Research in Health Science
ISSN 2470-6205 (Print) ISSN 2470-6213 (Online)
Vol. 10, No. 2, 2025
www.scholink.org/ojs/index.php/rhs

*Commentary*

# Research Challenges During Low Malaria Incidence in Eswatini: Implication for Achievement of Elimination Targets

Dlamini Sabelo Vusi[1], Mathobela[2], Mbongiseni N.[1], Hlophe Londiwe D.[1] & Nxumalo Sihle A.[1]

[1] University of Eswatini, Department of Environmental Health Science, Faculty of Health Sciences, Eswatini

[2] National Malaria Programme, Eswatini

***Abstract***

*In May 2015, governments endorsed the World Health Organisation's Global Technical Strategy for Malaria 2016 – 2030, a new technical roadmap for countries that are working to reverse and eliminate the spread of malaria. Research is an important component of the Global strategy to evaluate the impact of interventions and monitor progress towards achievement of elimination targets. However, there are critical issues when conducting research in areas with low malaria transmission, particularly identifying and eliminating parasites in asymptomatic infections. Accurate detection of micro-parasitaemic infections and achieving adequate sample sizes to derive statistical power remain a major challenge in the design and conduct of valuable research studies. To monitor progress towards elimination, it is often necessary to quantify Plasmodium infections and artemisinin resistant parasites among the border-crossing populations, including asymptomatic persons. Population movement from high to low transmission areas import infections and facilitate spread of drug resistance. Quantification of the problem of importation of parasites is difficult and complicated by the presence of informal entry points for infections including asymptomatic ones. Surveillance in the context of malaria elimination has to shift from measuring reductions in mortality and morbidity to detecting infections with or without symptoms. The capacity to assess trends and respond without delay has to be developed so that surveillance becomes an additional intervention process. Methods to determine and monitor importation of parasites among people with micro-parasitaemic infections require DNA based techniques such as nested Polymerase Chain Reaction (PCR) and Loop-mediated isothermal amplification (LAMP) which, despite their ability to identify very low parasitaemia, remain expensive for application in field studies. Early warning of the emerging of resistance is needed to identify new foci, predict their spread and inform prompt containment action to counter and slow down the spread of antimalarial and insecticide resistance. It is therefore critical to identify and deploy efficient methods to track signs of reduced efficacy to artemisinin-based combination drugs and insecticides used for indoor residual sprays at the earliest possible time. The efficacy of long lasting insecticide nets*

*(LLINs) in the field is not only determined by direct mortality, but also personal protection and deterrence, for which correlations are much weaker. All these requirements create a challenge in the capacity of the country to conduct comprehensive research to provide information required to maintain a positive drive towards malaria elimination.*

***Keywords***

*low malaria incidence, micro-parasitaemia, cross-border infections, migrant mobile populations, Swaziland*

**Background**

Malaria is a leading cause of mortality and morbidity globally among children (Naghavi, 2015) and its control and eradication has been a top global priority of the United Nations since 1955 (Mayo and Brady, 1955). The Roll Back Malaria (RBM) partnership, established in 1998 as part of a global drive to galvanize action to bring malaria under control and ultimately to eliminate the disease, has had significant impact in reducing malaria incidence in many countries in Sub-Saharan Africa. Eswatini is one of the countries that has benefitted from support of the RBM's efforts to expand anti-malaria interventions. The country has managed to achieve the malaria-focused targets of the Millennium Development Goals (MDGs), which call for halting malaria and reduce the incidence by 2015. The RBM has made an important contribution to this achievement helping forge consensus between partners, mobilising resources and catalysing action. As a result of the RBM efforts, baseline incidence rates of malaria in Eswatini were reduced to 2.34 per 1 000 population at risk in 2014 (MOSASWA, 2015) following implementation of strategies to eliminate the disease by 2018 (MOH, 2008). However, the need to review antimalarial drug's efficacy, impact of current intervention programmes such as indoor residual spray (IRS) and promotion of use of long lasting insecticide treated nets (LLINs), chemoprophylaxis for travellers to endemic areas and identification of asymptomatic cases for parasite clearance are still important areas required to determine whether current tools are still needed and to assess their impact. During progress towards malaria elimination, surveillance and response must be strengthened.

Integrated research requires systems thinking, outcome-oriented monitoring and evaluation approaches across disciplines, sectors and regions, multi-stakeholder engagement and sensitivity to social equity. The complexity of these challenges currently outpace the existing capacities of research teams, especially among countries trying to accelerate malaria control to elimination. The same countries also have commonly weak research capacities and compartmented institutional landscapes. Research in many countries in Sub-Saharan Africa is often led by experts from developed countries and often neglects the importance of building partnerships with local research institutions and personnel who have a good know-how of the local landscapes and who will remain to drive research agendas forward long after overseas researchers have departed. Local research, therefore, must also have the important objective of developing local capacity to carry over beyond international partnerships.

For the reasons stated above, it remains essential to evaluate the current methodological barriers that limit conduct of comprehensive studies of malaria in low transmission settings of low parasite prevalence and low parasite densities, and to assess the potential implication for contribution to malaria elimination. Research at this level is essential to develop evidence required to inform decision-making processes used to implement interventions likely to achieve the desired goal of malaria elimination.

**Assessment of diagnoses and case-management**

Detection of the presence of *Plasmodium* parasites is essential to guide case management. Improved diagnostic tools are required to enable targeted treatment of infected individuals. Also, field-ready diagnostic tools for mass screening and surveillance that can detect asymptomatic infections of very low parasite densities are needed to monitor transmission reduction and ensure elimination. However, the very low levels of transmission currently experienced in Eswatini present new challenges that will demand new diagnostic tools and strategies, in particular, a change from passive case detection to "active" case detection. As the elimination agenda is increasingly followed, assessment and improvements in current field diagnostics (microscopy and rapid diagnostic tests [RDTs]) for case management and new diagnostics that can detect very low levels of *Plasmodium* parasites in the blood of asymptomatic individuals who may contribute to continuing malaria transmission (Roper et al., 1996; Harries et al., 2010; Oudraogo et al., 2009; Shekalaghe et al., 2007). More specifically, to avoid onward transmission, elimination programmes for malaria increasingly need to focus on prompt and accurate detecting the highest possible fraction of infections in the general public through active rather than passive case detection. This change of focus is essential because *Plasmodium* infections can persist at low densities for different lengths of time with no significant symptoms (Collins and Jeffrey, 1999; Roper et al., 1996). In Eswatini, where successful sustained control has been achieved, the need to adjust diagnostic strategies as transmission continue to decline to low levels, and as elimination is considered, will become necessary. Importantly, until eradication of malaria is achieved (and diagnostics therefore no longer required), efforts to eliminate malaria will continue to require diagnostics strategies as reintroduction will remain a reality. In Eswatini, the risk of autochthonous or imported malaria remains a major challenge; hence diagnostics must be capable of rapidly and accurately detecting and quantifying parasitaemia in febrile patients. Pooling of knowledge and sometimes operational research is required to maximize the impact of diagnostic tools in case management. However, despite that very sensitive diagnostic tools need to be implemented, these tools must be suitable for use in resource-poor field settings to support prompt and accurate identification of parasite carriers.

During low malaria transmission, surveillance in the context of elimination has to shift from measuring reduced morbidity and mortality to prompt detection of both symptomatic and asymptomatic infections. Both symptomatic and asymptomatic infections are important in measuring and sustaining gains in malaria control and maintenance of gains towards elimination. Surveillance programmes have to develop tools able to promptly identify new infections, assess trends and response without delay i.e.

before outbreaks from imported parasites occur. While symptomatic infections are readily identifiable when patients seek healthcare in appropriate health facilities, many programmes face challenges in promptly identifying asymptomatic infections and offering treatment to remove source of infection for the susceptible population. The need to clearly understand the contribution of sub-patent infections to malaria transmission is very important in any country aiming at reducing local transmission. Identification of individuals with sub-potent infections and offering them treatment aimed at clearing the parasite reservoir remains a critical consideration for malaria elimination. Many countries that have lowered malaria transmission have engaged ReActive case detection (RACD) as a strategy to promptly identify symptomatic infections. In order to have the desired impact, reactive case detection must also be able to identify asymptomatic populations with sub-potent parasite reservoirs. However, RACD is an operationally demanding and resource intensive method (Sturrock et al., 2013). In Eswatini, microscopy and Rapid Diagnostic Tests (RDTs) are the most commonly used tools for diagnosing malaria infections. Microscopy can only detect 200 parasites per 100 000 population. The accepted standard for malaria diagnosis remains the evaluation of Giemsa-stained blood smears by light microscopy, which is labour intensive, slow, and requires trained personnel. The quality of microscopy tends to get reduced when laboratory personnel do not review malaria patient material regularly in low endemic areas. Periodic determination of the capacity of microscopists is required in order to identify skill gaps and strategies to fill those gaps up. The ability of microscopy or RDTs to detect parasites at very low densities is, therefore, poor and individuals with low parasitaemia are recorded as negative yet remain infectious to mosquitoes (Coleman et al., 2004; Manjurano et al., 2011; McMorrow et al., 2011). For achieving malaria elimination, we should not overlook the low-density and asymptomatic infections, hence Polymerase Chain Reaction (PCR) is preferred worldwide for the detection of *Plasmodium* parasites. However, the use of PCR currently is restricted to malaria diagnosis in research laboratories due to the technicalities and a higher cost. The use of DNA based testing techniques, such as Nested PCR and Loop-mediated isothermal amplification (LAMP), which have the ability to detect sub-patent infections is recommended as additional diagnostic tools among countries aiming to eliminate malaria. Nested Polymerase Chain Reaction (PCR) is often cited to have the ability to detect sub-patent infections at two parasites per millilitre of whole blood (Mahajan et al., 2011). However, DNA based techniques are expensive, require extensive training and are usable for research purposes only (Schachterle et al., 2011). At present, the application of PCR-based methods is restricted to well-equipped laboratories with specially trained technicians, partly because the need to avoid contamination (which leads to false-positive results) requires very high standards of laboratory practices. Loop Mediated Isothermal Amplification (LAMP), has the potential to reduce training and infrastructure requirements, the method has not been adequately field tested for wide-scale use or developed in a format suitable for the processes of high sample numbers. A study conducted by a Febrer-Sendra and colleagues (2023) evaluated LAMP clinically on blood samples in a resource-poor malaria-endemic area. The colorimetric LAMP proved to be more sensitive than microscopy and RDTs

for malaria diagnosis in field conditions. The possibility to use LAMP in a real-time format in a portable device reinforces the reliability of the assay for molecular diagnosis of malaria in resource-poor laboratories in endemic areas. Companies are manufacturing RDTs for malaria but most have not been carefully evaluated and the performance typically falls below the expected level. Also, use of antigen-based RDTs is likely to miss a significant proportion of asymptomatic cases in low transmission settings (Roper et al., 1996; Collins and Jeffrey, 1999). Research aimed towards increasing the sensitivity of existing RDTs may not change this situation because of the limitations of the currently available technology. RDTs also present some logistical challenges at the community level and in some private sector settings, particularly with regard to the potential risks of other blood-borne infections.

**Identification of asymptomatic cases**

In Eswatini, malaria is highly seasonal and occurs at low endemicity; and as such, many cases remain asymptomatic or minimally symptomatic while serving as reservoirs of infection for maintaining malaria transmission as the parasite can persist in these individuals from one season to the next (Bousema et al., 2012; Harris et al., 2010). Currently, the gold standard method for malaria diagnosis remains microscopic examination of thin and thick blood smears. Unfortunately, microscopy examination has many limitations. It is time-consuming and requires expert training in parasite morphology, and false-negative results can occur when parasitaemia is low. The problem is compounded by the fact that these individuals do not seek medical attention, making it difficult for them to be identified (Manjurano et al., 2011). Despite these challenges, identifying asymptomatically infected individuals and clearing these parasite reservoirs is critical to sustain local control and drive the elimination agenda forward (Karl et al., 2011; Sutcliffe et al., 2011). If control measures ignore the local parasite reservoirs in human populations, malaria resurge is likely to occur following successful control. Targeting gametocyte reservoirs has the benefit of halting transmission and should be integrated into the national malaria control programme. During low transmission seasons, malaria infections in Eswatini tend to cluster in geographically defined areas and later create hotspots of infection when vector populations resurge. Very often, malaria outbreaks in subsequent transmission seasons occur around these clusters that serve as the source of parasites (Bousema et al., 2011). The country has engaged RACD following identification and confirmation of an infected individual. During RACD, the surveillance unit of the National Malaria Programme (NMP) receives information about a confirmed case. The case is followed to its locality and all persons within a radius of 500m are tested for malaria using Rapid Diagnostic Tests (RDTs) or Microscopy. Unfortunately, the efficacy of the RACD strategy is limited by the low sensitivity of RDTs and microscopy in low transmission settings (Tedesse et al., 2015). Several studies have reported the presence and persistence of sub-microscopic infections in low transmission settings (Atkinson et al., 2012; Golassa et al., 2015). Sub-microscopic infections have been reported to contribute 20-50% towards human-to-mosquito transmissions (Okell et al., 2012). While molecular methods such as the Polymerase Chain Reaction (PCR) and

Loop-Mediated Isothermal Amplification (LAMP) have the ability to identify low parasitaemia up to 1 parasite per microlitre of blood, these methods have limitation for application to field situations because of equipment, infrastructure, and expertise required remain major limiting factors. Therefore, the absence of simple, cheap and sensitive methods to identify sub-microscopic parasitaemia suggests that persons with parasites will continue to be missed and will contribute to onward transmission of parasites and further delay elimination efforts.

**Mapping malaria risk spots**

As malaria transmission declines, it becomes very important to identify and map risk areas and use them to predict outbreak points. Knowing and mapping risk areas has the advantage of concentrating resources over smaller geographical areas and ensuring that preventive measures remain cost effective. However, malaria risk mapping poses significant challenges in low transmission settings and around the border areas of Eswatini. Uncontrolled human movements around border areas make it difficult to identify malaria 'hot spots'. Such challenges underscore the effectiveness of efforts aimed at understanding and predicting transmission patterns and implementing efforts aimed at reducing the risks of outbreaks to prevent malaria parasites from spreading over a wider geographical area and undermining elimination efforts.

**Insecticide and Antimalarial drug efficacy studies**

Workers in Ethiopia (Balkew et al., 2010; Yewhalaw et al., 2010; 2011; Abate and Haddis, 2011) reported increased resistance of *An. arabiensis* to insecticides belonging to the four classes. Given the small number of insecticides for IRS and LLINs, the Ministry of Health in Eswatini should routinely measure by formulating a policy as well as implementing insecticide resistance management within the framework of integrated vector management in Eswatini. The absence of experts to conduct in-depth studies to determine development and spread of resistance to current insecticides poses a huge threat for the elimination efforts. The rationale for new initiatives dedicated to develop integrated research partnerships on malaria in Eswatini stems from the need for a consolidated evidence base, enhanced capacities, and stronger advocacy for outcome oriented malaria research that transcends disciplinary and sectorial boundaries (Bazzani and Wiese, 2012). Development of insecticide resistance can be determined through monitoring and early identification of the appearance of genetic markers such as the *kdr* gene and conduct of drug sensitivity assays on local vectors. The current drugs recommended for treatment of uncomplicated malaria in Eswatini involve Artemether-lumefantrine + a single dose of 0.25mg of Primaquine (Swaziland Malaria Diagnosis and Case Treatment Guidelines, 2017). To assess the effectiveness of this intervention, adequate numbers of patients must be treated and followed up. With a low number of infected people in Eswatini, it is difficult to accumulate adequate numbers for follow up. The inclusion of Primaquine in the treatment regimen was delayed by concerns of the absence of evidence detailing the existence of G6PD-deficiency in the Eswatini population. While Primaquine has the benefits of reducing the population of mature gametocytes and therefore, reducing parasite transmission to mosquito vectors, treatment with Primaquine increases the risk of haemolysis

among G6PD-deficient patients. Primaquine also has the benefit of abolishing hypnozoites and prevent relapses in patients infected with *P. vivax* malaria. However, the absence of concrete evidence on the prevalence of G6PD deficiency has limited application of this strategy to the World Health Organisation recommended low dosage which was decided to avert or reduce the risk of haemolysis.

**Power of studies aimed at assessing effectiveness of interventions (LLINs, IRS)**

Studies aimed at adequately assessing the effectiveness of interventions such as Long Lasting Insecticide Treated Nets (LLINs) and Indoor Residual Sprays (IRS) require a large sample size in order to derive adequate study power. Study power in such studies may be derived by increasing the number of subjects, increasing the duration of data collection or increasing the geographical area to be investigated. However, these changes often come with significant additional costs and possible challenges of changes in seasonality or variation in the micro-epidemiology having an impact in the desired outcome (Greenwood, 1989; Smith et al., 2005). Currently, there is a relative dearth of evidence-based approaches to determine how funds should be divided between LLINs and IRS. Malaria intervention campaigns are not a one-size-fits-all solution, and tailored approaches are necessary to account for the heterogeneity of malaria epidemiology within a country where some areas may have very low transmission compared to other areas. For example, in 2014, the country of Mozambique was in the midst of developing an application to the Global Fund to support its malaria control programmes in which decisions needed to be made on what kinds of vector control strategies would be implemented and where. To facilitate decision-making by the Mozambique Ministry of Health, the Global Fund convened the Mozambique Modelling Working Group (MMWG) that used JANUS, a computational economic, operational, and clinical outcomes modelling platform connected with *Open Malaria*, a malaria transmission model to determine the value of changing LLIN and IRS coverage (Lee et al., 2017). The goal of the MMWG was to investigate the cost-effectiveness of differing allocation options of LLINs versus IRS, taking into account the heterogeneous transmission throughout the country, to inform the country's Global Fund application. Many individual countries pursuing the elimination agenda do not possess the capacity to perform such important and informative operational requirements to inform their decision-making processes.

**Refusal of participants to participate**

In low transmission areas, recruitment of participants is commonly a problem due to the low possibility of them being infected. When participants fail to view malaria as a significant problem, they are less likely to see any benefit of their participation in studies. Therefore, irrespective of the study design, epidemiologic studies have challenges in acquiring required sample sizes to derive statistical power. In order to derive statistical power and because of the low incidence of the disease, studies in low transmission settings have to follow up participants for lengthy periods which leads to loss-to-follow up due to lack of interest in the study. Long periods of follow could also result in unnecessary costs that low and middle income countries may not adequately fund.

**Mobile migrant population data**

Several studies have reported the importance of human mobility for malaria elimination in previous elimination attempts where malaria re-emerged due to failure of surveillance systems to account for movements of human populations (Pindolia et al., 2013; Martens and Hall, 2000). Malaria importation rates for African malaria elimination countries are much higher than those for countries that eliminated malaria in the past decades due to high mobility and highly endemic neighbours (Tatem, 2010). Current malaria control efforts typically target geographically stable groups i.e. the distribution of long lasting insecticide nets (LLINs) relies on intermittently updated census data and also indoor residual spraying (IRS) focuses on stable administrative villages, all of which fail to account for mobile migrant populations (Wessen, 1972; Wangroongsarb et al., 2011; Grietens et al., 2012). Human population movement between Eswatini and neighbouring countries present a real challenge for malaria elimination in Eswatini. Different types of mobility require different malaria control and elimination strategies. Profiling migrant populations at various points between original and final areas of destination to derive in-depth understanding of malaria risk in each group can lead to wastage of efforts and resources leading to delay in elimination. Existence of several informal entry points into the country as well as large numbers of illegal immigrants, language differences or a lack of familiarity with formal health care services present major challenges. Given the large socio-cultural differences between various types of mobile migrant populations, it can be expected that there is no standard way to address mobility in malaria control and elimination. In this regard, targeting itinerant workers (i.e. street vendors and traders in border regions) require radically different approaches compared to national and international migrants. Standard malaria control interventions in the country implicitly operate on the assumption that individuals and subpopulations are registered and therefore easy to access. Moreover, in biomedical and clinical research, mobile populations are often purposefully excluded so as to minimize poor compliance to treatment or losses to follow up. As a result, assessment of the effectiveness of standard interventions among mobile populations often yields poor results due to low power of studies. Short-term cross-border human population movements are common between Eswatini and Mozambique; and between Eswatini and South Africa. Populations living within the border communes are allowed to cross these borders without permits or travel documentation for one-day stays. Some cross to visit relatives or to attend ceremonies and funerals overnight. However, some of these stay for longer, and because of the absence of stringent monitoring mechanisms, they cannot be traced or tracked by immigration authorities. Several indigenous populations in Eswatini also travel to Mozambique to buy products, particularly clothes, from Maputo for resale within Eswatini. Because of the partial illegality of these movements and indefinite stay periods, it becomes difficult to obtain reliable information from travellers when conducting interviews. The complexity of these movements were also recently described in Northeast Cambodia where difficulties posed by informal human movements were reported to create challenges in the development of evidence to inform targeted malaria elimination processes (Grietens et al., 2015). Migrant populations moving daily across

formal border posts often have limited time for face-to-face interviews with researchers or to participate in focus group discussions because they cross very early in the morning in a hurry to complete their errands in the destination country and return very late when they need to return to their homes on time.

**Implications on Malaria Elimination in Eswatini**

The World Health Organization (WHO) defines malaria as eliminated in an area if there is zero incidence of locally acquired clinical cases in a defined geographical area during three consecutive years (WHO, 2008). To achieve this requirement in Eswatini, asymptomatically infected people must be identified through testing the population for persistent pockets of infections to ensure that malaria reservoirs are truly eliminated. With zero indigenous disease burden, prompt detection by health care providers is a major challenge, both amongst individuals returning from malaria endemic countries and the local population as malaria is low on the differential diagnosis of patients presenting with fever. Also, if high costs prevent implementation of diagnostic methods able to identify very low parasiteamia in the population, current diagnostic tools will not determine if malaria reservoirs have truly been eliminated. When cases occur, it becomes difficult to determine whether these are resulting from local malaria reservoirs or from sub-patent reservoirs from imported cases. The ability to accurately study populations that are likely responsible for sustaining malaria transmission from one season to the next will be critical to achieving malaria elimination in Eswatini, but are particularly difficult to assess with the diagnostic and epidemiological tools currently available (malERA, 2011; Manjurano et al., 2011). Voluntary screening and treatment mechanisms for malaria should be operational at all entry points into the country, formal or informal. However, voluntary screening largely depends on having a strong and effective health promotion programme in place which, unfortunately, is not the case in Eswatini. Active screening of immigrants, such as the Pakistani immigrants, refugees from endemic countries and foreign workers, such as those from neighbouring Mozambique is largely dependent on strong collaboration between institutions such as the United Nations High Commission for Refugees (UNHCR) or the international Organisation for Migration (IOM) and the Eswatini National Malaria Programme. Details of irregular immigrants are commonly not available with any of these organisations despite a requirement for them to be involved in the registration of immigrants. Also, new entrants in the country may settle down in the same location as their predecessors making it a daunting task to locate and access them for the purpose of implementation of customary malaria control interventions. A study by Koita and colleagues (2013), identified high risk social networks of immigrants and recommended targeting of control measures in these networks. However, individuals who have undergone a screening test once and tested negative are likely to develop a false sense of security than persuading for another repeat test later, or even when they develop fever, is likely to prove challenging. With malaria levels currently reaching record low levels, malaria diagnosis is likely to be delayed or reluctance of health care staff to perform repeat diagnoses among fever patients testing negative for malaria.

Finally, an examination of the history of malaria elimination efforts in Eswatini argues for the value of

intense efforts to use scientifically valid and up-to-date methods to monitor parasite and vectors, the status of parasite resistance to current antimalarial drugs, vector susceptibility to currently used insecticides and associated changes in vector species and behaviour, human movements and other socio-cultural aspects, all of which are worth exploring in greater detail in the ongoing malaria elimination phase in Eswatini. There is still a lack of the clear understanding of the maintenance of a residual proportion of locally acquired malaria for a significant number of years beyond the period of logarithmic decline in the number of cases, leading to failure of earlier projections to eliminate malaria by 2015 and considering that there are better tools available to complement efforts now. This underscores the need for integrating new technologies in the process of assessing the current and informing the future in order to achieve success in the elimination efforts in Eswatini.

**References**

Abate A., & Haddis M. (2011). Susceptibility of *Anopheles gambiae s.l.* to DDT, malathion, permethrin and deltamethrin in Ethiopia. *Tropical Medicine International Health*, *16*, 486-491.

Atkinson J.A., Johnson M.L., Wijesinghe R., Bobogare A., Losi L., O'Sullivan M., Yamaguchi Y., Kenilorea G., Vallely A., Cheng Q., Ebringer A., Bain L., Gray K., Harris I., Whittaker M., Reid H., Clements A., & Shanks D. (2012). Operational research to inform a sub-national surveillance intervention for malaria elimination in Solomon Islands. *Malar Journal*, *11*, 101.

Balkew M., Ibrahim M., Koekemoer L.L., Brooke B.D., Engers H., Aseffa A., Gebre-Michael T., & Elhassen I. (2010). Insecticide resistance in *Anopheles arabiensis* (Diptera: Culicidae) from villages in Central, Northern and South West Ethiopia and detection of *kdr* mutation. *Parasites and Vectors*, *3*, 40.

Bazzani R., & Wiese M. (2012). Poverty, Ecosystems, and Vector-Borne Diseases: Introduction. *In Ecohealth Research Practice*. Edited by Charron D. Ottawa IDRC and Springer 133-138.

Bousema T., Griffin J.T., Sauervwein R.W., Smith D.L., Churcher T.S., Takken W., Ghani A., Drakeley C., & Gosling R. (2012). Hitting hotspots: spatial targeting of malaria for control and elimination. *PLoSMedicine*, *9*, e1001165.

Collins W.E., & Jeffrey G.M. (1999). A retrospective examination of the patterns of recrudescence in patients infected with *Plasmodium falciparum*. *American Journal of Tropical Medicine and Hygiene*, *61*, 44-48.

Febrer-Sendra B., Crego-Vicente B., Nindia A., Martínez-Campreciós J., Aixut S., Mediavilla A., Silgado A., Oliveira-Souto I., Salvador F., Molina I., Muro A., Sulleiro E., Fernández-Soto P. (2023) First feld and laboratory evaluation of LAMP assay for malaria diagnosis in Cubal, Angola. *Parasites & Vectors* 16: 343.

Golassa L., Baliraine F.N., Enweji N., Erko B., Swedberg G., & Abraham Aseffa A. (2015). Microscopic and molecular evidence of the presence of asymptomatic Plasmodium falciparum and Plasmodium vivax infections in an area with low, seasonal and unstable malaria transmission in

Ethiopia. *BioMed Central Infectious Diseases*, *15*, 310.

Greenwood B.M. (1989). The micro epidemiology of malaria and its importance to malaria control. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *83*(Suppl), 25-29.

Grietens K.P., Gryseels C., Dierickx S., Bannister-Tyrrell M., Trienekens S., Phoeuk P., Suon S., Set S., Gerrets R., Hoibak S., Ribera J.M., Hausmann-Muela S., Tho S., Durnez L., Sluydts V., d'Alessandro U., Coosemans M., & Erhart A. (2015). Characterizing types of human mobility to inform differential and targeted malaria elimination strategies in Northeast Cambodia. *Scientific Reports*, *5*, 16837 http://doi.org/10.1038/srep16837

Grietens P.K., Xuan X.N., Ribera J.M., Duc T. N., Bortel W., Ba N. T., Van K. P., Xuan H. L., D'Alessandro U., & Erhart A. (2012). Social Determinants of Long Lasting Insecticidal Hammock-Use Among the Ra-Glai Ethnic Minority in Vietnam: Implications for Forest Malaria Control. *PloS ONE*, *7*, e29991. http://doi.org/10.1371/journal.pone.0029991.

Harris I., Sharrock W.W., Bain L.M., Gray K.A., Bobogare A., Boaz L., Lilley K., Krause D., Vallely A., Johnson M.L., Gatton M.L., Shanks G.D., & Cheng Q. (2010). 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*, *9*, 254.

Karl S., Gurarie D., Zimmerman P.A., King C.H., St Pierre T.G., & Davis T.M. (2011). A sub-microscopic gametocyte reservoir can sustain malaria transmission. *PLoS One*, *6*, e20805

Kitua A., Ogundahunsi O., Lines J., & Mgone C. (2011). Conquering malaria: enhancing the impact of effective interventions towards elimination in the diverse and changing epidemiology. *Journal of Global Infectious Diseases*, *3*, 161-165.

Koita K., Novotny J., Kunene S., Zulu Z., Ntshalintshali N., Gandhi M., & Gosling R. (2013) Targeting imported malaria through social networks: a potential strategy for malaria elimination in Swaziland. *Malaria Journal*, *12*, 219.

Lee B.Y., Bartsch S.M., Stone N.T.B., Zhang S., Brown S.T., Chatterjee C., DePasse J.V., Zenkov E., Briët O.J.T., Mendis C., Viisainen K., Candrinho B., & Colborn J. (2017). The Economic Value of Long-Lasting Insecticidal Nets and Indoor Residual Spraying Implementation in Mozambique*American Journal of Tropical Medicine and Hygiene*, *96*(6), 1430-1440.

malERA Consultative Group on Diagnoses and Diagnostics. (2011). A research agenda for malaria eradication: diagnosis and diagnostics. *PLoS Med*, *8*, e1000396.

Manjurano A., Okell L., Lukindo T., Reyburn H., Olomi R., Roper C., Clark T.G., Goseph S., Riley E.M., & Drakeley C. (2011). Association of sub-microscopic malaria parasite carriage with transmission intensity in north-eastern Tanzania. *Malaria Journal*, *10*, 370.

Martens P. & Hall L. (2000). Malaria on the move: human population movement and malaria transmission. *Emerging Infectious Disease*, *6*, 103.

Mayo C.W., Brady F.J. (1955). The eighth health assembly. *Public Health Reports* (1896-1970), *70*, 1057. http://doi.org/10.2307/4589280

Naghavi M. (2015). Global, regional and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the global burden of diseases study 2013. *Lancet*, *385*, 117-171.

Okell L.C., Bousema T., Griffin J.T., Ouedraogo A.L., Ghani A.C., & Drakeley C.J. (2012). Factors determining the occurrence of sub-microscopic malaria infections and their relevance for control. *Nature Communications*, *3*, 1237.

Ouédraogo A.L., Bousema T., Schneider P., de Vlas S.J., Ilboudo-Sanogo E., Cuzin-Ouattara N., Nébié I, Roeffen W., Verhave J.P., Luty A.J.F., & Sauerwein R. (2009). Substantial contribution of submicroscopical*Plasmodium falciparum* gametocyte carriage to the infectious reservoir in an area of seasonal transmission. *PLoS One*, *4*, e810. http://doi.org/10.1371/journal.pone.0008410.

Pindolia D.K., Garcia A.J., Huang Z., Smith D.L., Alegana V.A., Noor A.M., Snow R.W., & Tatem A.J. (2013). The demographics of human and malaria movement and migration patterns in East Africa. *Malaria Journal*, *12*, 10.1186. http://doi.org/10. 1186/1475-2875-12-397

Roper C., Elhassan I.M., Hviid L., Giha H., Richardson W., Babiker H., Satti G.M., Theander T.G., & Arnot D.E. (1996). Detection of very low level *Plasmodium falciparum* infections using the nested polymerase chain reaction and the reassessment of the epidemiology of unstable malaria in Sudan. *American Journal of Tropical Medicine and Hygiene*, *54*, 325-331.

Shekalaghe S.A., Bousema J.T., Kunei K.K., Lushino P., Masokoto A., Woleters L.R., Mwakalinga S., Mosha F.W., Sauerwein R.W., & Drakeley C.J. (2007). Submicroscopic*Plasmodium falciparum* gametocyte carriage is common in an area of low and seasonal transmission in Tanzania. *Tropical Medicine International Health*, *12*, 547-553.

Smith D.L., Dushoff J., Snow R.W., & Hay S.J. (2005). The entomological inoculation rate and Plasmodium falciparum infection in African children. *Nature*, *438*, 492-495.

Sturrock H.J.W., Novotny J.M., Kunene S., Dlamini S., Zulu Z., Cohen J.M., Hsiang M.S., Greenhouse B., & Gosling R.D. (2013). Reactive Case Detection for Malaria Elimination: Real-Life Experience from an Ongoing Program in Swaziland. *PLoS One*, *8*(5), e63830. http://doi.org/10.1371/journal.pone.0063830

Suitcliffe C.G., Kobayashi T., Hamapumbu H., Shields T., Kamanga A., Mharakurwa S., Thuma P.E., Glass G., & Moss W.J. (2011). *Malaria Journal*, *10*, 324.

Swaziland Malaria Diagnosis and Treatment Guidelines. (2017). *Ministry of Health* (Swaziland).

Swaziland Ministry of Health and Social Welfare. (2008). *Malaria elimination strategy 2008-2015*. Mbabane.

Tadesse F.G., Pett H., Baidjoe A., Lanke K., Grignard L., Sutherland C., Hall T., Drakeley C., Bousema T., & Hassen M. (2015). Sub-microscopic carriage of *Plasmodium falciparum* and Plasmodium vivax in a low endemic area in Ethiopia where no parasitaemia was detected by microscopy or

rapid diagnostic test. *Malaria Journal*, *14*, 303.

Tatem A.J. (2010). Ranking of elimination feasibility between malaria-endemic countries. *The Lancet*, *376*, 1579-1591.

Wangroongsarb P., Satimai W., Khamsiriwatchara A., Thwing J., Eliades J.M., Kaewkungwal J., & Delacollette C. (2011). Respondent-driven sampling on the Thailand-Cambodia border. II. Knowledge, perception, practice and treatment-seeking behaviour of migrants in malaria endemic zones. *Malaria Journal*, *10*, 10.1186. http://doi.org/10.1186/1475-2875-10-117.

Wessen A.F. (1972). Human ecology and malaria. *American Journal Tropical Medicine and Hygiene*, *21*, 658-662.

World Health Organisation. (2008). Global malaria control and elimination: report of a technical review. *World Health Organization*.

Yawhalaw D., Bortel V.W., Denis I., Coosemans M., Duchateau I., & Speybroeck N. (2010). First evidence of high knockdown resistance frequency in *Anopheles arabiensis* (Diptera: Culicidae) from Ethiopia. *American Journal of Tropical Medicine and Hygiene*, *3*, 122-125.

Yawhalaw D., Wassie F., Steurbaut W., Spanoghe P., Van Bortel W.V., Denis L., Tessema D.A., Getachew Y., Coosemans M., DuchateauL., & Speybroeck N. (2011). Multiple Insecticide Resistance: An impediment to insecticide-based malaria control program. *PLoS One*, *6*(1), e16066.