**28**:14-25

[68] Achee NL, Bangs MJ, Farlow R, Killeen GF, Lindsay S, Logan JG, et al. Spatial repellents: From discovery and development to evidence-based validation. Malaria Journal. 2012;**11**:164

[69] Mosqueira B, Soma DD, Namountougou M, Poda S, Diabaté A, Ali O, et al. Pilot study on the combination of an organophosphate-based insecticide paint and pyrethroid-treated

long lasting nets against pyrethroid resistant malaria vectors in Burkina Faso. Acta Tropica. 2015;**148**:162-169

[70] Benelli G. Plant-mediated biosynthesis of nanoparticles as an emerging tool against mosquitoes of medical and veterinary importance: A review. Parasitology Research. 2016; **115**:23-34

[71] Benelli G, Jeffries C, Walker T. Biological control of mosquito vectors: Past, present, and future. Insects. 2016;**7**:52

[72] Marten GG, Bordes ES, Nguyen M. Use of Cyclopoid Copepods for Mosquito Control. Ecology and Morphology of Copepods. Dordrecht: Springer Netherlands; 1994. pp. 491-496

[73] Bowatte G, Perera P, Senevirathne G, Meegaskumbura S, Meegaskumbura M. Tadpoles as dengue mosquito (*Aedes aegypti*) egg predators. Biological Control. 2013;**67**:469-474

[74] Murugan K, Benelli G, Panneerselvam C, Subramaniam J, Jeyalalitha T, Dinesh D, et al. *Cymbopogon citratus*-synthesized gold nanoparticles boost the predation efficiency of copepod *Mesocyclops aspericornis* against malaria and dengue mosquitoes. Experimental Parasitology. 2015;**153**:129-138

[75] Walshe DP, Garner P, Abdel-Hameed Adeel AA, Pyke GH, Burkot T. Larvivorous fish for preventing malaria transmission. In: Burkot T, editor. Cochrane Database of Systematic Reviews. Chichester, UK: John Wiley & Sons, Ltd; 2013

[76] Ōmura S, Crump A. Ivermectin and malaria control. Malaria Journal. 2017;**16**:172

[77] Chaccour C, Rabinovich NR. Ivermectin to reduce malaria transmission II. Considerations regarding clinical development pathway. Malaria Journal. 2017;**16**:166

[78] Chaccour C, Rabinovich NR. Ivermectin to reduce malaria transmission III. Considerations regarding regulatory and policy pathways. Malaria Journal. 2017;**16**:162

[79] Chaccour C, Hammann F, Rabinovich NR. Ivermectin to reduce malaria transmission I. Pharmacokinetic and pharmacodynamic considerations regarding efficacy and safety. Malaria Journal. 2017;**16**:161

[80] Beier JC, Keating J, Githure JI, Macdonald MB, Impoinvil DE, Novak RJ. Integrated vector management for malaria control. Malaria Journal. 2008;**7**:S4

[81] WHO. Global Strategic Framework for Integrated Vector Management; 2004. (In WHO/CDS/CPE/PVC/2004. pp. 12)

[82] WHO. Report of the WHO Consultation on Integrated Vector Management (IVM). World Health Organization, Geneva; 2007

[83] WHO. Handbook on Integrated Vector Management. Geneva: World Health Organization; 2012

[84] WHO. Malaria Vector Control and Personal Protection; 2006. WHO Technical Report Series No 936

[85] Lengeler C. Insecticide-treated bed nets and curtains for preventing malaria. In: Lengeler C, editor. Cochrane Database of Systematic Reviews. Chichester, UK: John Wiley & Sons, Ltd; 2004. p. CD000363

[86] Pluess B, Tanser FC, Lengeler C, Sharp BL. Indoor residual spraying for preventing malaria. In: Lengeler C, editor. Cochrane Database of Systematic Reviews. Chichester, UK: John Wiley & Sons, Ltd; 2010. p. CD006657

[87] Bhattarai A, Ali AS, Kachur SP, Mårtensson A, Abbas AK, Khatib R, et al. Impact of Artemisinin-based combination therapy and insecticide-treated nets on malaria burden in Zanzibar. PLoS Medicine. 2007;**4**:e309

[88] Kleinschmidt I, Torrez M, Schwabe C, Benavente L, Seocharan I, Jituboh D, et al. Factors influencing the effectiveness of malaria control in Bioko Island, Equatorial Guinea. The American Journal of Tropical Medicine and Hygiene. 2007;**76**:1027-1032

[89] Keating J, Locatelli A, Gebremichael A, Ghebremeskel T, Mufunda J, Mihreteab S, et al. Evaluating indoor residual spray for reducing malaria infection prevalence in Eritrea: Results from a community randomized control trial. Acta Tropica. 2011;**119**:107-113

[90] Protopopoff N, Van Bortel W, Marcotty T, Van Herp M, Maes P, Baza D, et al. Spatial targeted vector control is able to reduce malaria prevalence in the highlands of Burundi. The American Journal of Tropical Medicine and Hygiene. 2008;**79**:12-18

[91] WHO. Global Malaria Control and Elimination: Report of a Technical Review. Geneva: World Health Organization; 2008

[92] Killeen GF, Githure JI, Beier JC. Short report: Entomologic inoculation rates and *Plasmodium falciparum* malaria prevalence in Africa. The American Journal of Tropical Medicine and Hygiene. 1999;**61**:109-113

[93] Shaukat AM, Breman JG, McKenzie FE. Using the entomological inoculation rate to assess the impact of vector control on malaria parasite transmission and elimination. Malaria Journal. 2010;**9**:122

[94] Killeen GF. Characterizing, controlling and eliminating residual malaria transmission. Malaria Journal. 2014;**13**:330

[95] Bayoh MN, Mathias DK, Odiere MR, Mutuku FM, Kamau L, Gimnig JE, et al. *Anopheles gambiae*: Historical population decline associated with regional distribution of insecticide-treated bed nets in western Nyanza Province, Kenya. Malaria Journal. 2010;**9**:62

[96] Norris DE, Glass GE, Norris LC, Fornadel CM. Analysis of *Anopheles arabiensis* blood feeding behavior in southern Zambia during the two years after introduction of insecticide-treated bed nets. The American Journal of Tropical Medicine and Hygiene. 2010; **83**:848-853

[97] Russell TL, Govella NJ, Azizi S, Drakeley CJ, Kachur SP, Killeen GF. Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. Malaria Journal. 2011;**10**:80

[98] Derua YA, Alifrangis M, Hosea KM, Meyrowitsch DW, Magesa SM, Pedersen EM, et al. Change in composition of the *Anopheles gambiae* complex and its possible implications for the transmission of malaria and lymphatic filariasis in North-Eastern Tanzania. Malaria Journal. 2012;**11**:188

[99] Mwangangi JM, Mbogo CM, Orindi BO, Muturi EJ, Midega JT, Nzovu J, et al. Shifts in malaria vector species composition and transmission dynamics along the Kenyan coast over the past 20 years. Malaria Journal. 2013;**12**:13

[100] Naqqash MN, Gökçe A, Bakhsh A, Salim M. Insecticide resistance and its molecular basis in urban insect pests. Parasitology Research. 2016;**115**:1363-1373

[101] Tabashnik BE. Evolution of resistance to *Bacillus thuringiensis*. Annual Review of Entomology. 1994;**39**:47-79

[102] Hemingway J, Ranson H. Insecticide resistance in insect vectors of human disease. Annual Review of Entomology. 2000;**45**:371-391

[103] Enayati A, Hemingway J. Malaria management: past, present, and future. Annual Review of Entomology. 2010;**55**:569-591

[104] Ranson H, N'Guessan R, Lines J, Moiroux N, Nkuni Z, Corbel V. Pyrethroid resistance in African anopheline mosquitoes: What are the implications for malaria control? Trends in Parasitology. 2011;**27**:91-98

[105] Ranson H, Abdallah H, Badolo A, Guelbeogo W, Kerah-Hinzoumbé C, Yangalbé-Kalnoné E, et al. Insecticide resistance in *Anopheles gambiae*: Data from the first year of a multi-country study highlight the extent of the problem. Malaria Journal. 2009;**8**:299

[106] Charlwood JD, Pinto J, Sousa CA, Ferreira C, Do RVE. Male size does not affect mating success (of *Anopheles gambiae* in Sao Tome). Medical and Veterinary Entomology. 2002;**16**:109-111

[107] Cabrera M, Jaffe K. An aggregation pheromone modulates lekking behavior in the vector mosquito *Aedes aegypti* (Diptera: Culicidae). Journal of the American Mosquito Control Association. 2007;**23**:1-10

[108] Dabiré KR, Sawadogo PS, Hien DF, Bimbilé-Somda NS, Soma DD, Millogo A, et al. Occurrence of natural *Anopheles arabiensis* swarms in an urban area of Bobo-Dioulasso city, Burkina Faso, West Africa. Acta Tropica. 2014;**132**:S35-S41

[109] Diabaté A, Yaro AS, Dao A, Diallo M, Huestis DL, Lehmann T. Spatial distribution and male mating success of *Anopheles gambiae* swarms. BMC Evolutionary Biology. 2011; **11**:184

[110] Pitts RJ, Mozūraitis R, Gauvin-Bialecki A, Lempérière G. The roles of kairomones, synomones and pheromones in the chemically-mediated behaviour of male mosquitoes. Acta Tropica. 2014;**132**:S26-S34

[111] Oliveira-Ferreira J, Lacerda MV, Brasil P, Ladislau JL, Tauil PL, Daniel-Ribeiro CT. Malaria in Brazil: An overview. Malaria Journal. 2010;**9**:115

[112] malERA Refresh Consultative Panel on Health Systems and Policy Research. malERA: An updated research agenda for combination interventions and modeling in malaria elimination and eradication. PLoS Medicine. 2017;**14**:e1002453

[113] Rabinovich RN, Drakeley C, Djimde AA, Fenton Hall B, Hay SI, Hemingway J, et al. malERA: An updated research agenda for malaria elimination and eradication. PLoS Medicine. 2017;**14**:e1002456

[114] malERA Refresh Consultative Panel on Tools for Malaria Elimination. malERA: An updated research agenda for diagnostics, drugs, vaccines, and vector control in malaria elimination and eradication. PLoS Medicine. 2017;**14**:e1002455

[115] Lemon SM, Sparling F, Hamburg MA, Relman DA, Choffnes ER, Mack A. Vector-borne diseases: Understanding the environmental, human health, and ecological connections, Workshop Summary. 2008. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21452451

[116] Ferguson HM, Dornhaus A, Beeche A, Borgemeister C, Gottlieb M, Mulla MS, et al. Ecology: A prerequisite for malaria elimination and eradication. PLoS Medicine. 2010; **7**:1-7

[117] Wilson AL, Boelaert M, Kleinschmidt I, Pinder M, Scott TW, Tusting LS, et al. Evidence-based vector control? Improving the quality of vector control trials. Trends in Parasitology. 2015;**31**:380-390

[118] Becker N, Pluskota B, Kaiser A, Schaffner F. Exotic Mosquitoes Conquer the World. In: Mehlhorn H, editor. Arthropods as Vectors of Emerging Diseases. Parasitology Research Monographs. Berlin, Heidelberg: Springer; 2012;**3**:31-60. DOI: https://doi.org/10.1007/978-3-642-28842-5_2

[119] Sinka ME, Bangs MJ, Manguin S, Rubio-palis Y, Chareonviriyaphap T, Coetzee M, et al. A global map of dominant malaria vectors. Parasites & Vectors. 2012;**5**:69

[120] Benelli G, Beier JC. Current vector control challenges in the fight against malaria. Acta Tropica. 2017;**174**:91-96

Chapter 17

Entomological Surveillance as a Cornerstone of Malaria Elimination: A Critical Appraisal

Gerry F. Killeen, Prosper P. Chaki, Thomas E. Reed, Catherine L. Moyes and Nicodem J. Govella

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.78007

Abstract

Global capacity for developing new insecticides and vector control products, as well as mathematical models to evaluate their likely impact upon malaria transmission has greatly improved in recent years. Given that a range of new vector control products are now emerging that target a greater diversity of adult mosquito behaviours, it should soon be feasible to effectively tackle a broader range of mosquito species and settings. However, the primary obstacles to further progress towards more effective malaria vector control are now paucities of routine programmatic entomological surveillance, and capacity for data processing, analysis and interpretation in endemic countries. Well-established entomological methods need to be more widely utilized for routine programmatic surveillance of vector behaviours and insecticide susceptibility, the effectiveness of vector control products and processes, and their impacts on mosquito populations. Such programmatic data may also be useful for simulation analyses of mosquito life histories, to identify opportunities for pre-emptively intervening early in the life cycle of mosquitoes, rather than targeting transmission events occurring when they are older. Current obstacles to more effective utilization, archiving and sharing of entomological data largely centre around global inequities of analytical capacity. These prohibitive and unfair imbalances can be addressed by reorienting funding schemes to emphasize south-centred collaborations focused on malaria-endemic countries.

Keywords: malaria, entomological surveillance, mathematical modelling, capacity strengthening, vector control

1. Introduction

Elimination of malaria parasite transmission from most of the tropics will require scalable, affordable new vector control interventions, which improve upon long-lasting

insecticidal nets (LLINs) and indoor residual spraying (IRS) for population suppression of mosquitoes which feed or rest indoors, and also extend control of adult mosquitoes outdoors [1–3]. However, the greatest challenge that lies ahead is defining exactly which of these intervention options is necessary and optimal [4] in each of the diverse vector systems that support malaria transmission across the tropics [5–7]. Product developers and manufacturers need a manageably short list of ecologically-defined target product profiles to work with, based on quantitatively characterized traits of wild vector populations [6, 8]. Assuming an adequate arsenal of diverse and mutually-complementary vector control strategies can be made available [2, 3], malaria control programmes will then need to select the most effective subset of these options that they can afford and realistically implement [9], based on longitudinal, nationally-representative surveys of key behavioural and physiological traits [6–11].

As a result of long-term investments in the industrial development pipeline initiated over a decade ago [12], a diversity of new insecticide formulations for malaria vector control products are coming onto the market and entirely new insecticide classes will soon follow [13, 14]. It is also encouraging that a growing diversity of new or repurposed vector control methods are emerging which either use insecticides more efficiently and effectively, or even do entirely without them [2, 3]. Indeed, a range of new vector control technologies are now emerging for tackling a much wider range of mosquito behaviours and species in more diverse tropical settings [2, 3].

2. Knowledge and methodology limitations to improved vector control products and practices

Several detailed models of malaria transmission have been independently developed over the last decade, and integrated into collaborative ensemble platforms [15–21] that have successfully informed global policy [22]. However, as these models develop and improve, further progress is increasingly limited by lack of knowledge rather than global mathematical capacity:

> *Differences in the predicted impact size arise due to the different assumptions made about malaria transmission in each model, which represent realistic uncertainties in our understanding of this process* [22].

> *….assessment of the consequences of uncertainties in parameter values, are generally much more time-consuming and challenging than the modelling itself* [23].

Unfortunately, knowledge and data are most limiting in relation to the underlying entomological input parameters that these mathematical models are most sensitive to [24]. While the blood-stage dynamics of malaria parasites in humans are now simulated based on hundreds of observed time courses for individual human infections, and calibrated against tens of thousands of malaria prevalence data sets from the field, epidemiologically important variability in survival demographics between different mosquito populations [25] remains to be captured in commonly-used malaria transmission models. Given the

central importance of mosquito survival and gonotrophic cycle duration as targets for many vector control measures [24], it is remarkable that we know little about foraging and mortality processes occurring outside the artificial indoor environment of experimental huts (**Figure 1**). Only a handful of sites exist globally for which estimates of local vector survival, host preference, biting pattern, and adult emergence rates are all available, so that malaria transmission models can be explicitly tailored to the dynamic properties of local vector populations [26]. Indeed, several independently formulated families of models rely heavily on a single village in southern Tanzania for several of their most important vector parameter estimates [4, 15, 27, 28].

Many of the biggest knowledge gaps relating to malaria vector biology arise from our inability to observe, track or label mosquitoes over large, important parts of their life cycles that occur outdoors. Crucially, the outdoor environment represents a refuge for mosquitoes from currently prioritized indoor-targeted interventions like LLINs and IRS. Important limitations to existing entomological methodology includes: (1) representative sampling of outdoor-resting, blood-fed mosquitoes for surveying host choice, especially beyond the peri-domestic environment; (2) observing, tracing or tracking mosquitoes when they are not host-seeking, especially outdoors; (3) quantifying and mapping participation of males and females in mating swarms; (4) quantifying and mapping of oviposition behaviour and; (5) mapping dispersal between emergence, mating, feeding, resting, and oviposition sites.

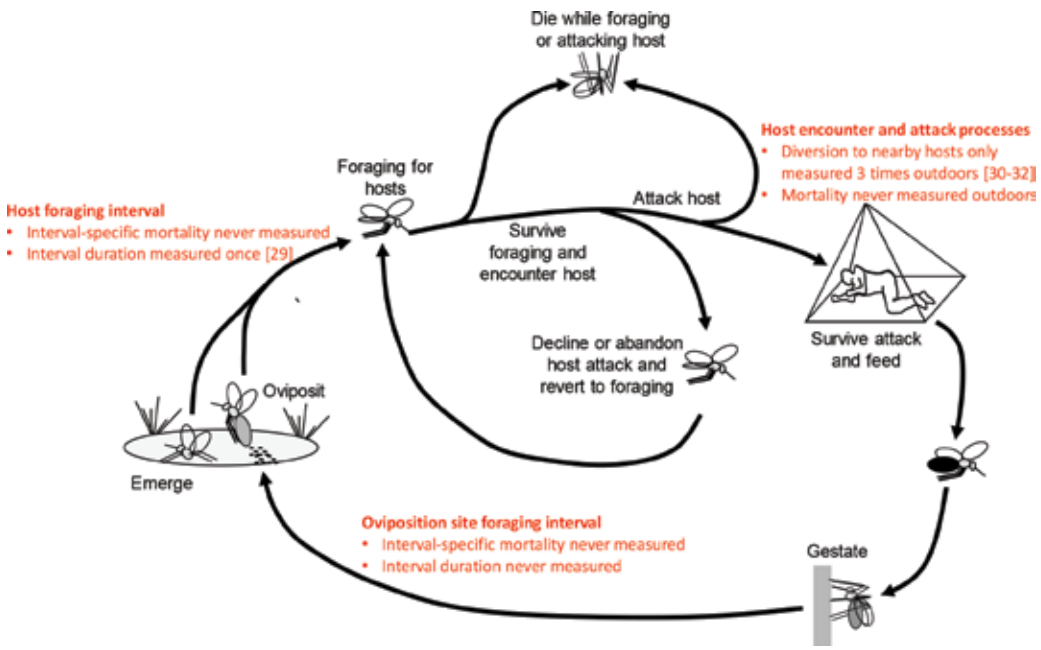


Figure 1. A schematic illustration of major gaps in knowledge about even the most simplistic conceptual model of a mosquito life cycle that are relevant to interventions targeting human-feeding mosquitoes. For simplicity, some common mosquito life history processes excluded, viz., include feeding on animals, feeding upon sugar, swarming and mating. *Source*: Ref. [29–32].

3. Underutilization of existing methodology for informing malaria vector control programmes

While the methodological limitations described above merit investment, a far bigger limitation is underutilization of long-established and widely-accepted methodologies to inform vector control product development, deployment and assessment. It is remarkable just how few study sites are available with consistently-collected, long-term legacy data that capture longitudinal trends for coverage with important interventions along with both entomological (human biting rate, sporozoite infection prevalence, entomologic inoculation rate) and epidemiological (parasitological incidence and prevalence, as well as disease burden) outcomes to enable satisfactory analysis. Dielmo is a rare exception on the vast continent of Africa, where the same entomological methods for monitoring vector population densities and malaria inoculation rates have been continuously applied in a consistent manner for more than two decades [33]. More recently, these vector population dynamics surveys have been supplemented with repeated characterisations of behavioural interactions between humans and mosquitoes [34]. These additional measurements of human exposure distribution across indoor and outdoor environments at different times of the day certainly help explain why robust residual transmission persists, and reveals worrying signs of a worsening situation [34, 35].

While this intensively studied village provides a valuable illustration of how informative such longitudinal surveillance can be, it is not necessarily representative of other parts of Senegal, much less any other country in Africa [35]. National malaria control or elimination programmes all need their own, nationally-representative set of surveillance sites like Dielmo, where malaria transmission is continually and indefinitely monitored using consistent methods. Such platforms are needed to reliably monitor the dynamics of malaria vector populations and transmission intensity across all major ecological and epidemiological strata, so that the limitations and failures of interventions can be identified, distinguished (**Figure 2**), investigated and responded to.

Looking more broadly at programmatically-relevant entomological measurements, insecticide susceptibility testing is now widespread, but measurements of important mosquito and human behaviours are remarkably sparse (**Figure 3**). The species identity of blood hosts that mosquitoes feed upon has long been recognized as a crucial determinant of malaria transmission intensity and an indicator of intervention impact [6, 36–39]. Although adequate field and laboratory methodology for surveying the blood meal choices of most vector species have been available for over 50 years, species-specific reports of this metric remain remarkably scarce for all but a few key vector species (**Figure 3B**). The principles of how to weight indoor and outdoor human landing catch data in proportion to survey results for where people spend each time of the night were first outlined by Garrett-Jones in 1964 [40], yet today less than a dozen such estimates of how human exposure is distributed have been reported, in most cases for undifferentiated mixtures of vector complexes or groups (**Figure 3C**). Only

http://dx.doi.org/10.5772/intechopen.78007

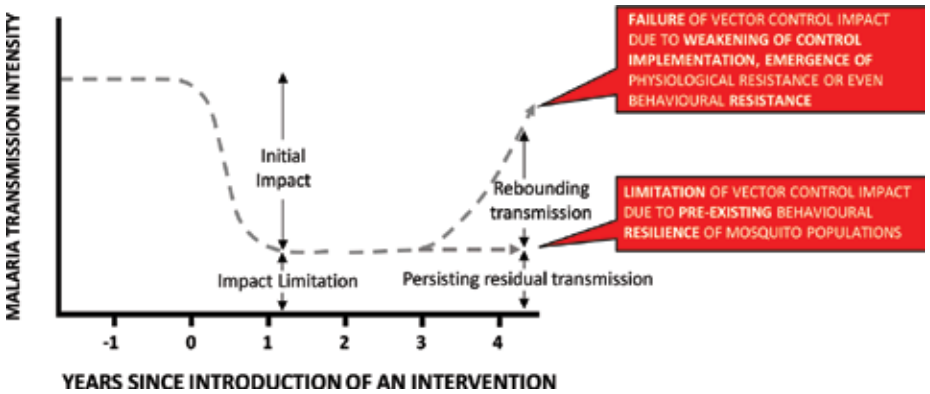


Figure 2. A schematic illustration of how the different trajectories of intervention limitations and failures can be captured and distinguished through longitudinal surveillance of vector population and transmission dynamics (adapted from [6]).

two of the four villages for which a full minimum set of parameters for modelling malaria transmission based on local measurements (**Figure 3D**) relate to a single species, neither of which relied on disaggregation of species-specific data, because only one sibling species from within the relevant complex was abundant.

There are also limitations to the quality of data collection, archiving and analysis that result in most available entomological measurements being reported at the level of species groups or complexes, rather than disaggregated on a species-by-species basis. For example, despite clear evidence for differences among species in both the mechanisms of insecticide resistance and in the prevalence of resistance phenotypes [41–44], the species-specific data presented in **Figure 3A** represents only 27% of the total available [45]. The remaining 73% of archived data represent aggregated mortality rates for mixtures of two or more undifferentiated species [45]. In some cases, species-level classification simply was not conducted. In others, species identification was conducted but the bioassay results were only provided for the pooled species. In other examples, the species data was compromised because only a subset of the mosquitoes assayed, for example only bioassay survivors, were identified to the species level. This lack of species-specific data reduces the power to investigate trends in insecticide resistance and to detect associations between resistance in wild vector populations and malaria transmission experienced by the human population. The data in **Figure 3B** represents only the small fraction (12%) of all available data on human blood indices that unambiguously relates to a single species rather than an undifferentiated mixture of two or more. Only three [46–48] of the small handful of estimates for the proportion of human biting exposure occurring indoors or while asleep (**Figure 3C**) relate to a single, clearly identified and disaggregated species.

Generic (e.g., Microsoft Access®) or freely available (e.g., mySQL®) relational database applications have been adopted as standard tools and used ubiquitously by almost all epidemiologists and field biologists for decades, but medical entomologists generally lag far behind, especially in low income countries. If links between data fields are lost they cannot

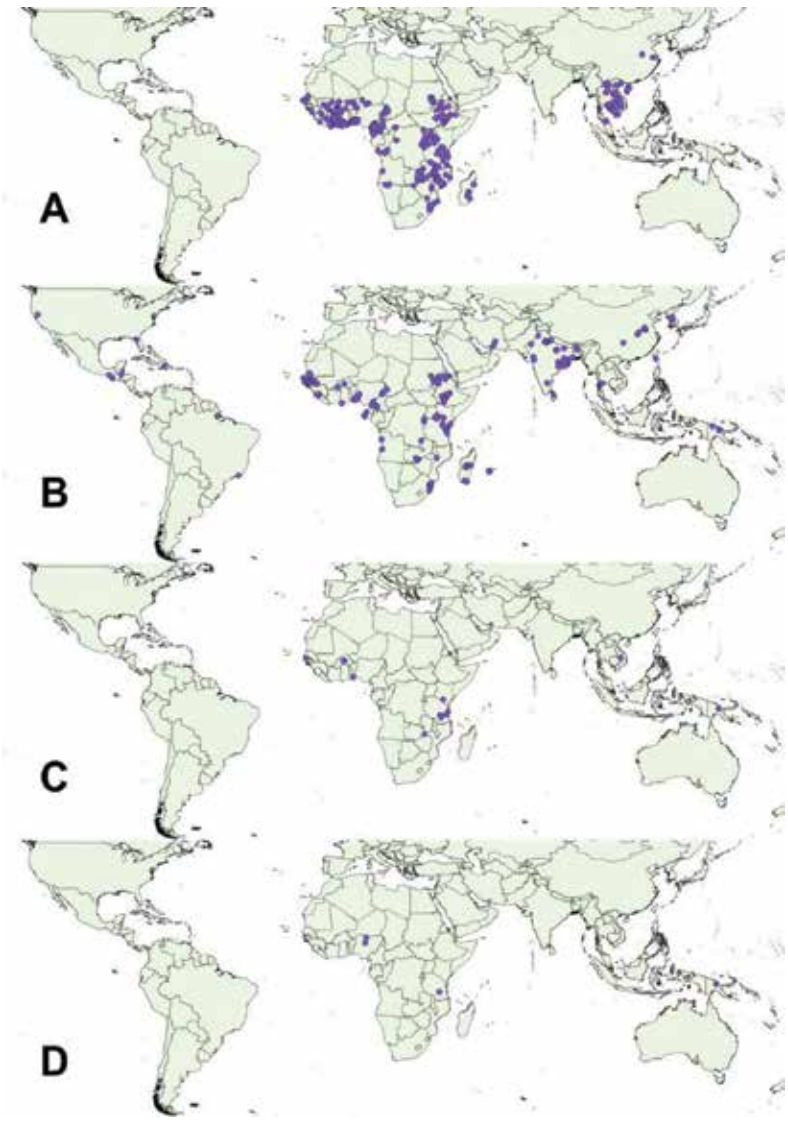


Figure 3. The global distribution of reported measurements for (A) insecticide resistance, (B) the proportions of bloodmeals obtained from humans, (C) the proportion of human exposure to bites occurring indoors, for *Anopheles* vectors of malaria, and (D) the only four locations, we are aware of, where estimates for the mean adult biting density, survival and human blood index for even a single vector, as well as human population size and infectiousness are all available, so that malaria transmission and control can be modelled in a site-specific manner based on local estimates of these parameters. Panels A and B respectively represent the species-specific subset of all insecticide susceptibility bioassay [45] and human blood index [7, 49] data collated by the time they were most recently published as dataset summary reports. The studies represented in Panel C include only three reports of species-specific estimates [46–48] for the proportion of human biting exposure occurring indoors, with the remaining handful all relating to undifferentiated mixtures of species in a complex or group [34, 50, 51]. Panel D represents only four locations in only three countries (Nigeria, Tanzania, Papua New Guinea), all of which were small rural villages with intense transmission and anthropophagic vectors (otherwise at least some of these parameters would probably have been impossible to measure), but nevertheless yielded remarkably different vector-parasite demography patterns and suitability for various complementary vector control measures beyond LLINs and IRS [26, 52].

http://dx.doi.org/10.5772/intechopen.78007

be reinstated and the utility of the unlinked data is severely reduced. The most obvious and common example is a collection of morphologically indistinguishable mosquitoes that are used in an insecticide bioassay, then separated into live and dead mosquitoes, and tested for species and sporozoite infection. If unique identifiers are not assigned to each mosquito and linked to data on bioassay survival, species, sporozoite presence and blood meal for that individual mosquito, then any analysis of the relationships among species, insecticide susceptibility, infection and blood meals is severely hampered. Too often, all that is reported is the sporozoite rate, species composition, etc., for the whole sample, i.e., the aggregate value for all the mosquitoes in the original collection even though they comprise a mixture of two or more different species. This is probably the single most important limiting factor when it comes to species-specific measurements for the variables that matter most, or the interactions between them.

In addition to being so limited in quantity and quality, the utility of existing vector bionomic data is also compromised by the fact that it has mostly been collected haphazardly and opportunistically with project-based research funding. As a result, it has typically been collected on scales varying from villages to districts, over only a few years at a time. Different research studies in any given setting typically have different objectives that often necessitate different sampling and trapping approaches, so collating data sets from multiple projects often yields a patchwork of data with substantial temporal gaps and methodological inconsistencies that confound unambiguous interpretation [6–8, 11].

4. Addressing data deficits through programmatic entomological surveillance platforms

The latest guidelines from WHO for entomological surveillance, monitoring and evaluation offer an excellent new framework for comprehensively applying existing field and analytical methods, and for conducting operational research into improving their use practices in the future [53]. In order for national programmes to make evidence-based decisions about what vector control measures to deploy, and evaluate their ongoing impact, the remarkably diverse arsenal of entomological methods already at our disposal [54] now need to be adapted to programmatic surveillance platforms that are nationally representative of all major ecological and epidemiological strata in the country [6–8, 11]. Such programmatic platforms should emphasize the absolute minimum number of essential entomological metrics, with strong data quality control and assurance processes to maximize confidence and minimize ambiguity of interpretation (**Figure 4**).

Data quantity and quality obviously trade off against each other, especially when working across very large geographic areas, so it is often necessary to select the smallest number of surveillance sites required to adequately represent all major epidemiological and ecological strata in the country. Based on our experience, a minimum of five surveillance sites per stratum is suggested to adequately capture variation within each stratum. The recent WHO guideline of one sentinel site per million people [53] also represents a good benchmark that

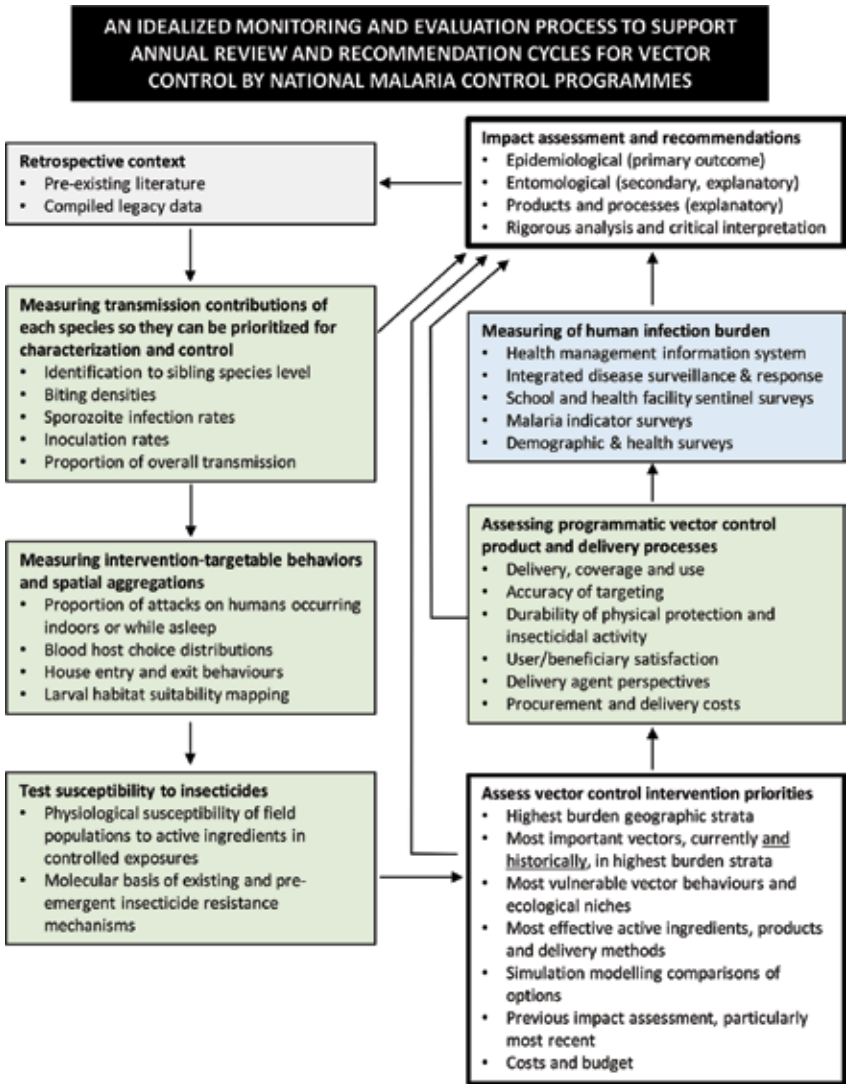


Figure 4. A suggested flow diagram for cyclical collection and assessment of programmatic surveillance data to inform malaria vector control on national scales. Formal annual review cycles may be supplemented by *ad hoc* review meetings at short notice, whenever surprizing or alarming trajectories are observed through ongoing data monitoring.

can be modified according to need by countries that are particularly large, small or diverse. In addition to routine surveys of established surveillance sites, *ad hoc* spot checks and focus investigations are also recommended as ways to further improve vector surveillance and control [53]. Additionally, much more intensive, finer-scale surveillance of vector population dynamics is required wherever pro-active mosquito abatement methods, specifically larvicide application or space spraying, are deployed. These vertically-managed methods for delivering insecticides across large areas need to be repeated on a regular basis, often as frequently as every week. In order for mosquito population density measurements to be useful for monitoring purposes, they need be collected at spatial scales fine enough to identify

operational coverage gaps as soon as they arise. Entomological monitoring to inform daily operations of such area-wide insecticide applications therefore need to be repeated on a correspondingly regular basis, and on geographic scales approximately matched to the scale at which insecticide application succeeds or fails. While active larval searches can be useful for monitoring the effectiveness of insecticide applications, they are prone to large observational biases, especially towards over-representing the most obvious and accessible habitats most likely to be effectively covered. Adult mosquitoes, however, fly and gravitate towards stimuli so they can be passively monitored with traps. Adult mosquito density measurements are therefore a far more reliable way to verify impact and inform operational implementation of larviciding or space-spraying programmes that actively deliver relatively short-lived insecticides across large areas. For such pro-active area-wide interventions, much higher spatial and temporal resolution is required than for more passive human-targeted approaches like LLINs and IRS, which rely on mosquitoes being attracted to protected individuals and households.

National surveillance platforms need to not only monitor the most useful predictors and indicators of successful vector control, but also the products and delivery processes that are essential to achieving impact in practice. Biologically-rational selection of an optimal overall intervention approach, such as LLINs, IRS or larviciding, does not in itself guarantee success. First, it is essential that the most efficacious products within that class are procured, and that they are then effectively delivered. WHO pre-qualification and centralized procurement help target investments towards reliable products. Subsequently, laboratory-based efficacy testing of products sampled from various stages of the supply chain are an invaluable means to quality assure, and even quality control, product supplies. However, beyond successful procurement and supply, it is also essential to ensure that vector control products are satisfactorily delivered and remain effective over the lifetimes required of them. While the coverage of area-wide, frequently re-applied products like larvicides can be difficult to quantify objectively [55, 56], coverage indicators for human-targeted measures like LLINs and IRS can be readily incorporated into questionnaires for routine epidemiological surveys. Reliable, standardized methodology has long been available for measuring the extent and durability insecticidal activity of walls, roofs and ceilings treated with IRS, and have been applied to great effect across multi-country scales, to demonstrate programmatically-relevant variations in product performance [57]. Standardized methods for assessing the physical and insecticidal durability of LLINs reveal similarly important variations in performance, including some notable shortfalls relative to the requirements for recommendation of a '3-year net' [58]. Regardless of how well they are developed, manufactured and tested before they are delivered, products do not always 'do what they say on the tin', so their effectiveness in the field needs to be regularly assessed and re-assessed on nationally representative scales.

5. Directly interpretable entomological metrics of mosquito behaviours, insecticide susceptibility, intervention effectiveness, and intervention impact

Entomological monitoring to inform routine programme implementation needs to yield measurements that can be directly and informatively interpreted, so that those collecting the

data in the field can readily use and quality control it. As discussed in the section that follows, simple summary metrics of mosquito behaviour and insecticide susceptibility do have limitations that need to be addressed with simulation models, but nevertheless need to have decision-making value in their own right.

Despite their limitations, existing simple insecticide bioassays provide an excellent example. Some insecticides can induce delayed but nevertheless invaluable mortality among mosquitoes that are classified as highly resistant based on the 24-hour holding period traditionally used in standard susceptibility assays [59]. Nevertheless, complete and rapid mortality within a day of exposure can only be a good thing and favours the selection of an insecticide verified to do so. Once interventions like LLINs or IRS have been deployed, it is always encouraging if they can be verified to exhibit durable insecticidal efficacy in the field, using well-established cone or wire ball assays with fully-susceptible insectary-reared mosquitoes.

For measuring impact, reduced biting densities and sporozoite prevalence rates can be directly interpreted as indicators of intervention success. Also, vector population rebounds can be identified by directly examining simple graphs of longitudinal trends in density and infection prevalence (**Figure 2**). Any such suspected intervention failure should trigger careful examination of all the above vector behaviour and insecticide susceptibility metrics, as well as indicators of effective vector control products and delivery processes in the field.

On the behavioural front, high estimates for the proportion of human exposure to mosquito bites occurring indoors is always an encouraging indicator that LLINs should at least provide strong personal protection [6]. They may also achieve vector population control if they are also susceptible to the insecticidal active ingredients and obtain a large proportion of blood meals from humans [1, 6–8]. Once high LLIN use has been achieved, high proportions of residual transmission may occur outdoors, and the vector may become more reliant upon livestock as a source of blood, indicating that spatial insecticide emanators or veterinary endectocides may be considered as possible supplementary interventions [7]. While surveys of bloodmeal sources among samples of engorged mosquitoes are always biased to overrepresent the indoor-resting and human-feeding fraction of the vector population [36, 37], very high estimates of the human blood index are nevertheless a strong indicator of both vectorial capacity and vulnerability to attack with human-centred approaches [1, 6–8, 38]. Conversely, where large proportions of blood meals are found to originate from livestock, this is an encouraging indicator that veterinary formulations of endectocides could be useful as a supplementary vector control tool [6–8].

Perhaps the most important reason for entomological surveillance data to be readily and directly interpretable is so that data interrogation begins with the front-line staff who collect it in the field. The closer to the point of collection that data is examined and interpreted, the sooner it is acted upon and the sooner it is queried for completion and correctness. Even within our specialized research groups, we have recently achieved huge improvements in entomological data quality simply by having it entered by the people who collected it on the day it was recorded. Entomological surveillance indicators that can be directly and intuitively interpreted in the field are much easier to quality control and quality assure, especially through decentralized data collection platforms.

Appropriate graphical tools are particularly important for helping programme staff to accurately interpret data. For example, many entomologists directly interpret the results of indoor and outdoor human landing catches without weighting these biting rate measurements in proportion to estimates of where people spend various times of the night (**Figure 5A** and **B**). This common misinterpretation is even endorsed by the latest WHO guidelines [53], which recommend numerical expression in the form of an *endophagy index*, comprising the mean indoor biting rate divided by the sum of the mean indoor and outdoor biting rates. This approach usually grossly overestimates the outdoor fraction of transmission exposure because participants in human landing catches behave in a deliberately misrepresentative manner, spending an average of half their time indoors and half outdoors across all times of the night. In the vast

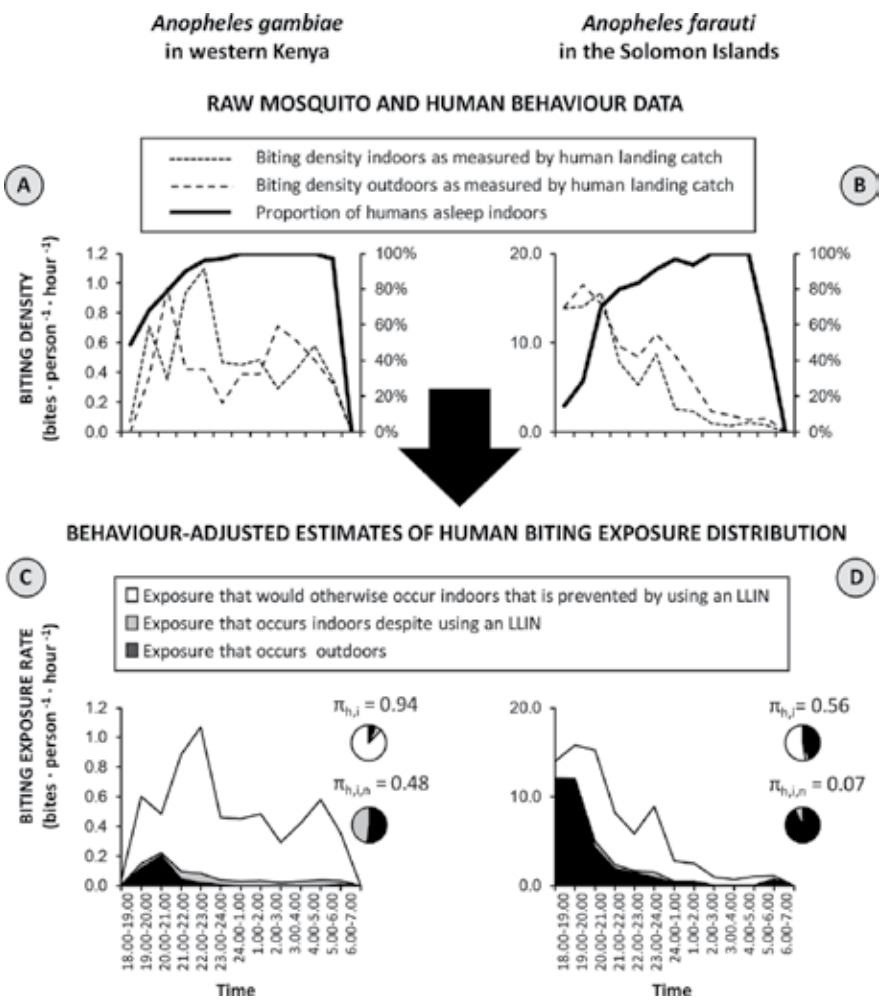


Figure 5. Two examples of how raw mosquito and human behaviour data must be combined with simple analytical models to allow visualization and quantification of where and when human exposure actually occurs as a behavioural interaction [6, 47, 61, 62].

majority of human populations, most people sleep indoors at night for security reasons, so very little of the biting activity measured outdoors is relevant to normal human exposure patterns [60]. Taking the major African malaria vector *An. gambiae* as an example, the traditional narrative describing it as *endophilic* is inaccurate, because their biting rates indoors and outdoors are usually similar and they have no strong or consistent preference for attacking people in either location [50]. It is the timing of biting activity that caused most historical exposure to occur indoors. This human-specialized mosquito species usually exhibits biting activity peaks that occur in the middle of the night when most people are asleep indoors (**Figure 5A**), and therefore vulnerable to attack unless protected with an LLIN. It is therefore more accurate to say that these vectors are highly *nocturnal*, feeding mostly at times when humans exhibit strong *endophilic* tendencies [35], and that is why most human exposure occurred indoors at night before the scale up of LLIN use (**Figure 5C**).

Contrasting with vector populations like those of *Anopheles farauti* in the Solomon Islands, where humans are mostly exposed to outdoors, the most important feature of this behavioural interaction is again the timing of host-seeking activity. By feeding predominantly in the evening, this species can readily attack humans indoors or outdoors while they are still awake and active so bed net use is impractical (**Figure 5B** and **D**). Again, biting densities are similar indoors and outdoors (**Figure 5B**), so it is inaccurate to describe this vector as *exophagic* in the strict sense, and much more important to emphasize that so much exposure occurs outdoors because it is *crepuscular*, with feeding activity that peaks at dusk when people are awake, active and cannot use bed nets.

The overall exposure distribution estimates represented by the areas under the curves in **Figure 5C** and **D**, can then be combined with direct field estimates for the proportion of bloodmeals obtained from humans to visualize the maximum limit of *biological coverage* [63] achievable with human-targeted measures like LLINs as simple box graphs (**Figure 6**).

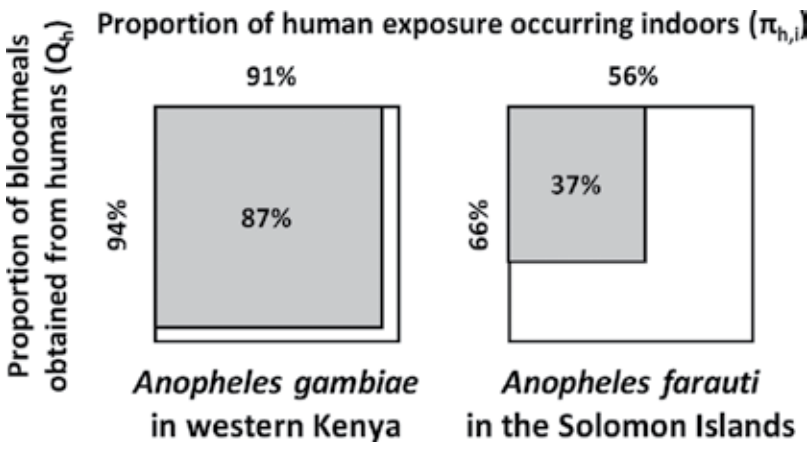


Figure 6. Box diagrams illustrating how the two different vectors described in **Figure 5** differ in terms of their overall behavioural vulnerability to population suppression with long-lasting insecticidal nets, expressed as the maximum achievable *biological coverage* of blood resources used by the mosquitoes [6].

Presenting measured behavioural interactions between mosquitoes and humans in such intuitive graphical formats is important for enabling accurate interpretation, and can be facilitated with user-friendly spreadsheet templates or automated visualization options in surveillance data dashboards.

6. Life history analyses to identify otherwise non-obvious vector control challenges and opportunities

The use of models to look at entire life histories of mosquitoes was central to the very earliest work of Ross [64], and to the ethos of *epidemiological entomology* defined by Garrett-Jones half a century ago [65]. While direct interpretation of simple indicators should provide the essential core of evidence used to inform programmatic decisions, astute application of analytical models to examine the life histories of mosquito populations can also yield important insights that would not otherwise be obvious.

For example, the slow-acting toxicity of pyrethroids to mosquitoes that are clearly resistant to this insecticide class was only recently identified as being central to the sustained impacts of LLINs [59]. While most African *Anopheles* populations are now sufficiently resistant against pyrethroids to survive immediately after exposure, they do suffer increased mortality over the longer term, essentially all of which occurs within the 10 days required for the parasite to complete sporogonic development [59]. As a result, while pyrethroid resistance clearly does compromise the impacts of LLINs [66], it falls far short of abrogating them entirely, so they remain an invaluable tool for malaria vector control [59].

Also, fitting process-explicit models of mosquito population dynamics to vector density trends may yield some insights that cannot be obtained by direct interpretation. Such mechanistic modelling analyses have been successfully applied to field data to identify negative density-dependence of mosquito reproduction, which make vector populations more robust to control than would otherwise be expected [67]. Similar models have been fitted to the population trajectories of self-propagating populations in large cages, which were experimentally exposed to different vector control measures and combinations thereof. These biologically-informative analyses quantified impacts on specific target parameters like survival and fecundity, helped confirm that near-extinction of these small populations was achieved, and revealed a surprising mode of action for one of these emerging technologies (Ng'habi et al., Unpublished). Such approaches could be readily extended to data from routine population dynamics monitoring, allowing the complementarities, synergies and redundancies achieved by combinations of vector control measures to be understood at an unprecedented level of detail.

Relatively simple deterministic models have also been used to illustrate how insecticide resistance traits and intervention avoidance behaviours can interact synergistically, allowing resilient mosquito populations to persist despite widespread LLIN use without necessitating any major adaptations of their preferred feeding times [68]. By foraging cautiously and repeatedly inside houses, to maximize their feeding opportunities while minimizing

their contact with LLINs, even nocturnal species like *An. arabiensis* can continually search around from one house to the next until an unprotected non-user is located [68]. By combining endophagy with exophily in this way, *An. arabiensis* can achieve feeding success rates despite high LLIN coverage that are only a quarter lower than in the absence of nets [68]. Furthermore, the resilience of such nocturnal but behaviourally plastic species may be further enhanced by physiological resistance to insecticides and opportunistically feeding upon animals, resulting in redistribution of feeding activity onto a combination of livestock and humans who either lack nets or are encountered outdoors at times when they are unprotected [68, 69].

However, life history analyses of how resilient mosquito species survive despite high LLIN coverage also identifies some exciting intervention opportunities that would not otherwise be obvious. For example, the most direct corollary of the observation that mosquitoes forage cautiously through several houses to find an unprotected human is that this creates enhanced opportunities to kill them if more effective indoor control methods can be deployed [7, 68, 69]. Emerging options for doing just that range from insecticidal eave tubes [70] and eave baffles [71] to untreated entry traps [72] and three-dimensional window screening [73].

More detailed consideration of life history distributions for the same vector population also reveals an even more counter-intuitive opportunity for such housing modifications to have an impact upon residual transmission. By the time a female *An. arabiensis* is old enough to have incubated malaria parasites through to infectious sporozoites, she will usually have completed at least 4 gonotrophic cycles, during which time she will most probably have been inside a house at least once [69]. So even though approximately half of all transmission events occur outdoors, they are all preceded by at least one house-entry event during which the guilty mosquito may be killed [69]. It is therefore possible to reduce levels of malaria transmission occurring outdoors using interventions that target mosquitoes when they enter or attempt to enter houses [69].

More strategically, this particular simulation analysis [69] also suggests a thematic perspective that may be useful to apply more broadly to life history analyses. It may often be more valuable to look for opportunities to intervene early in the life cycle of mosquitoes rather than targeting transmission events occurring when they are far older. The life histories of adult mosquitoes are cyclical so targeting mosquitoes when they engage in frequently repeated behaviours, in this case house entry, can have far greater impact than would be obvious from face-value interpretation of the fraction of single feeding events that occur indoors.

7. Global inequities of data handling and analytical capacity

So why are most reported entomological data not linked to explicit species identification data, and why are insightful analytical approaches so underutilized by control programmes? The simple answer is that most of the existing global capacity for advanced analysis of malaria-related data is in the wrong places, predominantly located at centres of excellence in high income countries with no local malaria transmission (**Figure 7**).

http://dx.doi.org/10.5772/intechopen.78007

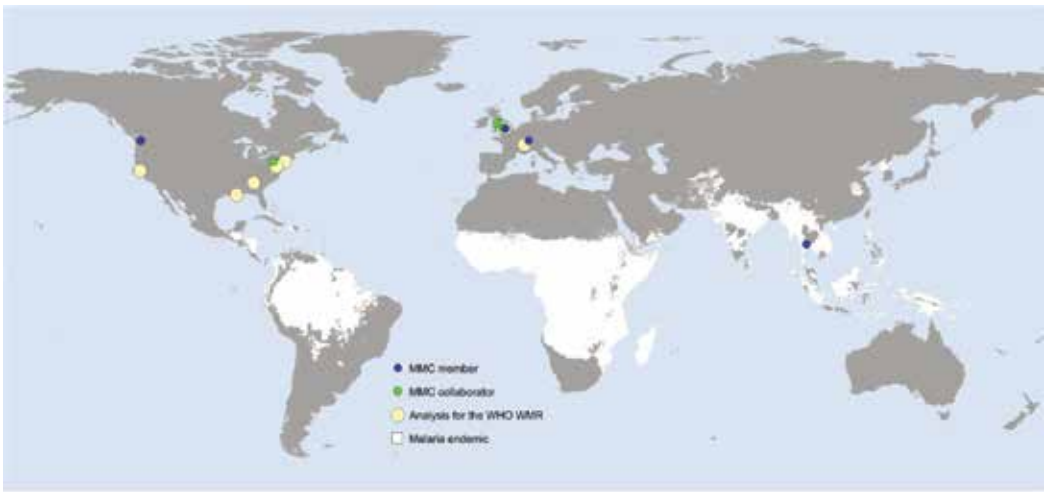


Figure 7. The global geographic distribution of current members and collaborators in the Malaria Modelling Consortium (MMC), as well as analytical contributors to the World Health Organization 2015–2017 World Malaria Report (WHO-WMR), overlaid upon a map with contemporary malaria endemicity (white).

To a large extent, these geographic inequities of data analysis capacity are an understandable consequence of pre-existing global poverty, education and opportunity patterns. However, accepting the *status quo* illustrated in **Figure 7** is not consistent with the 'think global, act local' ethos of successful malaria elimination programmes, and these global capacity inequities need to be addressed urgently if malaria is ever to be eradicated.

If the strategic vision presented by the global modelling community in **Figure 8** continues to be implemented in the context of the world map in **Figure 7**, several consequences are inevitable:

1. Malaria-related data will be collected in low-income countries but largely analyzed in high-income countries with no malaria problem to speak of.
2. Collectors of malaria-related data will have insufficient opportunity and training support to analyze their own data, develop their analytical skills and influence policy and practice. The data interrogation processes essential to timely use and effective quality control of surveillance data will remain underdeveloped where they are needed most.
3. Analysts of malaria data will continue to live far away from the point of data collection and the programme staff who collect it in the field, so their ability to critically analyze and interpret it will remain limited by lack of hands-on field experience and direct access to those who have it.
4. These two communities will remain separated by thousands of kilometers, as well as their very different roles and perspectives (**Figure 9A**). The synergistic interface required between human beings to achieve optimal data collection processes, critical analyses and appropriate programmatic responses (**Figure 9B**) will not be realized.

5. Ongoing geographic separation of data collection and analysis functions will continue to exacerbate recent trends towards overspecialization and excessive compartmentalization of entomologists, epidemiologists and mathematical modellers. Generalist but nevertheless expert *malariologists*, as exemplified by the working competence in entomology, epidemiology and process-explicit modelling of Ross or Garrett-Jones (**Figure 9B**), will remain a rare breed.

A particularly worrisome issue, which we doubt will spontaneously self-resolve, is the inability of programmes in malaria endemic countries to critically appraise the reliability and relevance of advanced modelling studies carried out at a distance. Some of the greatest mistakes in the history of global malaria policy and practice have arisen from over-confident interpretation of models that were very useful but nevertheless imperfect [75]. In the vast majority of endemic countries today, neither the national malaria control programmes nor the national universities and research institutes they should be able look to for locally-available expert

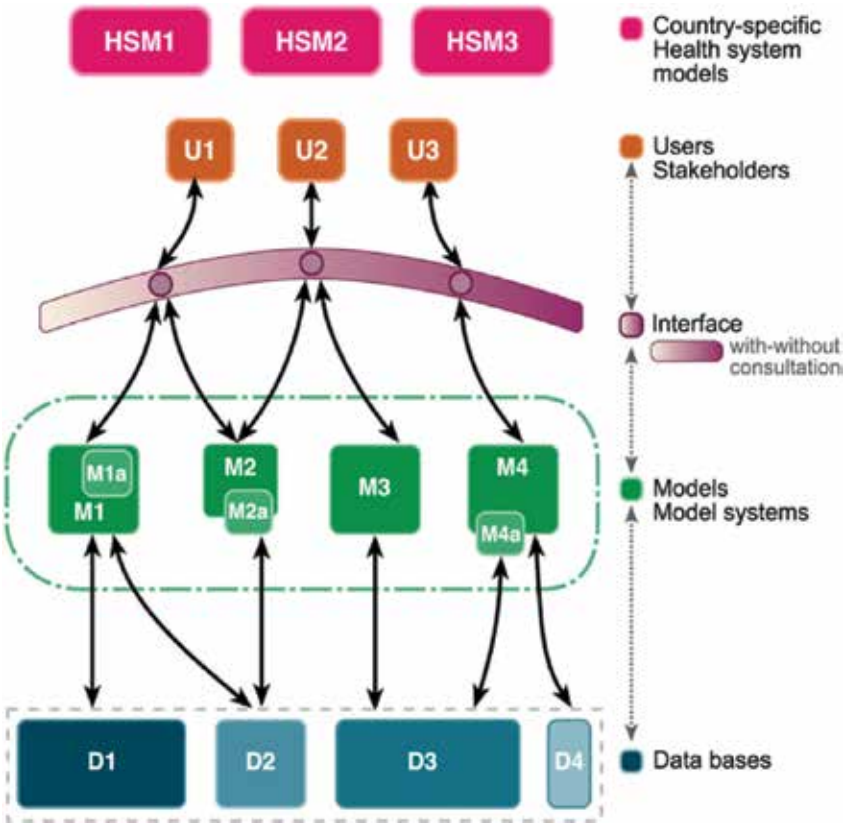


Figure 8. The schematic illustration of the comprehensive framework for malaria modelling presented by the malERA Consultative Group on Malaria Modelling in 2011 [74]. Consultations will allow policy makers, research scientists, and other stakeholders (U, users/stakeholders) from different country-specific health systems (HSM, country-specific health system models) to draw advice and analysis from multiple, independently derived models (M) grounded on data collected (D, data bases) from research on vector ecology, malaria epidemiology, and control through an interface that emphasizes direct engagement between modellers or modelling groups and end users.

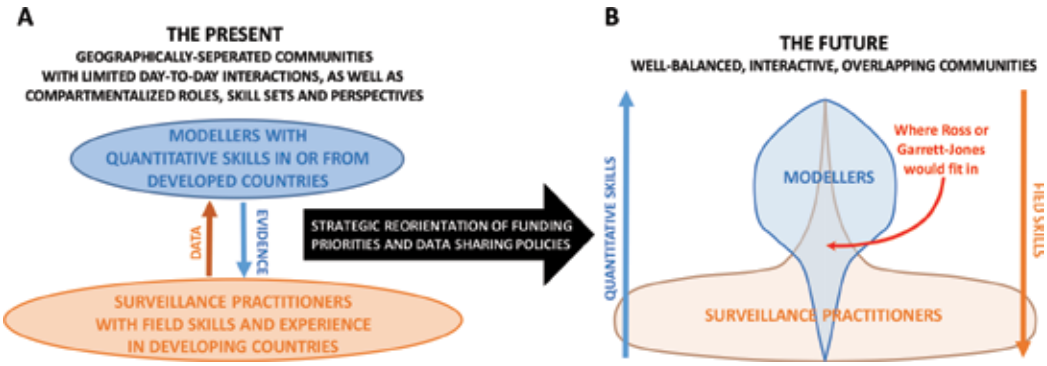


Figure 9. A schematic illustration of how data collection and analysis roles are currently distributed (A), and how they should be actively reformed going forward (B).

advice, currently have sufficient capacity to appraise the merits and limitations of state-of-the-art modelling analyses.

While analytical and predictive models can add considerable value to any data interpretation exercise, they also have some fundamental limitations that need to be considered. Even the most complex mathematical model is a deliberately simplified conceptual representation of reality. It is therefore important to critically understand what the limitations of both the models and the data themselves are, and how those uncertainties limit confidence in their interpretation:

> *… fitting complex models to multiple types of data is challenging, and model predictions are always likely to be unreliable at very high spatial resolution. The twin objectives of understanding the dynamics and making quantitative predictions can also be in conflict, because the push to include all relevant factors in a locally calibrated predictive model rapidly leads to complex behaviour that can no longer be explained* [23].

One of the most important reasons to develop a cadre of expert modellers in endemic countries is so they can advise their national programmes based on a full understanding of the uncertainties and inaccuracies of model-generated evidence. Expert modellers working at locally-owned and governed institutions in malaria-endemic countries have a vital role to play in guiding critical appraisal by their non-specialist colleagues who might otherwise be tempted to either disregard the results of modelling analyses they do not understand, or accept them at face value based on a level of trust that may not be warranted:

> *' … it is challenging for a non-specialist to distinguish modelling that is useful from poor quality modelling that may support misguided policies'* [23].

8. Epidemiological implications of the *Portfolio Effect*: Malaria transmission systematically tends to be more stable than it appears

Mosquito dispersal, human movement, heterogeneities in the intensity of transmission, and over-dispersed distributions of parasite infection durations have all been recognized as factors

that stabilize malaria transmission and frustrate efforts to eliminate it [23]. Indeed, the importance of all these phenomena has been illustrated with a range of different mathematical models. However, all models are systematically biased to underestimate the stability of complex biological systems simply because they are models [23, 76, 77]. Conservation biologists have recently adopted the *portfolio effect* concept from economics, to guide their thinking in relation to ecosystem management. Diversification stabilizes investment portfolios, thereby reducing risks of catastrophic losses [78]. The same is true of complex, biologically and environmentally diverse ecosystems, which are always more stable than any of their component species, habitats or subsets thereof [79]. Mathematical models are deliberately designed to be simpler than the biological system they are intended to mimic [23, 76, 77], so they systematically underestimate their complexity, stability and resilience. Malaria transmission therefore tends to be more stable and less vulnerable to control than face-value interpretation of predictive mathematical models suggest (Killeen & Reed, Unpublished).

The extent to which portfolio effects make malaria transmission resilient against vector control is probably impossible to quantify. Nevertheless, simply being mindful of the overall principle can help moderate expectations of intervention impacts upon guilds of multiple vectors distributed across highly heterogeneous environments. The diversity of overlapping transmission dynamics these complex biological and environmental interactions generate result in malaria transmission that is far more resilient to programmatic-scale control than any single characterized species or location. In many tropical settings, elimination of malaria will probably necessitate elimination of its most efficient vectors [80], possibly including key vectors of residual transmission which readily, flexibly and opportunistically feed upon either humans or animals [1]. Malaria parasite populations that spread their reproductive bets across two or more vectors with different behaviours, ecological niches, seasonal dynamics and vulnerability to specific control measures will always be more difficult to eliminate, and will usually require more diverse intervention packages, than in settings with a single vector species. Furthermore, where individual vector species spread their own reproductive bets across multiple aquatic habitat types, resting sites or blood sources, this creates refugia that limit the impact of any given vector control measure applied in any given time and place. No matter how much detail we try to capture in our mathematical models of vector biology and malaria transmission, they will always under-represent the full complexity and diversity of those interactions, so they are biased towards under-estimating the resilience of malaria transmission against vector control. Whatever vector population response trajectory is expected following introduction of a new vector control measure, the portfolio effect will tend to flatten it out to some unknown extent. The only sensible way to integrate the implications of the portfolio effect into our efforts is to interpret entomological surveillance data and simulation models with considerable restraint (Killeen & Reed, Unpublished).

9. A healthier future for malaria surveillance data collection, ownership and utilization

Global inequities of capacity and opportunity are a difficult but massively important issue to discuss [81]. We have no wish to offend any of our colleagues based at prestigious institutes

in wealthier, cooler, malaria-free countries, nor do we suggest that the capacities they bring to the table are anything less than invaluable. However, the existing *status quo* is neither effective nor fair, and will persist until it is deliberately addressed with far more south-centred funding schemes and productive data sharing mechanisms (**Figure 9**). The time has come for the systematic redistribution of funding investment, to unambiguously prioritize locally-owned and governed institutions in the low-income countries struggling with malaria on an ongoing basis.

And data governance structures that incentivize productive *south–south* and *south–north* (as distinct from *north–south*) collaborations are equally important. For many surveillance staff, investigators and institutions in developing countries, ownership of their data and the analytical opportunities it provides constitute their most important means leverage when negotiating fair conditions in collaborations through which they can develop their data handling and analytical capacities. South-centred platforms for archiving and sharing data, that empower data collectors and incentivize development-friendly collaborations with expert partners from high-income countries, are urgently needed. Looking beyond entomological surveillance, invaluable lessons may be learned from the encouraging experiences of regional and global networks for monitoring anti-malarial drug resistance [82].

The funding and data sharing policies that have shaped the global capacity distribution illustrated in **Figure 7** need to be progressively and aggressively reformed. Vocal advocacy for such strategic changes are a job for everyone in the malaria surveillance community. Each of us are, in our own way, responsible for the landscape as it stands today, and have no-one to blame but ourselves if such inequities and inefficiencies are allowed to persist. Unless we all play our part in actively finding solutions, we must accept that we are passively perpetuating the problem.

10. Conclusions

Considerable progress towards development and deployment of a much broader diversity of vector control tools can be achieved through far more widespread adaptation of established entomological field methods to programmatic surveillance platforms. However, ensuring such data are effectively collected, analyzed, interpreted and acted upon will require that current geographic inequities of analytical capacity are decisively addressed. Specifically, funding and data sharing systems need to be re-oriented to prioritize south-centred collaborations that enable low-income malaria-endemic countries to develop and institutionalize their own expertise base.

Acknowledgements

We thank numerous colleagues for invaluable discussions over the years that have contributed to this perspective, particularly Tom Smith, Samson Kiware and John Gimnig for sharing their experiences with entomological informatics, as well as Mafalda Viana, Jason Matthiopoulos and Heather Ferguson for their insights on life history modelling.

Conflict of interest

The authors declare no competing interests.

Acronyms

LLIN: long lasting insecticidal nets

IRS: indoor residual spraying

MMC: Malaria Modelling Consortium

malERA: Malaria Eradication Consultative Group on Malaria Modelling

WHO: World Health Organization

WHO-WMR: World Health Organization-World Malaria Report

Author details

Gerry F. Killeen[1,2]*, Prosper P. Chaki[1], Thomas E. Reed[3], Catherine L. Moyes[4] and Nicodem J. Govella[1]

*Address all correspondence to: gkilleen@ihi.or.tz

1 Department of Environmental Health and Ecological Sciences, Ifakara Health Institute, Ifakara and Dar es Salaam, United Republic of Tanzania

2 Department of Vector Biology, Liverpool School of Tropical Medicine, Liverpool, United Kingdom

3 School of Biological, Earth and Environmental Sciences, University College Cork, Cork, Republic of Ireland

4 Big Data Institute, Li Ka Shing Centre for Health Information and Discovery, University of Oxford, Oxford, United Kingdom

References

[1] Killeen GF, Kiware SS, Okumu FO, Sinka ME, Moyes CL, Massey NC, Gething PW, Marshall JM, Chaccour CJ, Tusting LS. Going beyond personal protection against mosquito bites to eliminate malaria transmission: Population suppression of malaria vectors that exploit both human and animal blood. BMJ Global Health. 2017;**2**:e000198. DOI: 10.1136/bmjgh-2016-000198

[2] Killeen GF, Tatarsky A, Diabate A, Chaccour C, Marshall J, Okumu FO, Brunner S, Newby G, Williams Y, Malone D, Tusting L, Gosling R. Developing an expanded vector control toolbox for malaria elimination. BMJ Global Health. 2017;**2**:e000211

[3] Barreaux P, Barreaux AMG, Sternberg ED, Suh E, Waite JL, Whitehead SA, Thomas MB. Priorities for broadening the malaria vector control tool kit. Trends in Parasitology. 2017; **33**:763-774. DOI: 10.1016/j.pt.2017.06.003

[4] Kiware SS, Chitnis N, Tatarsky A, Wu S, Castellanos HMS, Gosling R, Smith D, Marshall JM. Attacking the mosquito on multiple fronts: Insights from the vector control optimization model (VCOM) for malaria elimination. PLoS One. 2017;**12**:e0187680. DOI: 10.1371/journal.pone.0187680

[5] Sinka ME, Bangs MJ, Manguin S, Rubio-Palis Y, Chareonviriyaphap T, Coetzee M, Mbogo CM, Hemingway J, Patil AP, Temperley WH, Gething PW, Kabaria CW, Burkot TR, Harbach RE, Hay SI. A global map of dominant malaria vectors. Parasites & Vectors. 2012;**5**:69. DOI: 10.1186/1756-3305-5-69

[6] Killeen GF. Characterizing, controlling and eliminating residual malaria transmission. Malaria Journal. 2014;**13**:330. DOI: 10.1186/1475-2875-13-330

[7] Killeen GF, Marshall JM, Kiware SS, South AB, Tusting LS, Chaki PP, Govella NJ. Measuring, manipulating and exploiting behaviours of adult mosquitoes to optimize malaria vector control impact. BMJ Global Health. 2017;**2**:e000212

[8] Killeen GF, Seyoum A, Gimnig JE, Stevenson JC, Drakeley CJ, Chitnis N. Made-to-measure malaria vector control strategies: Rational design based on insecticide properties and coverage of blood resources for mosquitoes. Malaria Journal. 2014;**13**:146

[9] The malERA refresh consultative panel on combination interventions and modelling: An updated research agenda for combination interventions and modelling in malaria elimination and eradication. PLoS Medicine. 2017;**14**:e1002453. DOI: 10.1371/journal.pmed.1002453

[10] Gatton ML, Chitnis N, Churcher T, Donnelly MJ, Ghani AC, Godfray HC, Gould F, Hastings I, Marshall J, Ranson H, Rowland M, Shaman J, Lindsay SW. The importance of mosquito behavioural adaptations to malaria control in Africa. Evolution. 2013;**67**:1218-1230. DOI: 10.1111/evo.12063

[11] Govella NJ, Chaki PP, Killeen GF. Entomological surveillance of behavioural resilience and resistance in residual malaria vector populations. Malaria Journal. 2013;**12**:124. DOI: 10.1186/1475-2875-12-124

[12] Hemingway J, Beaty BJ, Rowland M, Scott TW, Sharp BL. The innovative vector control consortium: Improved control of mosquito-borne diseases. Trends in Parasitology. 2006;**22**:308-312. DOI: 10.1016/j.pt.2006.05.003

[13] Hemingway J, Ranson H, Magill A, Kolaczinski J, Fornadel C, Gimnig J, Coetzee M, Simard F, Roch DK, Hinzoumbe CK, Pickett J, Schellenberg D, Gething P, Hoppe M,

Hamon N. Averting a malaria disaster: Will insecticide resistance derail malaria control? Lancet. 2016;**387**:1785-1788. DOI: 10.1016/S0140-6736(15)00417-1

[14] Ranson H, Lissenden N. Insecticide resistance in African *Anopheles* mosquitoes: A worsening situation that needs urgent action to maintain malaria control. Trends in Parasitology. 2016;**32**:187-196. DOI: 10.1016/j.pt.2015.11.010

[15] Smith T, Ross A, Maire N, Chitnis N, Studer A, Hardy D, Brooks A, Penny M, Tanner M. Ensemble modeling of the likely public health impact of a pre-erythrocytic malaria vaccine. PLoS Medicine. 2012;**9**:e1001157. DOI: 10.1371/journal.pmed.1001157

[16] Galactionova K, Tediosi F, Camponovo F, Smith TA, Gething PW, Penny MA. Country specific predictions of the cost-effectiveness of malaria vaccine RTS,S/AS01 in endemic Africa. Vaccine. 2017;**35**:53-60. DOI: 10.1016/j.vaccine.2016.11.042

[17] Penny MA, Pemberton-Ross P, Smith TA. The time-course of protection of the RTS,S vaccine against malaria infections and clinical disease. Malaria Journal. 2015;**14**:437. DOI: 10.1186/s12936-015-0969-8

[18] Cameron E, Battle KE, Bhatt S, Weiss DJ, Bisanzio D, Mappin B, Dalrymple U, Hay SI, Smith DL, Griffin JT, Wenger EA, Eckhoff PA, Smith TA, Penny MA, Gething PW. Defining the relationship between infection prevalence and clinical incidence of *Plasmodium falciparum* malaria. Nature Communications. 2015;**6**:8170. DOI: 10.1038/ncomms9170

[19] Penny MA, Galactionova K, Tarantino M, Tanner M, Smith TA. The public health impact of malaria vaccine RTS,S in malaria endemic Africa: Country-specific predictions using 18 month follow-up phase III data and simulation models. BMC Medicine. 2015;**13**:170. DOI: 10.1186/s12916-015-0408-2

[20] Pemberton-Ross P, Smith TA, Hodel EM, Kay K, Penny MA. Age-shifting in malaria incidence as a result of induced immunological deficit: A simulation study. Malaria Journal. 2015;**14**:287. DOI: 10.1186/s12936-015-0805-1

[21] Brady OJ, Slater HC, Pemberton-Ross P, Wenger E, Maude RJ, Ghani AC, Penny MA, Gerardin J, White LJ, Chitnis N, Aguas R, Hay SI, Smith DL, Stuckey EM, Okiro EA, Smith TA, Okell LC. Role of mass drug administration in elimination of *Plasmodium falciparum* malaria: A consensus modelling study. Lancet Global Health. 2017;**5**: e680-e6e7. DOI: 10.1016/S2214-109X(17)30220-6

[22] Okell L, Slater H, Ghani A, Pemberton-Ross P, Smith TA, Chitnis N, Penny M, Wenger E, Gerardin J, Maude R, Aguas R, White LJ, Brady OJ, Hay SI, Okiro E, Kern S: Consensus Modelling Evidence to Support the Design of Mass Drug Administration Programmes. Geneva: World Health Organization; 2015. 20p. Available from: http://www.who.int/malaria/mpac/mpac-sept2015-consensus-modelling-mda.pdf [Accessed: 2018-04-16]

[23] Smith TA, Chitnis N, Penny M, Tanner M. Malaria modeling in the era of eradication. Cold Spring Harbor Perspectives in Medicine. 2017;**7**:313-332. DOI: 10.1101/cshperspect.a025460

[24] Brady OJ, Godfray HC, Tatem AJ, Gething PW, Cohen JM, McKenzie FE, Perkins TA, Reiner RC Jr, Tusting LS, Sinka ME, Moyes CL, Eckhoff PA, Scott TW, Lindsay SW,

Hay SI, Smith DL. Vectorial capacity and vector control: Reconsidering sensitivity to parameters for malaria elimination. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2016;**110**:107-117. DOI: 10.1093/trstmh/trv113

[25] Clements AN, Paterson GD. The analysis of mortality and survival rates in wild populations of mosquitoes. Journal of Applied Ecology. 1981;**18**:373-399

[26] Killeen GF, McKenzie FE, Foy BD, Schieffelin C, Billingsley PF, Beier JC. A simplified model for predicting malaria entomologic inoculation rates based on entomologic and parasitologic parameters relevant to control. American Journal of Tropical Medicine and Hygiene. 2000;**62**:535-544

[27] Killeen GF, Okumu FO, N'Guessan R, Coosemans M, Adeogun A, Awolola S, Etang J, Dabiré RK, Corbel V. The importance of considering community-level effects when selecting insecticidal malaria vector products. Parasites & Vectors. 2011;**4**:160. DOI: 10.1186/1756-3305-4-160

[28] Eckhoff PA. Mathematical models of within-host and transmission dynamics to determine effects of malaria interventions in a variety of transmission settings. American Journal of Tropical Medicine and Hygiene. 2013;**88**:817-827. DOI: 10.4269/ajtmh.12-0007

[29] Charlwood JD, Smith T, Billingsley PF, Takken W, Lyimo EOL, Meuwissen JHET. Survival and infection probabilities of anthropophagic anophelines from an area of high prevalence of *Plasmodium falciparum* in humans. Bulletin of Entomological Research. 1997; **87**:445-453

[30] Moore SJ, Davies CR, Hill N, Cameron MM. Are mosquitoes diverted from repellent-using individuals to non-users? Results of a field study in Bolivia. Tropical Medicine & International Health. 2007;**12**:532-539. DOI: 10.1111/j.1365-3156.2006.01811.x

[31] Sangoro O, Turner E, Simfukwe E, Miller JE, Moore SJ. A cluster-randomized controlled trial to assess the effectiveness of using 15% DEET topical repellent with long-lasting insecticidal nets (LLINs) compared to a placebo lotion on malaria transmission. Malaria Journal. 2014;**13**:324. DOI: 10.1186/1475-2875-13-324

[32] Ogoma SB, Mmando A, Kyeba JS, Horstmann S, Malone D, Killeen GF. A low technology emanator treated with the volatile pyrethroid Transfluthrin confers long term protection against outdoor-biting vectors of lymphatic filariasis, arboviruses and malaria. PLoS Neglected Tropical Diseases. 2017;**11**:e0005455

[33] Trape JF, Tall A, Sokhna C, Ly AB, Diagne N, Ndiath O, Mazenot C, Richard V, Badiane A, Dieye-Ba F, Faye J, Ndiaye G, Diene Sarr F, Roucher C, Bouganali C, Bassene H, Toure-Balde A, Roussilhon C, Perraut R, Spiegel A, Sarthou JL, da Silva LP, Mercereau-Puijalon O, Druilhe P, Rogier C. The rise and fall of malaria in a West African rural community, Dielmo, Senegal, from 1990 to 2012: A 22 year longitudinal study. Lancet Infectious Diseases. 2014;**14**:476-488. DOI: 10.1016/S1473-3099(14)70712-1

[34] Sougoufara S, Thiaw O, Cailleau A, Diagne N, Harry M, Bouganali C, Sembène PM, Doucoure S, Sokhna C. The impact of periodic distribution campaigns of long-lasting

insecticide-treated bed nets on malaria vector dynamics and human exposure in Dielmo, Senegal. American Journal of Tropical Medicine and Hygiene. In press, 2018;**98**:1343-1352. DOI: 10.4269/ajtmh.17-0009

[35] Killeen GF. A revival of epidemiological entomology in Senegal. American Journal of Tropical Medicine and Hygiene. In press, 2018;**98**:1216-1217. DOI: 10.4269/ajtmh.18-0162

[36] Garrett-Jones C, Boreham P, Pant CP. Feeding habits of anophelines (Diptera: Culicidae) in 1971-1978, with reference to the human blood index: A review. Bulletin of Entomological Research. 1980;**70**:165-185

[37] Silver JB. Blood feeding and its epidemiological significance. Mosquito Ecology: Field Sampling Methods. Dordrecht, the Netherlands: Springer; 2008. pp. 677-769

[38] Garrett-Jones C. The human blood index of malarial vectors in relationship to epidemiological assessment. Bulletin of the World Health Organization. 1964;**30**:241-261

[39] Kiswewski AE, Mellinger A, Spielman A, Malaney P, Sachs SE, Sachs J. A global index representing the stability of malaria transmission. American Journal of Tropical Medicine and Hygiene. 2004;**70**:486-498

[40] Garrett-Jones C. A method for estimating the man-biting rate. Geneva: World Health Organization; 1964. Document No.: WHO/Mal/450

[41] Kawada H, Dida GO, Ohashi K, Komagata O, Kasai S, Tomita T, Sonye G, Maekawa Y, Mwatele C, Njenga SM, Mwandawiro C, Minakawa N, Takagi M. Multimodal pyrethroid resistance in malaria vectors, *Anopheles gambiae s.s.*, *Anopheles arabiensis*, and *Anopheles funestus s.s.* In western Kenya. PLoS One. 2011;**6**:e22574. DOI: 10.1371/journal.pone.0022574

[42] Reimer L, Fondjo E, Patchoke S, Diallo B, Lee Y, Ng A, Ndjemai HM, Atangana J, Traore SF, Lanzaro G, Cornel AJ. Relationship between kdr mutation and resistance to pyrethroid and DDT insecticides in natural populations of *Anopheles gambiae*. Journal of Medical Entomology. 2008;**45**:260-266

[43] Nkya TE, Akhouayri I, Poupardin R, Batengana B, Mosha F, Magesa S, Kisinza W, David JP. Insecticide resistance mechanisms associated with different environments in the malaria vector *Anopheles gambiae*: A case study in Tanzania. Malaria Journal. 2014;**13**:28. DOI: 10.1186/1475-2875-13-28

[44] Ondeto BM, Nyundo C, Kamau L, Muriu SM, Mwangangi JM, Njagi K, Mathenge EM, Ochanda H, Mbogo CM. Current status of insecticide resistance among malaria vectors in Kenya. Parasites & Vectors. 2017;**10**:429. DOI: 10.1186/s13071-017-2361-8

[45] Coleman M, Hemingway J, Gleave KA, Wiebe A, Gething PW, Moyes CL. Developing global maps of insecticide resistance risk to improve vector control. Malaria Journal. 2017;**16**:86. DOI: 10.1186/s12936-017-1733-z

[46] Govella NJ, Okumu FO, Killeen GF. Insecticide-treated nets can reduce malaria transmission by mosquitoes which feed outdoors. American Journal of Tropical Medicine and Hygiene. 2010;**82**:415-419. DOI: 10.4269/ajtmh.2010.09-0579

[47] Bugoro H, Cooper RD, Butafa C, Iro'ofa C, Mackenzie DO, Chen CC, Russell TL. Bionomics of the malaria vector *Anopheles farauti* in Temotu Province, Solomon Islands: Issues for malaria elimination. Malaria Journal. 2011;**10**:133. DOI: 10.1186/1475-2875-10-133

[48] Moiroux N, Damien GB, Egrot M, Djenontin A, Chandre F, Corbel V, Killeen GF, Pennetier C. Human exposure to early morning *Anopheles funestus* biting behavior and personal protection provided by long-lasting insecticidal nets. PLoS One. 2014;**9**:e104967. DOI: 10.1371/journal.pone.0104967

[49] Massey NC, Garrod G, Wiebe A, Henry AJ, Huang Z, Moyes CL, Sinka ME. A global bionomic database for the dominant vectors of human malaria. Scientific Data. 2016;**3**: 160014. DOI: 10.1038/sdata.2016.14

[50] Huho BJ, Briët O, Seyoum A, Sikaala CH, Bayoh N, Gimnig JE, Okumu FO, Diallo D, Abdulla S, Smith TA, Killeen GF. Consistently high estimates for the proportion of human exposure to malaria vector populations occurring indoors in rural Africa. International Journal of Epidemiology. 2013;**42**:235-247. DOI: 10.1093/ije/dys214

[51] Gryseels C, Durnez L, Gerrets R, Uk S, Suon S, Set S, Phoeuk P, Sluydts V, Heng S, Sochantha T, Coosemans M, Peeters Grietens K. Re-imagining malaria: Heterogeneity of human and mosquito behaviour in relation to residual malaria transmission in Cambodia. Malaria Journal. 2015;**14**:165. DOI: 10.1186/s12936-015-0689-0

[52] Killeen GF, McKenzie FE, Foy BD, Schieffelin C, Billingsley PF, Beier JC. The potential impacts of integrated malaria transmission control on entomologic inoculation rate in highly endemic areas. American Journal of Tropical Medicine and Hygiene. 2000; **62**:545-551

[53] WHO. Malaria Surveillance, Monitoring and Evaluation: A Reference Manual. Geneva: World Health Organization; 2018. 105 p. Available from: http://apps.who.int/iris/bitstream/handle/10665/272284/9789241565578-eng.pdf?sequence=1&isAllowed=y [Accessed: 2018-04-16]

[54] Silver JB. Mosquito Ecology: Field Sampling Methods. 3rd ed. Dordrecht, the Netherlands: Springer; 2008. 1494 p

[55] Chaki PP, Dongus S, Kannady K, Fillinger U, Kelly A, Killeen GF. Community-owned resource persons for malaria vector control: Enabling factors and challenges in an operational programme in Dar es Salaam, Tanzania. Human Resources for Health. 2011;**9**:21

[56] Chaki PP, Govella NJ, Shoo B, Hemed A, Tanner M, Fillinger U, Killeen GF. Achieving high coverage of larval-stage mosquito surveillance: Challenges for a community-based mosquito control programme in urban Dar es Salaam, Tanzania. Malaria Journal. 2009;**8**:311. DOI: 1475-2875-8-311 [pii] 10.1186/1475-2875-8-311

[57] Dengela D, Seyoum A, Lucas B, Johns B, George K, Belemvire A, Caranci A, Norris LC, Fornadel CM. Multi-country assessment of residual bio-efficacy of insecticides used for indoor residual spraying in malaria control on different surface types: Results from program monitoring in 17 PMI/USAID-supported IRS countries. Parasites & Vectors. 2018;**11**:71. DOI: 10.1186/s13071-017-2608-4

[58] Massue DJ, Moore SJ, Mageni ZD, Moore JD, Bradley J, Pigeon O, Maziba EJ, Mandike R, Kramer K, Kisinza WN, Overgaard HJ, Lorenz LM. Durability of Olyset campaign nets distributed between 2009 and 2011 in eight districts of Tanzania. Malaria Journal. 2016;**15**:176. DOI: 10.1186/s12936-016-1225-6

[59] Viana M, Hughes A, Matthiopoulos J, Ranson H, Ferguson HM. Delayed mortality effects cut the malaria transmission potential of insecticide-resistant mosquitoes. Proceedings of the National Academy of Sciences of the United States of America. 2016;**113**:8975-8980. DOI: 10.1073/pnas.1603431113

[60] Lindblade KA. Does a mosquito bite when no-one is around to hear it? International Journal of Epidemiology. 2013;**42**:247-249. DOI: 10.1093/ije/dyt004

[61] Bayoh MN, Walker ED, Kosgei J, Ombok M, Olang GB, Githeko AK, Killeen GF, Otieno P, Desai M, Lobo NF, Vulule JM, Hamel MJ, Kariuki S, Gimnig JE. Persistently high estimates of late night, indoor exposure to malaria vectors despite high coverage of insecticide treated nets. Parasites & Vectors. 2014;**7**:380

[62] Killeen GF. A second chance to tackle African malaria vector mosquitoes that avoid houses and don't take drugs. American Journal of Tropical Medicine and Hygiene. 2013;**88**:809-816. DOI: 10.4269/ajtmh.13-0065

[63] Kiware SS, Chitnis N, Devine GJ, Moore SJ, Majambere S, Killeen GF. Biologically meaningful coverage indicators for eliminating malaria transmission. Biology Letters. 2012;**8**:874-877. DOI: 10.1098/rsbl.2012.0352

[64] Ross R. The Prevention of Malaria. London: Murray; 1911. 669 p

[65] Garrett-Jones C, Shidrawi GR. Malaria vectorial capacity of a population of *Anopheles gambiae*: An exercise in epidemiological entomology. Bulletin of the World Health Organization. 1969;**40**:531-545

[66] Protopopoff N, Mosha JF, Lukole E, Charlwood JD, Wright A, Mwalimu CD, Manjurano AFWM, Kisinza W, Kleinschmidt I, Rowland M. Effectiveness of a long-lasting piperonyl butoxide-treated insecticidal net and indoor residual spray interventions, separately and together, against malaria transmitted by pyrethroid-resistant mosquitoes: A cluster, randomised controlled, two-by-two factorial design trial. Lancet. 2018;**391**:1577-1588. DOI: 10.1016/S0140-6736(18)30427-6

[67] Russell TL, Lwetiojera D, Knols BGJ, Takken W, Killeen GF, Ferguson HM. Linking individual phenotype to density-dependent population growth: The influence of body size on the population dynamics of malaria vectors. Proceedings of the Royal Society B. 2011;**278**:3142-3151

[68] Killeen GF, Chitnis N. Potential causes and consequences of behavioural resilience and resistance in malaria vector populations: A mathematical modelling analysis. Malaria Journal. 2014;**13**:97

[69] Killeen GF, Govella NJ, Lwetoijera DW, Okumu FO. Most outdoor malaria transmission by behaviourally-resistant *Anopheles arabiensis* is mediated by mosquitoes that have previously been inside houses. Malaria Journal. 2016;**15**:225. DOI: 10.1186/s12936-016-1280-z

[70] Sternberg ED, Ng'habi KR, Lyimo IN, Kessy ST, Farenhorst M, Thomas MB, Knols BG, Mnyone LL. Eave tubes for malaria control in Africa: Initial development and semi-field evaluations in Tanzania. Malaria Journal. 2016;**15**:447. DOI: 10.1186/s12936-016-1499-8

[71] Killeen GF, Masalu JP, Chinula D, Fotakis EA, Kavishe DR, Malone D, Okumu F. Control of malaria vector mosquitoes by insecticide-treated combinations of window screens and eave baffles. Emerging Infectious Diseases. 2017;**23**:782-789. DOI: 10.3201/eid2305.160662

[72] Diabate A, Bilgo E, Dabire RK, Tripet F. Environmentally friendly tool to control mosquito populations without risk of insecticide resistance: The Lehmann's funnel entry trap. Malaria Journal. 2013;**12**:196. DOI: 10.1186/1475-2875-12-196

[73] Khattab A, Jylha K, Hakala T, Aalto M, Malima R, Kisinza W, Honkala M, Nousiainen P, Meri S. 3D mosquito screens to create window double screen traps for mosquito control. Parasites & Vectors. 2017;**10**:400. DOI: 10.1186/s13071-017-2322-2

[74] MalERA Consultative Group on Modeling. A research agenda for malaria eradication: Modeling. PLoS Medicine. 2011;**8**:e1000403. DOI: 10.1371/journal.pmed.1000403

[75] Garrett-Jones C. Prognosis for interruption of malaria transmission through assessment of the mosquito's vectorial capacity. Nature. 1964;**204**:1173-1175

[76] McKenzie FE. Why model malaria? Parasitology Today. 2000;**16**:511-516

[77] McKenzie FE, Samba EM. The role of mathematical modeling in evidence-based malaria control. American Journal of Tropical Medicine and Hygiene. 2004;**71**:94-96

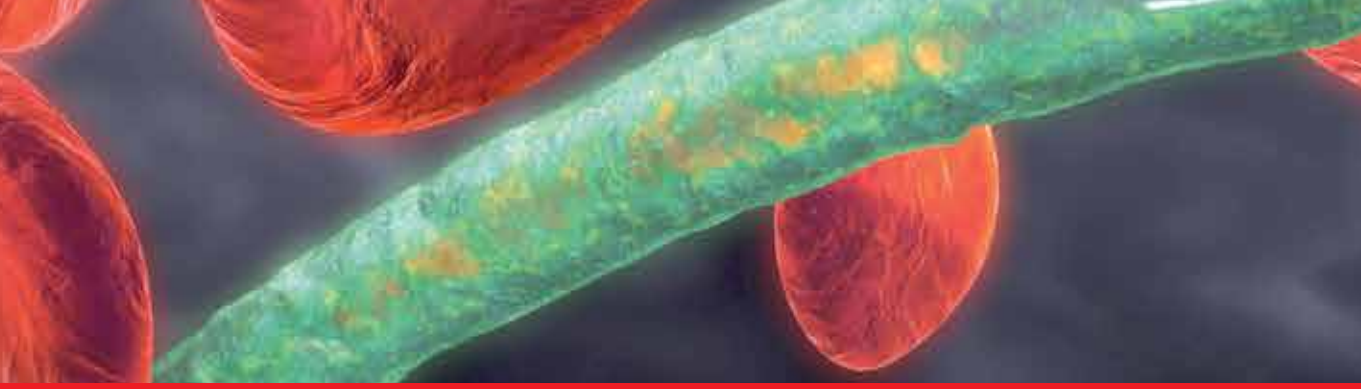
[78] Markowitz H. Portfolio selection. Journal of Finance. 1952;**7**:77-91

[79] Schindler DE, Reed TE, Armstrong J. The portfolio concept in ecology and evolution. Frontiers in Ecology and Evolution. 2013;**13**:257-263. DOI: 10.1890/140275

[80] Killeen GF, Seyoum A, Sikaala CH, Zomboko AS, Gimnig JE, Govella NJ, White MT. Eliminating malaria vectors. Parasites & Vectors. 2013;**6**:172. DOI: 10.1186/1756-3305-6-172

[81] Geissler PW, Okwaro F. Discuss inequality. Nature. 2014;**513**:303. DOI: 10.1038/513303a

[82] Merson L, Gaye O, Guerin PJ. Avoiding data dumpsters-toward equitable and useful data sharing. New England Journal of Medicine. 2016;**374**:2414-2415. DOI: 10.1056/NEJMp1605148



Edited by Sylvie Manguin and Vas Dev

Towards Malaria Elimination - A Leap Forward was started to mark the occasion for renewed commitment to end malaria transmission for good (the WHO's call for 'Malaria Free World' by 2030). This book is dedicated for the benefit of researchers, scientists, program and policy managers, students and anyone interested in malaria and other mosquito-borne diseases with the goal of sharing recent information on success stories, innovative control approaches and challenges in different regions of the world. Some main issues that emerged included multidrug-resistant malaria and pandemic risk, vaccines, cross-border malaria, asymptomatic parasite reservoir, the threat of Plasmodium vivax and Plasmodium knowlesi, insecticide resistance in Anopheles vectors and outdoor malaria transmission. This book is one little step forward to bring together in 17 chapters the experiences of malaria-expert researchers from five continents to present updated information on disease epidemiology and control at the national/regional level, highlighting the constraints, challenges, accomplishments and prospects of malaria elimination.

Published in London, UK

IntechOpen

ISBN 978-1-83881-413-7