
**Faculty of Medicine and Health Sciences**

# Towards malaria elimination: Innovative tools and interventions to accelerate interruption of malaria transmission in The Gambia

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I, Edgard Diniba Dabira, declare that this Thesis is my original work. It is being submitted for the degree of Doctor of Medical Sciences at the University of Antwerp, Belgium. It has not been submitted before for any degree or examination at this or any other University, and all the sources used or quoted have been acknowledged by references.

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## Samenvatting

De malaria endemiciteit is in ‘The Gambia’ de afgelopen twee decennia aanzienlijk afgenomen. Ondanks de hoge dekking van standaard controle-interventies is de overdracht van malaria echter niet onderbroken, vooral in Oost-Gambia. Dit noodzaakt ons om innovatieve hulpmiddelen en interventies te exploreren om de situatie te consolideren en de malariaoverdracht verder te verminderen en uiteindelijk eliminatie te bereiken.

In een omgeving met lage transmissie zoals The Gambia, wordt het onderbreken van malariatransmissie bemoeilijkt door het verborgen menselijke infectiereservoir, meestal vertegenwoordigd door sub-patente infecties i.c. infecties die alleen detecteerbaar zijn met moleculaire diagnostische methoden. Dergelijke infecties onderhouden de resterende overdracht. De huidige routine diagnostische middelen d.w.z. microscopie en malaria sneltesten (RDT), zijn niet in staat om dergelijke infecties met een lage parasietdichtheid te detecteren, missen tot 80% van de infecties. Deze infecties moeten worden aangepakt met innovatieve hulpmiddelen en interventies. Dit kan bijvoorbeeld door massale toediening van geneesmiddelen (MDA) aan risicopopulaties met een effectief antimalariamiddel, meestal een op artemisinine gebaseerde combinatiebehandeling (ACT), mogelijks aangevuld met andere geneesmiddelen (ivermectine, primaquine). Ten tweede, het inzetten op het terrein van verbeterde diagnostische hulpmiddelen zoals zeer gevoelige malaria diagnostische tests voor massale screening en behandeling. Bovendien kunnen innovatieve studie ontwerpen zoals studies met gecontroleerde menselijke malaria-infectie (CHMI) de ontwikkeling van effectieve malariavaccins en -behandelingen versnellen.

Dit doctoraatsproject onderzoekt in de Gambiaanse context enkele van de mogelijke interventies om malaria-eliminatie-inspanningen te ondersteunen. Het richt zich op massabehandelingsstrategieën om transmissiereductie te versnellen en op de capaciteiten van zeer gevoelige op antigeen gebaseerde diagnostische tests voor verbeterde surveillance en groepstest en -behandeling. Het onderzoekt ook de aanvaardbaarheid door de lokale gemeenschappen van onderzoek met behulp van CHMI-modellen, omdat deze laatsten de evaluatie van nieuwe behandelingen en vaccins versnellen. Voor de eerste doelstelling van dit doctoraatsproject bepaalden we de impact van massale toediening van dihydroartemisinine-piperaquine en ivermectine op de malariaoverdracht. De interventie werd geëvalueerd door een community-based cluster-gerandomiseerde studie uit te voeren die 32 dorpen omvatte die geloot waren naar de interventie of de controlegroep (n=16 per groep). De interventie verminderde de malariaprevalentie met ongeveer 60% (odds ratio [OR]: 0,30; 95%BI:0,16-0,59; p<0,001) en vectordichtheid met 58% (OR:0,39, 95%BI:0,20- 0,74,

p<0,005); hoewel het geen invloed had op vectorpariteit (OR:0,90; 0,66-1,25; p=0,54), een proxy van muggenoverleving. De meeste bijwerkingen waren van lichte intensiteit en geen van de 11 ernstige bijwerkingen was gerelateerd aan de interventie. Kortom, de interventie was veilig en werd goed verdragen en zou mogelijk een aanvulling kunnen zijn op andere malariabestrijdingsinstrumenten.

Voor het tweede doel hebben we een klinische studie uitgevoerd om de werkzaamheid en veiligheid van pyronaridine-artesunaat (PA) bij verschillende doseringen (volledige of onvolledige behandeling) bij asymptomatische P. falciparum-geïnfecteerde personen te beoordelen. Pyronaridine-artesunaat (PA) is een gestandaardiseerde ACT die kan worden gebruikt voor MDA-campagnes. Ondanks het eenvoudige doseringsschema, één dosis per dag gedurende drie dagen, nemen mensen mogelijk niet de hele behandeling tijdens een MDA-campagne, omdat de meesten van hen gezonde proefpersonen zullen zijn. De parasietdichtheid bij asymptomatische malaria-geïnfecteerde personen is echter meestal laag en een onvolledige behandeling kan voldoende zijn om de infectie te verwijderen. Een totaal van 303 deelnemers werden geïncludeerd en gerandomiseerd naar de 3-daagse, 2-daagse of 1-daagse regimes. Dag 28 PCR-gecorrigeerde adequate parasitologische respons was 100% voor zowel de 3-daagse (98/98) als de 2-daagse regimes (96/96), en 96,8% (89/94) voor het 1-daagse regime. Er was geen verschil in bijwerkingen tussen de drie studiegroepen; De meeste bijwerkingen waren van lichte of matige intensiteit (85% [136/160]). Deze studie suggereert dat PA kan worden gebruikt voor gemeenschapsgerichte malariabestrijdingsinterventies, in combinatie met andere controle activiteiten.

Voor de derde doelstelling werd kwalitatief onderzoek uitgevoerd naar de perceptie en aanvaardbaarheid van de gemeenschappen van de gecontroleerde humane malaria-infectie (CHMI) modellen na de implementatie van de eerste CHMI-studie in Gambia. Belangrijke motivaties voor deelname waren de financiële compensatie, uitgebreide gezondheidscontroles en de bereidheid om malariaonderzoek te ondersteunen. De risico's verbonden aan studiedeelname werden als laag beschouwd. Er werd onder meer bezorgdheid geuit over de frequentie van bloedafnames en het verzamelde bloedvolume. De studie toont een positief beeld over CHMI, wat aangeeft dat dergelijke studies aanvaardbaar zijn voor Gambiaanse gemeenschappen.

Voor het laatste doel werden de terreinprestaties van een zeer gevoelige sneltest (HS-RDT) bij asymptomatische malaria-geïnfecteerde personen met een lage parasietendichtheid beoordeeld. Inderdaad, in een context van malaria-eliminatie is de beschikbaarheid van eenvoudig te gebruiken, goedkope en in het terrein inzetbare tests die asymptomatische

malaria-geïnfecteerde personen kunnen identificeren, essentieel voor massale screening- en behandelingscampagnes. Deze laatste zijn gericht zijn op het verminderen van het menselijke reservoir van infectie. Dergelijke tests zouden ook nuttig zijn bij het opsporen, karakteriseren en monitoren van malariagevallen in de context van malariasurveillance. De HS-RDT-gevoeligheid was laag in vergelijking met qPCR, waardoor de toegevoegde waarde ervan voor malariasurveillance en massale screening en behandeling mogelijks beperkt is.

De bevindingen van dit doctoraatsproject dragen bij aan het groeiende bewijs over de impact van massabehandelingsstrategieën. Deze zouden een sleutelrol spelen in de eliminatie-inspanningen en moeten worden geëvalueerd door het National Malaria Control Programme (NMCP) en snel worden geïntegreerd naast de bestaande interventies. Ze moeten worden ondersteund door diagnostische tests met een betere gevoeligheid dan diegene die momenteel beschikbaar zijn, om te worden gebruikt voor malariasurveillance en voor het volgen van trends in overdracht. Helaas waren de prestaties van de geëvalueerde HS-RDT slecht.

Onderzoek naar aanvullende middelen, waaronder diagnostica, vaccins en geneesmiddelenproducten is lopende. CHMI-studies zijn belangrijk voor de ontwikkeling van dergelijke middelen en worden in toenemende mate uitgevoerd in Afrika ten zuiden van de Sahara. In The Gambia zijn dergelijke studies aanvaardbaar voor lokale gemeenschappen.

## Summary

The malaria burden in The Gambia has decreased substantially over the last two decades. However, despite the high coverage of standard control interventions, malaria transmission has not been interrupted, especially in eastern Gambia, underlining the need for innovative tools and interventions to consolidate gains and further decrease malaria transmission and eventually achieve elimination.

In a low transmission setting such as The Gambia, interrupting malaria transmission is challenged by the hidden human reservoir of infection, mostly represented by sub-patent infections that are detectable only by molecular diagnostic methods. Such infections maintain residual transmission. Current routine diagnostic tools, i.e., microscopy and malaria rapid diagnostic tests (RDT), are unable to detect such low parasite density infections, missing up to 80% of them. These infections should be targeted with innovative tools and interventions, including mass drug administration (MDA) of at-risk populations with an effective antimalarial, usually an artemisinin-based combination treatment (ACT), possibly complemented by other drugs (ivermectin, primaquine); field deployment of improved diagnostic tools such as highly sensitive malaria diagnostic tests for mass screening and treatment. Additionally, innovative tools such as controlled human malaria infection (CHMI) model can accelerate the development of efficacious malaria vaccines and treatments.

This doctoral project explores in the context of The Gambia some of the potential interventions to support malaria elimination efforts. It focuses on mass treatment strategies to accelerate transmission reduction and on the performance of highly sensitive antigen-based diagnostic tests for improved surveillance and mass testing and treatment. It also explores the local communities' acceptance of research using CHMI models as these can be used to evaluate new treatments and vaccines. For the first objective of this doctoral project, we determined the impact of mass drug administration of dihydroartemisinin-piperaquine and ivermectin on malaria transmission. The intervention was evaluated by implementing a community-based cluster-randomized trial that included 32 villages randomized to either the intervention or the control group (n=16 per group). The intervention decreased malaria prevalence by about 60% (odds ratio [OR] 0.30, 95% CI 0.16–0.59; p<0.001) and vector density by 58% (OR: 0.39, 95% CI 0.20- 0.74, p<0.005); although it did not affect vector parity (OR: 0.90, 0.66–1.25; p=0.54), a proxy of mosquito survival. Most adverse events were of mild intensity, and none of the 11 serious adverse events were related to the intervention. In conclusion, the intervention was safe and well-tolerated and could potentially complement other malaria control tools.

For the second objective, we implemented a clinical trial to assess the efficacy and safety of pyronaridine-artesunate (PA) at different dosages (full or incomplete treatment) in asymptomatic *P. falciparum*-infected individuals. Pyronaridine-artesunate (PA) is a fixed-dose ACT that could be used for MDA campaigns. Despite its simple dosing schedule, one dose per day for three days, people may not take the whole treatment during an MDA campaign as most of them would be healthy subjects. Nonetheless parasite density in asymptomatic malaria-infected individuals is usually low and an incomplete treatment may be sufficient to clear the infection. A total of 303 participants were included and randomized to the 3-day, 2-day or 1-day regimens. Day 28 PCR-adjusted Adequate Parasitological Response was 100% for both the 3-day (98/98) and 2-day regimens (96/96), and 96.8% (89/94) for the 1-day regimen. There was no difference in adverse events between the three study groups; most adverse events were of mild or moderate intensity (85% [136/160]). This study suggests that PA could be used for community-based malaria control interventions, in conjunction with other tools.

For the third objective, the field performance of a highly sensitive RDT (HS-RDT) in asymptomatic malaria-infected individuals with low-parasites density was assessed. Indeed, in a malaria elimination context, the availability of easy-to-use, cheap, and field-deployable tests able to identify asymptomatic malaria-infected individuals is essential for mass screening and treatment campaigns aiming at reducing the human reservoir of infection. Such tests would also be useful in detecting, characterizing, and monitoring malaria cases in the context of malaria surveillance. HS-RDT sensitivity was low when compared to qPCR, possibly limiting its use for malaria surveillance and mass screening and treatment.

For the last objective, a qualitative study on the communities' perception and acceptability of controlled human malaria infection (CHMI) models was carried out following the implementation of the first CHMI study in The Gambia. Key motivating factors for participation were the financial compensation, comprehensive health checks, and willingness to support malaria research. Risks associated with participation were considered low. Concerns raised included the frequency of bleeding and the blood volume collected. The study shows a positive view about CHMI, indicating that such studies are acceptable to Gambian communities.

The findings of this doctoral project contribute to the growing evidence on the impact of mass treatment strategies. These would play a key role in the elimination efforts and should be evaluated by the National Malaria Control Programme (NMCP) and rapidly integrated within the existing interventions. They must be supported by diagnostic tests with better sensitivity

than those currently available, to be used for malaria surveillance and for tracking trends in transmission. Unfortunately, the performance of the HS-RDT we evaluated was poor.

Research for additional tools, including diagnostics tools, vaccines candidates and drugs products are ongoing. CHMI studies are important for developing such tools and are increasingly carried out in sub-Saharan Africa. In The Gambia, such studies are acceptable by local communities.

Acronyms and abbreviations

| | |
|---|---|
| ACT | Artemisinin-based combination therapy |
| CHMI | Controlled Human malaria infection |
| CRR | Central River Region |
| DDT | Dichloro-diphenyl trichloroethane |
| DP | Dihydroartemisinin-piperaquine |
| EIR | Entomological inoculation rate |
| GDP | Gross domestic product |
| GTS | Global Technical Strategy |
| IPT | Intermittent preventive treatment during pregnancy |
| IPTsc | Intermittent preventive treatment in school aged children |
| IRS | Indoor Residual Spraying |
| ITN | Insecticide-treated bed nets |
| LLIN | long-lasting insecticide-treated bed net |
| LRR | Lower River Region |
| MDA | Mass drug administration |
| MEG | Malaria Elimination Group |
| MRC at LSHTM | Medical Research Council Unit The Gambia at London School of Hygiene and Tropical Medicine |
| NBER | North Bank East Region |
| NBWR | North Bank West Region |
| NMCP | National Malaria Control Programme |
| PA | Pyronaridine -artesunate |

| | |
|---|---|
| PCR | Polymerase Chain Reaction |
| PDMC | Post- discharge malaria chemoprevention |
| PMC | Perennial malaria chemoprevention |
| PQ | Primaquine |
| RBM | Roll Back Malaria |
| RDT | Rapid diagnostic test |
| SMC | Seasonal Malaria chemoprevention |
| SSA | Sub-Saharan Africa |
| URR | Upper River Region |
| VHW | Village health worker |
| WHO | World Health Organisation |
| WHR1 | West Coast Health Region 1 |
| WHR2 | West Coast Health Region 2 |

# Chapter 1 Introduction

## 1.1 Global burden of malaria

Malaria is an important global public health problem, with nearly half of the world's population at risk of infection and disease (1). From 2000 to 2015, the wide-scale implementation of available malaria interventions, namely insecticide-treated bed nets (ITNs), indoor residual spraying and artemisinin combination therapies (ACTs), has resulted in a substantial decrease of malaria morbidity and mortality. Between 2000 and 2015, malaria incidence declined by 27%, from 80 to 58 cases per 1000 population at risk; malaria deaths decreased from 736,000 to 436,000 (2).

Nevertheless, over the last 6-7 years progress has stalled as the number of cases between 2015 and 2019 decreased by less than 2%. In 2017, WHO reported that the number of malaria cases had levelled off and increased in some countries, mainly in sub-Saharan Africa. In 2021, there were an estimated 247 million malaria cases in 84 malaria endemic countries, an increase of 2 million cases compared with 2020. The estimated number of malaria deaths stood at 619 000 in 2021 compared to 625 000 in 2020 (1). The COVID 19 pandemic exacerbated malaria morbidity and mortality. During the pandemic, in 2020 and 2021, more than 13.4 millions cases and 63,000 malaria deaths were attributed to the service disruptions caused by COVID 19 (1).

Malaria also has a significant impact on the economy of endemic countries as "where malaria prospers most, human societies have prospered least" (3). The economic burden of malaria affects households, health systems, economic development and growth. Between 1965 and 1990, countries with a large proportion of their population living in malaria endemic areas experienced an average growth in per-capita gross domestic product (GDP) of 0.4% per year, whereas average growth in other countries was 2.3% per year (3). The direct and indirect economic costs of malaria are enormous, but the overall economic impact of malaria is likely to be higher than suggested by estimates (3).

## 1.2 Malaria burden and transmission in sub-Saharan Africa

Sub-Saharan Africa (SSA) bears the greatest malaria burden, both in terms of morbidity and mortality. The WHO Africa Region, with an estimated 234 million cases in 2021, accounted for about 95% of all cases globally. In this region, between 2019 and 2020, estimated malaria cases increased from 218 million to 232 million, and deaths from 544 000 to 599 000. Children aged under 5 years were disproportionally affected, with 78.9% of all deaths in this age group (1). Four African countries accounted for nearly half of all malaria cases globally – Nigeria

(26.6%), the Democratic Republic of Congo (12.3%), Uganda (5.1%) and Mozambique (4.1%). Additionally, Burkina Faso accounted for 3.3%, Mali 3.1% and Ghana 2.2% (1).

Four species are responsible for almost all human infections but in SSA *P. falciparum* is the most common species, and responsible for most severe malaria cases and deaths. The long lifespan and strong anthropophilic behavior of the African malaria vectors explain the high malaria burden in SSA. Although it is preventable and treatable, malaria continues to have a devastating impact on people's health and livelihoods.

## 1.3 Malaria control and elimination efforts in The Gambia

### 1.3.1 The setting: The Gambia

The Gambia is located in the Sahel zone of West Africa, with a population estimated at 2.7 million in 2022 (4). It is the smallest country in mainland Africa, with a total area of 11,300 square km, surrounded by Senegal except from its coastline, bordered by Atlantic ocean (4,5). Most of the population lives around the coast, and the life expectancy at birth is 64 years. Almost half of the working population are engaged in agriculture as its primary means for economic activity, generating 20% of GDP. Malaria transmission is seasonal, between July and December, and a few months after the rains.

The Gambia's health care system is organized into a hierarchical three-tier system. At the primary level, healthcare village posts are clustered into circuits and services delivered by village health workers and traditional birth companions. The secondary level includes major and minor health centres, and service deliveries consist of routine preventive and curative care with some surgical and obstetrics practices. Besides the teaching hospital- Edward Francis Small Teaching Hospital in Banjul, the tertiary level is represented by general, specialized and district hospitals.

A few private, commercial, community-funded, non-for-profit clinics are available, but the public health service accounts for more than 80% of healthcare delivery and is heavily subsidized by the Government. Reproductive and child health services, including malaria, are offered free of charge. Table 1 shows the number and type of health facilities in The Gambia.

Table 1: Number and type of health facilities, by region in The Gambia, 2013.

| Facility type | WHR1 | WHR2 | NBWR | NBER | LRR | CRR | URR | Total |
|---|---|---|---|---|---|---|---|---|
| Hospital | 4 | 1 | 0 | 1 | 0 | 1 | 0 | 7 |
| Major health centre | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 6 |
| Minor health centre | 5 | 4 | 4 | 6 | 5 | 7 | 10 | 41 |
| Non-government clinic | 5 | 4 | 2 | 1 | 2 | 0 | 4 | 18 |
| Private clinic | 6 | 9 | 0 | 0 | 1 | 2 | 5 | 23 |
| Community-funded clinic | 7 | 9 | 6 | 5 | 4 | 8 | 1 | 40 |
| Villages Post health | 26 | 92 | 100 | 95 | 92 | 159 | 70 | 634 |
| Total services points | 54 | 120 | 113 | 108 | 105 | 178 | 91 | 769 |

WHR1: West Coast Health Region 1; WHR2: West Coast Health Region 2; NBWR: North Bank West Region; NBER: North Bank East Region, LRR: Lower River Region, CRR: Central River Region, URR: Upper River Region

### 1.3.2 Progress towards elimination

Over the past two decades, the malaria burden has decreased substantially in The Gambia. According to the National Malaria Indicator Survey, the prevalence of malaria infection among the general population decreased from 4% in 2010 to 2% in 2017 and further down to 0.1% in 2017 (6). Clinical malaria incidence has dropped five-fold, from 275 cases per 1000 population in 2010 to 57 cases per 1000 population in 2017. The number of confirmed malaria cases declined by 68%, from 166,232 in 2014 to 53,136 in 2019 (6,7). Such a dramatic change in the burden of malaria has been achieved thanks to the scale-up of malaria control interventions by the National Malaria Control Programme (NMCP). These include core vector control interventions, namely ITNs and indoor residual spraying, which is integrated with malaria case management and chemoprevention (intermittent preventative treatment during pregnancy, and seasonal malaria chemoprevention). The proportion of households with access to at least one ITN was 65% in 2017, while in the general population, 57% of the population slept under an ITN the night before the survey. The proportion of children who slept under an ITN the night before the survey was 83% in 2014 but dropped to 62% in 2017. A similar trend has been observed in pregnant women's utilization of ITNs as it dropped from 84.8% in 2014 to 69% in 2017. In the same period, the proportion of the population in target

areas protected by indoor residual spraying was 97% while the percentage of targeted structures sprayed in the last 12 months was 96% (5).

As a result of this remarkable progress in malaria control, The Gambia is moving towards malaria elimination and set this target for the year 2030. To achieve this, the strategic plan introduces the concept of stratification, based on WHO guidelines modified to suit the local context. Based on the epidemiological profiles, three strata i.e., very low, low, and moderate transmission have been defined for the implementation of the appropriate combination of interventions to effectively address and accelerate the interruption of malaria transmission. Interventions for malaria elimination will be implemented progressively, with high impact interventions adapted to each epidemiological stratum, to achieve zero local cases by 2025.

## 1.4 Objectives and organization of the thesis

The Gambia sets the ambitious goal of eliminating malaria by 2030. However, despite the high coverage of standard control interventions, malaria transmission has not been interrupted, especially in eastern Gambia, underlining the need for innovative tools and interventions to consolidate gains and accelerate interruption of transmission and ultimately achieve subnational and national elimination.

The aim of this doctoral research is to evaluate innovative tools and interventions to support malaria elimination efforts in The Gambia and beyond. Specific objectives are as follows:

1. Determine the impact of mass drug administration of ivermectin and dihydroartemisinin-piperaquine on malaria transmission in Gambian communities with high coverage of vector control interventions;
2. Determine the efficacy and safety of three different treatment regimens of pyronaridine – artesunate in malaria-infected asymptomatic individuals;
3. Determine the diagnostic accuracy of a highly sensitive Rapid Diagnostic Test: Alere™ Malaria Ag *P.f* in malaria-infected asymptomatic individuals with low parasite density and
4. Assess the perception and acceptability of Controlled Human Malaria Infection (CHMI) studies in Gambian communities.

The thesis of the doctoral research is organized in seven chapters.

**Chapter 1** outlines the current burden of malaria globally and its transmission in sub-Saharan Africa and provides an overview of malaria control and elimination activities in The Gambia.

**Chapter 2** is a review of the literature focusing on the historical perspective of malaria elimination and eradication, antimalarial medicines and vaccines targets, existing and novel tools and interventions for malaria elimination.

**Chapter 3**

**Chapter 3** presents the results of a cluster-randomised trial evaluating the impact of three monthly rounds of mass drug administration of ivermectin and dihydroartemisinin-piperaquine on malaria prevalence and vectors' parous rate in a region with high coverage of vector control interventions

**Chapter 4** reports the results of an individually randomized trial on the safety and efficacy of three different treatment regimens of pyronaridine – artesunate in malaria-infected asymptomatic individuals.

**Chapter 5** reports the results of a cross-sectional survey to determine the field performance of the highly sensitive RDT in detecting asymptomatic malaria infections.

**Chapter 6** presents the results of a qualitative study assessing the acceptability and perception of controlled human malaria infection studies in Gambians' communities.

**Chapter 7** is a general discussion on how the research results fit within the context of malaria elimination. It focusses on mass treatment strategies and on the performance of highly sensitive diagnostic tests and the community acceptability of CHMI and perspectives for malaria elimination in The Gambia.

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# Chapter 2 Tools and interventions for malaria control and elimination

## 2.1 Historical perspectives

Malaria elimination is defined as the interruption of local transmission (reduction to zero incidence of indigenous cases) of a specified malaria parasite species in a defined geographical area as a result of deliberate activities and malaria eradication is the permanent reduction to zero of the incidence of infection caused by all human malaria parasite species worldwide as a result of deliberate activities. Interventions are no longer required once eradication has been achieved (1).

Efforts to control malaria date back to the late 19th century. However, during the first half of the 20th century, little progress was made, partly due to the disruption caused by World Wars I and II. At the end of World War II, the development of new tools, including the insecticide dichloro-diphenyl trichloroethane (DDT), and antimalarial drugs such as chloroquine, amodiaquine, proguanil, improved the prospect of malaria control, with some countries such as Italy and Greece achieving malaria elimination (2,3), which stimulated the hopes for a malaria-free world. In May 1955, the WHO launched the Global Malaria Eradication Programme (GMEP) with the following statement "*The World Health Organization should take the initiative, provide technical advice, and encourage research and coordination of resources in the implementation of a program having as its ultimate objective the worldwide eradication of malaria*"(4).

Following mixed successes and failures, the programme was interrupted in July 1969. Although several reasons for failing to eradicate malaria were identified, three critical elements emerged. These were the insufficient recognition of the heterogeneity of malaria transmission, partly due to early successful elimination campaigns, the universal approach that assumed a single strategy would work everywhere —''one size fits all''— and the insufficient research and inadequate local application of research findings. The eradication programme shifted to a short-term strategic plan aiming at controlling malaria and limiting as much as possible malaria cases and deaths. However, in the 1970s and 1980s, the malaria burden increased considerably worldwide following the emergence and spread of resistance to insecticides (DDT) and antimalarial treatments (chloroquine), and the lack of financial investments.

The adoption of the Global Malaria Control Strategy in 1992 marked a new global focus on malaria control (5). In 1998, the WHO launched the Roll Back Malaria (RBM) partnership as an initiative for improving control interventions and increasing financial investment in malaria control. In 2000, the Abuja Declaration defined the progressive intervention coverage targets. Since 2003, the Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM), the US

President’s Malaria Initiative (PMI) and several organisations have been supporting the scale-up of malaria control interventions, including long-lasting insecticide-treated bed nets (LLINs), indoor residual spraying (IRS), the use of malaria rapid diagnostic tests (RDTs), and the provision of efficacious artemisinin-based combination treatments (ACT). In October 2007, The Bill and Melinda Gates Foundation called for malaria eradication which was rapidly endorsed by the WHO and RBM Partnership (6,7). Several meetings were held by international organisations, including the WHO, RBM and the established Malaria Elimination Group (MEG), to evaluate the implication of this change in strategy. As a result of the scale up of control interventions, a remarkable decrease of malaria burden was observed worldwide, renewing the interest for national and regional elimination, and global eradication as the ultimate goal. In 2015, the WHO adopted the Global Technical Strategy (GTS) for 2016 -2030 which sets milestones and goals for malaria elimination with a vision of “A world free of Malaria”(1,5,8).

## 2.2 Medicine and vaccine targets

Malaria control and elimination tools and interventions target the parasite life cycle either in the human host or the vector. The parasite life cycle is broadly divided into an asexual stage in humans and sexual stages in the vector.

The asexual life cycle begins when sporozoites are injected during blood meal by an anopheline mosquito into the host’s body. Sporozoites are carried to the liver by the circulatory system where they invade hepatocytes, multiply and produce merozoites in a process called exo-erythrocytic schizogony. Mature merozoites exit liver cells and invade red blood cells where they undergo mitotic division forming new merozoites. When these merozoites mature they are released into the blood stream following the rupture of the infected red blood cell, and invade other red blood cells. This cycle of invasion, replication and release is called erythrocytic schizogony and is linked to clinical symptoms. During the erythrocytic schizogony, some merozoites differentiate into male or female sexual forms, gametocytes, which can be ingested by mosquitoes during a blood meal. In the mosquito gut, male mosquitoes rapidly undergo a process of exflagellation to produce sexually competent gametes that fuse with the female gamete to form an ookinete. The ookinete passes through the wall of the mid-gut, developing into an oocyst that ruptures, releasing sporozoites that migrate to the salivary gland, ready to be injected into humans during a blood meal (Figure 1).

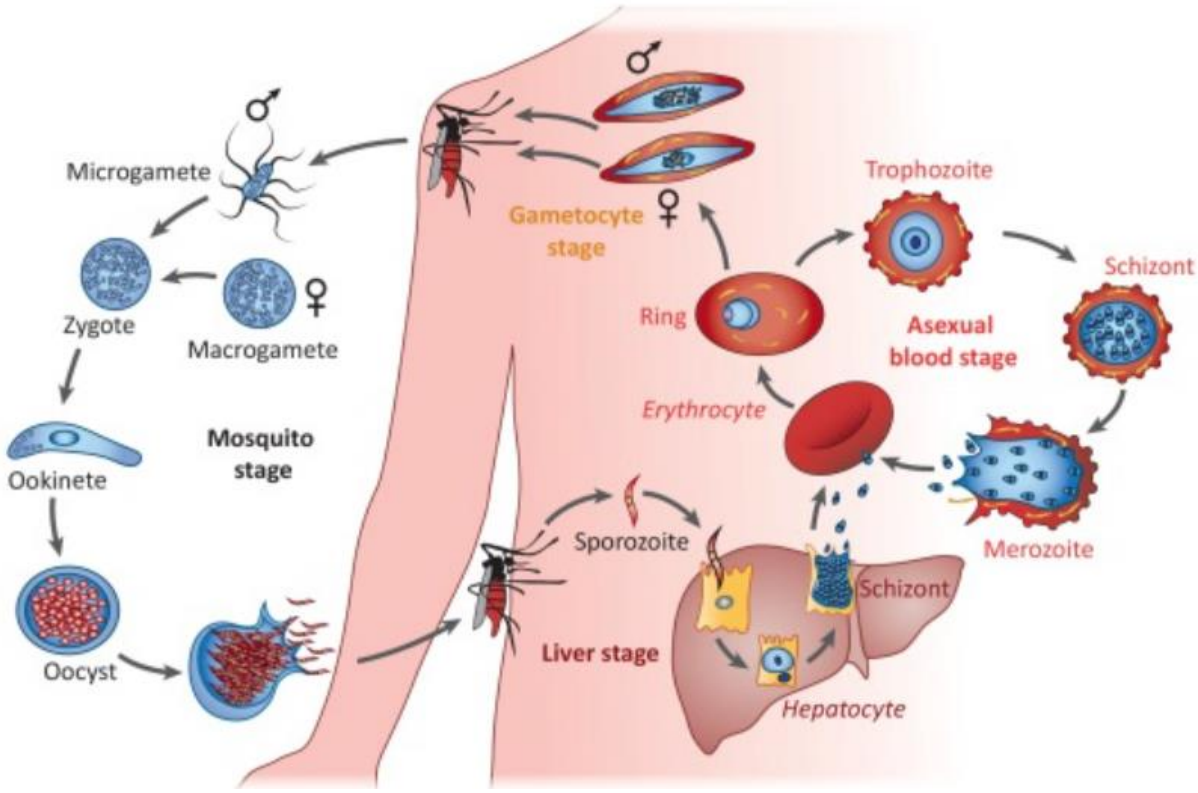


Figure 1: Life cycle of the malaria parasite (source: Maier, Alexander G. et al. "*Plasmodium Falciparum*." Trends in parasitology 35.6 (2019): 481–482. )(9).

Antimalarial drugs such as quinolines and ACTs target the asexual phase of the parasite development while primaquine and tafenoquine target the sexual stages (10). Artemisinin is a potent and fast-acting blood schizonticidal. Primaquine, besides being gametocytocidal, is effective against the hypnozoites of *Plasmodium vivax* and *Plasmodium ovale* (10).

Based on the target, three types of vaccines are under clinical evaluations. The pre-erythrocytic vaccines, including RTS,S/AS01, R21/Matrix M, and the falciparum sporozoite vaccine (PfSPZ) target the sporozoites and/or hepatic stages (11). Vaccines against blood-stage parasites such as AMA1 and EBA-175 target the asexual stage (mostly merozoites) of the parasite to clear parasitaemia and prevent clinical disease. The transmission-blocking vaccines target surface proteins expressed on gametocytes, zygotes, and ookinetes to prevent infection of mosquitoes and interrupt malaria transmission (2,11). Some of transmission-blocking vaccines include the gametocyte antigens (Pfs48/45 and Pfs230) and the falciparum ookinete surface antigens (Pfs25 and Pfs28) (11).

## 2.3 Existing tools for malaria control and elimination

Progress towards malaria elimination requires optimal coverage and uptake of malaria interventions. Mathematical models can be used to inform targeted interventions for malaria control and elimination. Interventions include early diagnostic and prompt treatment of malaria cases with ACTs, chemoprevention-based interventions (intermittent preventive treatment for pregnant women, seasonal malaria chemoprevention, intermittent preventive treatment in school aged children), interventions that reduce human–vector contact, such as indoor residual spraying (IRS) or insecticide-treated bed nets (ITNs), and a robust surveillance system.

### 2.3.1 Mathematical modelling for malaria control and elimination

Mathematical models for malaria control are derived from the basic Ross-MacDonald' model which is compartmentalized into susceptible (S), exposed (E), infected (I) and recovered (R) populations. The key assumptions of the model are that: i) the intensity of transmission is related to the number of infectious bites that would arise from all the mosquitoes that bite a fully infectious human in one day (vectorial capacity); ii) the number of human infections that occurs is proportional to the number of infectious bites. These assumptions are presented as the basic reproductive number (Ro). The Ro defines the expected number of secondary cases produced in a completely susceptible population, by a typical infective individual, and is expressed mathematically as: Ro = $\frac{ma^2p^n}{r-(lnp)}$

where " m " is the ratio of female mosquitoes to humans, " a " the biting rate (number of bites on a human/mosquito/day), " p " the proportion of anopheline surviving 1 day, " n " the duration of sporogony in days, and " r " the recovery rate (proportion of infected people who revert to the uninfected state in one day). Consequently, the value of Ro can predict if the disease will persist or will be interrupted. When Ro <1 the number of infected individuals decreases over time until the disease is interrupted. Conversely, when Ro >1, the number of infected cases increases, and Ro = 0 corresponds to a state with zero infected individual. These parameters can be modified by specific interventions, resulting in changes in the intensity of transmission (12,13).

### 2.3.2 Malaria diagnosis

The WHO recommends microscopic examination and rapid diagnostic tests (RDT) as primary diagnostic tools for confirmation and management of suspected malaria cases in all transmission settings, including areas of low transmission (1). RDTs are immuno-chromatographic tests for detecting parasite-specific antigens in a finger-prick blood sample. They are field deployable tests, easy to use by trained community health workers, and allow

detection of parasite antigens; some tests differentiate parasite species (14–16). Microscopic examination is the “gold standard” for the laboratory confirmation of malaria. It requires well-trained staff and laboratory support, and allows direct visualization of parasites, determination of species, and stages, and quantification of the parasite density. Both RDTs and microscopy examinations are excellent for managing patients with clinical malaria but unable to detect asymptomatic infection with low parasite density or the dormant liver stages of *P*. vivax and *P. ovale* (1). Routine RDTs detect parasite density in the order of 100- 200 parasites/µl, which is similar to the sensitivity of routine microscopic examination (17,18).

Serology and molecular-based techniques are other diagnostic tools. The serology test is based on the detection of antibodies against malarial parasites, using either indirect immunofluorescence (IFA) or enzyme-linked immunosorbent assay (ELISA). Serology does not detect current infection but rather measures past exposure. Serology tests are useful for screening asymptomatic individuals and identifying foci of recent infection but are inappropriate for case management.

The two molecular techniques used for identifying malaria parasites are polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP). Both techniques can identify low parasite densities. Molecular techniques are not recommended for routine use but are useful research tools in epidemiological studies, clinical trials, and for the detection of molecular markers of drug resistance.

### 2.3.3 Malaria medicines

Malaria medicines are used for case management, chemoprevention, and mass drug strategies (1,15). They can be grouped into four classes based on the molecular structure and biological activity: (1) quinoline-based antimalarials include the 4-aminoquinolines (chloroquine, amodiaquine and piperaquine) and 8-aminoquinolines (primaquine and tafenoquine); (2) arylaminoalcohols include quinine, mefloquine, halofantrine, and lumefantrine; (3) antifolate compounds comprising pyrimethamine, proguanil, dapsone, and sulfadoxine; (4) artemisinin and its derivatives, the first generation (dihdyroartemisinin, artesunate, arteether, and artemether) and second generation (artemisone) (19,20).

### 2.3.4 Curative chemotherapies: case management

Malaria case management consists of early detection and prompt treatment with an effective antimalarial drug. The WHO recommends the treatment of adults and children with uncomplicated *P. falciparum* malaria (including infants, pregnant women in their second and third trimesters and breastfeeding women) with an ACT (15). In 2022, the recommendation was updated to include pregnant women in their first trimester. Available ACTs are artemether-

lumefantrine, artesunate-amodiaquine, artesunate-mefloquine, dihydroartemisinin-piperaquine, artesunate-sulfadoxine–pyrimethamine (SP), artesunate-pyronaridine (15). In areas where non-falciparum parasites are resistant to chloroquine, the WHO recommends ACTs also for *P. vivax, P. ovale, P. malariae* and *P. knowlesi*. Atovaquone-proguanil may be considered for the treatment of uncomplicated malaria in travellers outside malaria-endemic areas.

Intravenous and intramuscular artesunate are currently the most effective and well-tolerated treatments for severe malaria, with rectal artesunate recommended for pre-referral treatment of children who cannot quickly access hospital care (15).

ACTs are generally well tolerated and highly effective. They combine a rapidly acting artemisinin derivative with a longer-acting (more slowly eliminated) partner drug. The artemisinin component rapidly clears parasites from the blood (reducing parasite density) while the longer-acting partner drug clears the remaining parasites and provides chemoprotection for a variable length of time, depending on the drug pharmacokinetic profile.

### 2.3.5 Preventive chemotherapies

Chemoprevention consists in the use of antimalarial medicines to prevent malaria infection and disease. Chemoprevention uses full therapeutic courses of antimalarial medicines at prescheduled times, irrespective of infection status, to treat existing infections and prevent new infections (15). Current WHO recommendations for chemoprevention include the intermittent preventive treatment of malaria in pregnancy (IPTp), perennial malaria chemoprevention (PMC), previously known as intermittent preventive treatment in infants (IPTi), seasonal malaria chemoprevention (SMC), intermittent preventive treatment in school aged children (IPTsc), post- discharge malaria chemoprevention (PDMC), mass drug administration (MDA) to reduce the transmission and burden of malaria, and mass relapse prevention (15). IPTp consists of the administration of sulfadoxine-pyrimethamine to pregnant women at antenatal care visits, regardless of their infection status. PMC is the administration to infants of a full treatment course of sulfadoxine-pyrimethamine during routine immunization. SMC is recommended in areas of seasonal malaria transmission. It consists of administering sulfadoxine-pyrimethamine and amodiaquine to children 3-59 months during the malaria transmission season, deployed in 3 or 4 monthly rounds per season. IPTsc is the administration of a full treatment course of an antimalarial at regular intervals to treat and prevent malaria infections in children who are old enough to attend school. PDMC is the administration of a full antimalarial treatment course at regular intervals to children admitted with severe anaemia.

MDA for malaria is the administration of a full therapeutic course of an antimalarial treatment to the whole population in a defined geographical area, regardless of their malaria infection status. MDA rapidly reduces the prevalence and incidence of malaria but its impact is short-lived (21–23). It is recommended for areas approaching elimination, malaria epidemics and complex emergencies (15,23). Because of its simple dosing schedule, longer half-live and efficacy, dihydroartemisinin-piperaquine is generally chosen for MDA (22).

## 2.4 Malaria vaccines

In October 2021, the WHO recommended the first ever malaria vaccine, RTS,S/AS01 also know by the brand name Mosquirix for use in children living in regions with moderate to high transmission. RTS,S/AS01 is a pre-erythrocytic recombinant protein vaccine, based on the RTS,S recombinant antigen. It comprises the hybrid polypeptide RTS, in which regions of the *P. falciparum* circumsporozoite protein known to induce humoral (R region) and cellular (T region) immune responses are covalently bound to the hepatitis B virus surface antigen (S). The vaccine is currently produced as a two-dose RTS,S powder to be reconstituted with a two-dose AS01 adjuvant system suspension. WHO recommends that the first dose of vaccine is administered from 5 months of age with a minimum interval of four weeks between doses. The vaccine should be administered in a three-dose primary schedule, with a fourth dose provided 12–18 months after the third dose to prolong the duration of protection. RTS, S/AS01 has been piloted in Ghana, Kenya and Malawi and delivered to children through routine immunization services (24). The vaccine is a potential major boost for malaria control and elimination. Nonetheless, malaria vaccines are currently envisaged as a complementary intervention that should not replace the package of existing interventions (15).

## 2.5 Vector control

Vector control interventions significantly reduce malaria transmission. The WHO currently recommends two core vector control strategies, insecticide-treated nets (ITNs) and indoor residual spraying (IRS) for populations at risk for malaria (15). These strategies consist of the use of mosquito-killing insecticide either on the bed nets (ITNs) or sprayed on the wall of sleeping rooms (IRS). Additionally, bed nets act as a physical barrier against mosquitoes that reduce the contact between vectors and humans. ITNs and IRS are core interventions for reducing the human biting rate and vector survival, which significantly reduce vectorial capacity and transmission (1,5). While high coverage and use of these interventions are essential to ensure maximal effectiveness, their impact is temporary and depends on their maintenance. Premature removal of these interventions is likely to result in a rebound of malaria transmission to pre-existing levels (25,26). High coverage of ITNs can be achieved and maintained most rapidly by a combination of mass free-distribution campaigns and

continuous distribution channels including antenatal, child health and vaccination services; schools, places of worship and community networks; and the private or commercial health sector. Larval source management should be implemented on the principle of integrated vector management to supplement ITNs and/or IRS.

## 2.6 Surveillance and response

As malaria transmission declines, cases become clustered in at-risk populations (“hot pops”) or locations (“hot spots”). Elimination efforts require a robust surveillance system to identify where transmission occurs for targeted and effective responses. For elimination efforts, surveillance and response should become a core intervention, focusing on case characterization, treatment and investigation (1,27).

Reporting and case investigations are key components of surveillance. Cases can be identified by passive case detection (PCD), at the time patients attend health facilities; and by active case detection (ACD), which requires extension of testing to high-risk, vulnerable groups, hard-to-reach populations or low-transmission settings; and reactive case detection (RCD), which involves an active response to a case detected by either PCD or ACD (1). RCD is a targeted intervention conducted in response to a local or imported case, with the assumption that in a low transmission setting, malaria cases are clustered.

Current tools and interventions can accelerate interruption of transmission and achieve a greater gain, including elimination in some areas. However, new tools and interventions are needed to achieve malaria elimination and eventually eradication (2,11,24,28).

## 2.7 Innovative tools and technologies for malaria elimination

New tools are required to address major challenges for malaria elimination. These challenges are the emergence and spread of resistance to insecticides and antimalarial drugs, the low sensitivity of routine diagnostic tests to detect the low-density asymptomatic infections, residual malaria transmission (outdoor biting mosquitoes), and the hard-to-reach populations. Several promising leading diagnostics, drugs, vaccines and vector control tools and technologies are under development.

### 2.7.1 Malaria diagnostics tests

In an elimination setting, detection of all infections, including foci of asymptomatic infected individuals with low-parasite density for targeted interventions and assessment of progress, are essential to accelerate interruption of local transmission and avoid resurgence. Current diagnostic methods (RDTs and microscopy) are generally adequate for routine case

management. However, elimination efforts require improved and field deployable RDTs to increase diagnostic accuracy and sensitivity, and strengthen active surveillance.

A highly sensitive diagnostic test was launched in 2017. This point-of-care, *Plasmodium falciparum* histidine-rich protein 2 (HRP2–based) RDT (Alere™/Abbott Malaria Ag P.f RDT) with a ten-fold improved analytical sensitivity as compared to average routine RDTs was prequalified by the WHO (29). The test may improve the detection of infected individuals with low-density parasites and strengthen surveillance, mass screening and treatment strategies. However, parasite deletion of the genes pfhrp2 and pfhrp3 has been implicated in false-negative results using HRP2-based RDTs. HRP2 deletions have been reported form countries in sub-Saharan Africa including Nigeria, Sudan, and South Sudan. Given the increasing frequency of PfHRP-2 and PfHRP-3 gene deletion, the next generation of highly sensitive diagnostic tests should address this issue (2). The diagnostic research pipeline includes point-of-care testing, hemozoin detection; spectroscopic approaches and nucleic acid amplifications techniques (30) (Figure 2).

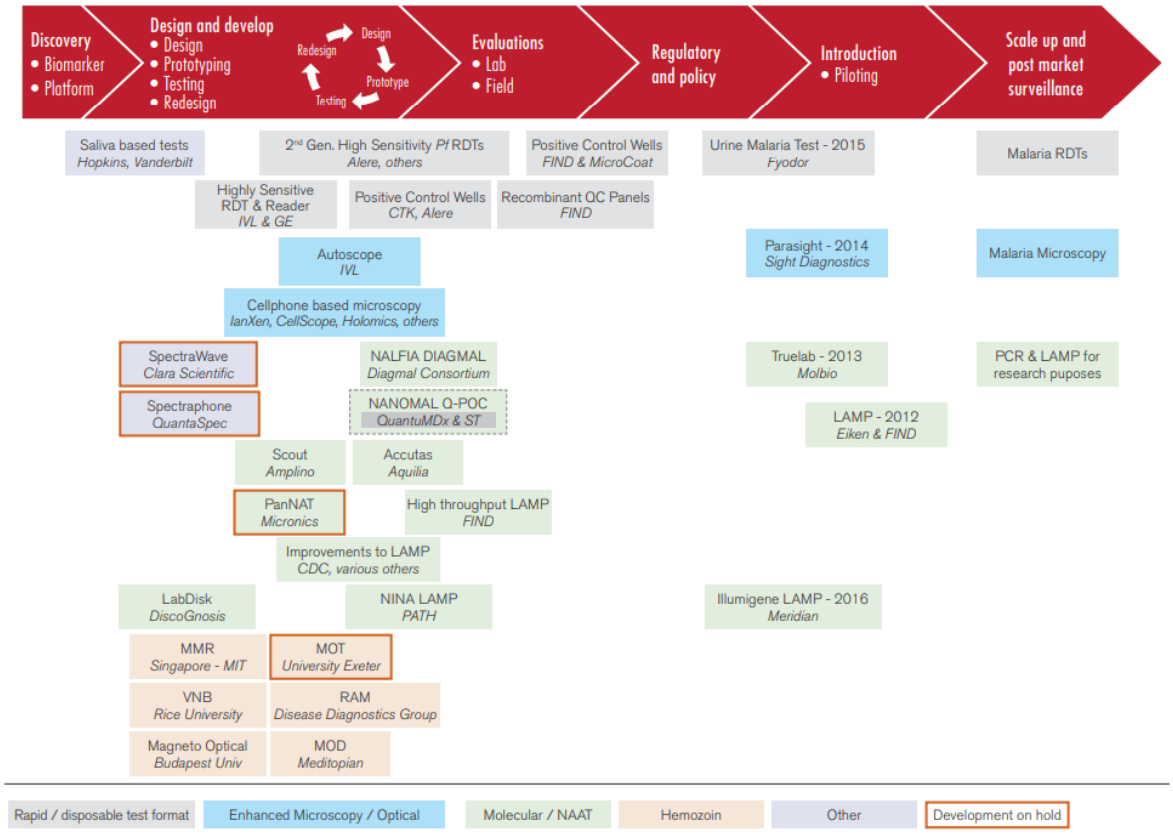


Figure 2: Overview of malaria diagnostics pipeline (source: WHO malaria diagnostics technology and market landscape, 2016).

### 2.7.2 Antimalarial treatment

Malaria elimination and worldwide eradication will require new medicines active against emerging drug resistant parasites, all parasite lifecycle stages, including hypnozoites, and a simplified regimen, easily deployable at the population level (2,11). The ideal drug for malaria, i.e., the target product profile is a single exposure radical cure and prophylaxis, where a single tablet could target all lifecycle stages of all human malaria parasites (1,2,11). The target product profile is unlikely to be available soon. Nonetheless, the research and development pipeline includes next generation of combination therapies (OZ439/PQP, triple ACTs, azithromycin-chloroquine), endectocides (ivermectin), and monoclonal antibodies.

Combination therapies with ozonide (OZ439) are at the final stage of clinical evaluations and are potentially useful substitutes for ACTs. Tafenoquine, a primaquine-analog that can be given in a single dose is active against gametocytes and hypnozoites. It is expected to play a pivotal role in the regional elimination of *P vivax* malaria from Asia-Pacific and the Americas (2,10). Ivermectin, an antiparasitic and endectocide, toxic to mosquitoes that feed on treated humans and animals is a potential complementary tools to target residual transmission (31–33). Ivermectin is currently under field trial evaluations (31,34,35). Majority of monoclonal antibody candidates target sporozoite stage antigens, particularly the CSP antigen. As of 2022, three malaria monoclonal antibodies candidates are being tested in clinical trials. These include two CSP-targeting antibodies (CIS43LS and L9LS) and one antibody (TB31F) targeting the gametocyte surface protein Pfs48/45 to block human-to-mosquito transmission. Monoclonal antibodies are at the early stage of development but have the potential to significantly reduce transmission in highly endemic areas in Africa (2,11,36). Antimalarial medicines are at different phases of development (37) (Table 1).

Table 1: Current antimalarial medicines clinical trials pipeline (adapted from Belete, Tafere Mulaw. “Recent Progress in the Development of New Antimalarial Drugs with Novel Targets.” Drug design, development and therapy 14 (2020): 3875–3889. Web).

| **Drug name** | **Developers** | **Trial phase** |
|---|---|---|
| KAE609 (cipargamin) | Novartis | 2a |
| M5717 (DDD498) | Merck, KGaA, Darmstadt | 1 |
| Albitiazolium (SAR9727) | CNRS/University of Montpellier/Sanofi 1 | 2 |
| SJ733 | St Jude/Eisai (Rutgers) | 1 |
| KAF156(Ganaplacide)/ lumefantrine | Novartis | 2b |
| DSM265 | Takeda (Univ.of Texas Southwestern University | 2a |
| Methylene Blue | University of Heidelberg | 2 |
| Sevuparin (DF02) | Dilaforette- Karolinska Institute | 2 |
| P218 | Medicines for Malaria Venture | 1 |
| MMV048 | University of Cape Town | 2a |
| MMV390048 | University of Cape Town | 2a |
| Artefenomel (oz439) + Piperaquine | Medicines for Malaria Venture | 3 |
| AQ 13 | Tulane University and University of Bamako | 2 |
| Fosmidomycin + piperaquine | Medicines for Malaria Venture and Jomaa Pharma GmbH | 2a 2b |

### 2.7.3 Malaria vaccines

Malaria vaccine development has been a long, expensive, and challenging journey. Nonetheless, malaria vaccine research continues to be a top priority as an efficacious vaccine against malaria may be an important endgame tool to be added to the arsenal of antimalarial interventions. Several vaccine candidates, to prevent *P. falciparum and P. vivax* infections and with different modes of action, are at various stages of development.

Two vaccine candidates are approaching late-stage clinical evaluation: the R21/Matrix M vaccine candidate targeting Pf CSP protein and the attenuated whole sporozoite vaccine Pf SPZ (38,39). Field trial evaluations of R21/Matrix M showed high-level efficacy. A phase 2 trial of R21/Matrix M conducted in Burkina Faso was safe, very immunogenic and reduced clinical malaria in children aged 5-17 months by 77%, achieving the WHO-specified goal of 75% or greater efficacy. As a result, WHO recently recommended the use of R21/Matrix M for prevention of malaria in children. Additional candidates targeting other malaria life-cycle stages include the Rh5 blood-stage vaccine candidate and Pfs25 and Pfs230 vaccine candidates targeting sexual-stage antigens to prevent human-to-mosquito transmission. New technologies, such as DNA and mRNA-based vaccines, the ongoing development of adjuvants, and delivery platforms such as virus-like particles (VLPs; the delivery platform used for RTS,S/AS01) and vesicle-based technologies are being explored for use in malaria vaccines (40,41). Malaria vaccine development includes various targets and is at different stages of evaluation (42).

Table 2: Malaria vaccine in clinical evaluations (adapted from Global malaria vaccines pipeline, 2022)

| **Vaccine name** | **Vaccine target** | **Vaccine target by antigens** | **Trial phase** |
|---|---|---|---|
| Ad35.CS.01 | Pre-erythrocytic stage | Pf CSP | Phase I |
| Ad35.CS.01 | Pre-erythrocytic stage | Pf CSP | Phase I |
| BK-SE36 | Blood stage | PfSERA5 | Phase I |
| BK-SE36/Alhydrogel | Blood stage | PfSERA5 | Phase I |
| ChAd63-MVA Pfs25-IMX313 | Sexual stage | PfAMA1 | Phase I |
| ChAd63-MVA PvDBP | Blood stage | PfAMA1 | Phase I |
| ChAd63-MVA PvDBP | Blood stage | PfAMA1 | Phase II |
| FMP014/ALFQ | Pre-erythrocytic stage | Pf CSP | Phase I |
| GMZ2 | Blood stage | PfGLURP | Phase II |
| PfSPZ Vaccine | Pre-erythrocytic stage | Whole sporozoite | Phase I/II |
| RTS,S/AS01E | Pre-erythrocytic stage | Pf CSP | Phase IV |
| VCL2510 | Pre-erythrocytic stage | Pf CSP | Phase I/II |
| VLPM01 | Pre-erythrocytic stage | Pf CSP | Phase I |
| VMP001/AS01B | Pre-erythrocytic stage | | Phase I/II |

### 2.7.4 Vector control insecticides

Current research and development are focused on new insecticide ingredients and tools to address insecticide-resistance and outdoor biting as well as developing longer lasting insecticides (1,2).

Clothianidin and chlorfenapyr insecticides with novel mode of action are available for IRS. Dual-ingredient LLIN, pyrethroids and piperonyl butoxide are promising leads. Innovative strategies to delay emergence of resistance to insecticide include rotation or combination of insecticides.

Longer-lasting insecticides would potentially reduce the need for ITNs replacement and the frequency of IRS, contributing to a substantial cost savings as these interventions account for more 50% of malaria programme costs (11). Currently available, pirimiphos-methyl (Actellic 300CS), a slow release chemical, is a reformulated organophosphate that doubles the longevity of the effective treatment period (43,44). Actellic 300CS is becoming a major innovative tool for IRS (11).

Endectocides drugs such as ivermectin are innovative vector control tools targeting both indoor and outdoor biting mosquitoes while Attractive Sugar Targeted Baits (ATSB) is also an innovative intervention targeting outdoors biting mosquitoes. ATSB, which consists of luring mosquitoes to a toxic bait and killing them (45,46), is currently evaluated in field trials (35,45,46). These innovative tools could address malaria residual transmission.

Gene drive technologies have progressed rapidly over the past five years and are promising vector control tools. These consist of genetically modified genes that confer a specific trait to offspring which becomes increasingly common within a specific species (2,47). Gene drive mosquito releases can either aim to reduce (population suppression) or to modify (population replacement) the vector population (2,47). Vector control pipeline includes new generation of ITNs, attractive targeted baits, gene drive mosquito, indoor residual wall treatments (48).

Table 3: Vector control intervention in the pipeline, 2020 (adapted from WHO, Global Observatory on Health R&D, Nov 2022)

| Intervention | Application | Research and Development stage |
|---|---|---|
| Aquastrike | Larvicide | WHO assessment |
| ATSB®, mosquitoes' bait station | Attractive targeted baits | Data generation |
| DuraNet Plus | Insecticide-treated nets (ITN) | WHO assessment |
| Friendly Mosquitoes | Genetic-Engineering- Self limiting male mosquitoes | Data generation |
| Imergard WP | Indoor Residual Wall treatments | WHO assessment |
| In2Care®EaveTube | House modification | Field evaluations |
| Interceptor G2 | Insecticide-treated nets (ITN) | Field evaluations |
| Ivermectin repurposed for malaria | Systemic insecticide and endectocide | Field evaluations |
| Kansai Anti-Mosquito Paint and Inesfly Insecticidal Paint | Indoor Residual Wall treatments | Data generation |
| MkitoNet | Insecticide-treated nets (ITN) | WHO assessment |
| Perimeter Eto Insect Guard | Personal protection | Field evaluations |
| Pirikool 200 CS-PE | Indoor Residual Wall treatments | Data generation |
| Pirikool 300 CS | Indoor Residual Wall treatments | Data generation |

| | | |
|---|---|---|
| Population alteration /reduction – gene-drive approach | Genetic manipulation of vectors-population replacement or modification | Data generation |
| Push pull strategy (devices to lure and repel) | Peridomestic combined repeland lure device | Field evaluations |
| Reliefnet | Insecticide-treated nets (ITN) | Data generation |
| Royal Guard | Insecticide-treated nets (ITN) | Field evaluations |
| Sylando 240SC | Indoor Residual Wall treatments | WHO assessment |
| Transfluthrin passive emanator | Spatial Repellent | clinical trials |
| Tsara | Insecticide-treated nets (ITN) | WHO assessment |
| VitalNet | Insecticide-treated nets (ITN) | Data generation |
| Yorkool | Insecticide-treated nets (ITN) | Data generation |
| Zero vector durable lining | Indoor Residual Wall treatments | Data generation |

## 2.8 Supporting tools and technologies for malaria elimination

### 2.8.1 Information technology

Information technologies when strategically applied are powerful tools for malaria surveillance, microplanning, prevention, diagnosis, and management (2,49), which are essential requirements for elimination efforts. Information technology, including smartphone and software applications, powerful computers, satellite images can provide useful information on people location, movement and interactions. These technologies can enable health workers to access and interact with data, improve community participation and timely reporting.

The GIS-based spatial decision support system (SDSS) is a web-based technology that allows to automatically locate and map the distribution of confirmed malaria cases, rapidly classify active transmission foci, and guide targeted responses in elimination zones. The Mobile-based Surveillance Quest using IT (MoSQuIT) is being used to automate and streamline malaria

surveillance for all stakeholders involved, from health workers to medical officers and public health decision-makers (49).

### 2.8.2 Mathematical modelling

Mathematical modelling aims at describing, explaining, or predicting behaviors or phenomena in the real world (50). Malaria mathematical modelling is an important tool to guide interventions deployment and estimate cost-effectiveness, optimize resource allocation and inform policy decision-making. It provides an insight of the expected impact of various interventions against malaria, either individually or in combination. Mathematical modelling can build on available data, test multiple scenarios, and make predictions on the expected outcomes of various interventions (12,50,51). Several mathematical models exist, from the simple compartmental (SIR) to more complex models with several parameters such as antimalarial drugs and coverage, transmission intensity, the variability in vector species, composition and associated bionomic (51). Malaria mathematical modelling would support elimination efforts as a tool for strategic planning and decision making.

### 2.8.3 Controlled human malaria infection (CHMI): a model for the development of new tools

Controlled human malaria infection (CHMI) studies consist of deliberately infecting healthy volunteers with malaria parasites, to either study their immune response or assess the efficacy of vaccines or treatments (52,53). These well-controlled proof of concept studies allow to rapidly screen for potential vaccine and drug candidates. Therefore, CHMI studies are valuable tools to accelerate the global antimalarial drug and vaccine development portfolio and support elimination efforts (54).

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# Chapter 3 Mass drug administration of ivermectin and dihydroartemisinin-piperaquine against malaria in settings with high coverage of standard control interventions: a cluster-randomised controlled trial in The Gambia

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## 3.1 Summary

**Background** Although the malaria burden has substantially decreased in sub-Saharan Africa, progress has stalled. We assessed whether mass administration of ivermectin (a mosquitocidal drug) and dihydroartemisinin–piperaquine (an antimalarial treatment) reduces malaria in The Gambia, an area with high coverage of standard control interventions.

**Methods** This open-label, cluster-randomised controlled trial was done in the Upper River region of eastern Gambia. Villages with a baseline *Plasmodium falciparum* prevalence of 7–46% (all ages) and separated from each other by at least 3 km to reduce vector spillover were selected. Inclusion criteria were age and anthropometry (for ivermectin, weight of ≥15 kg; for dihydroartemisinin–piperaquine, participants older than 6 months); willingness to comply with trial procedures; and written informed consent. Villages were randomised (1:1) to either the intervention (ivermectin [orally at 300–400 µg/kg per day for 3 consecutive days] and dihydroartemisinin–piperaquine [orally depending on bodyweight] plus standard control interventions) or the control group (standard control interventions) using computer-based randomisation. Laboratory staff were masked to the origin of samples. In the intervention group, three rounds of mass drug administration once per month with ivermectin and dihydroartemisinin–piperaquine were given during two malaria transmission seasons from Aug 27 to Oct 31, 2018, and from July 15 to Sept 30, 2019. Primary outcomes were malaria prevalence by qPCR at the end of the second intervention year in November 2019, and Anopheles gambiae (s l) parous rate, analysed in the intention-to-treat population. This trial is registered with ClinicalTrials.gov, NCT03576313.

**Findings** Between Nov 20 and Dec 7, 2017, 47 villages were screened for eligibility in the study. 15 were excluded because the baseline malaria prevalence was less than 7% (figure 1). 32 villages were enrolled and randomised to either the intervention or control group (n=16 in each group). The study population was 10 638, of which 4939 (46%) participants were in intervention villages. Coverage for dihydroartemisinin–piperaquine was between 49·0% and 58·4% in 2018, and between 76·1% and 86·0% in 2019; for ivermectin, coverage was between 46·9% and 52·2% in 2018, and between 71·7% and 82·9% in 2019. In November 2019, malaria prevalence was 12·8% (324 of 2529) in the control group and 5·1% (140 of 2722) in the intervention group (odds ratio [OR] 0·30, 95% CI 0·16–0·59; p<0·001). *A gambiae (s l)* parous rate was 83·1% (552 of 664) in the control group and 81·7% (441 of 540) in the intervention group (0·90, 0·66–1·25; p=0·537). In 2019, adverse events were recorded in 386 (9·7%) of 3991 participants in round one, 201 (5·4%) of 3750 in round two, and 168 (4·5%) of 3752 in round three. None of the 11 serious adverse events were related to the intervention.

**Interpretation** The intervention was safe and well tolerated. In an area with high coverage of standard control interventions, mass drug administration of ivermectin and dihydroartemisinin–piperaquine significantly reduced malaria prevalence; however, no effect of ivermectin on vector parous rate was observed.

**Funding** Joint Global Health Trials Scheme

## 3.2 Research in context

**Evidence before this study:** We searched PubMed from March 23, 2021, to March 27, 2021, with no language or date restrictions for studies assessing the effect of ivermectin on malaria transmission using the search terms “ivermectin”, “malaria”, and anopheles”. Retrieved studies showed that ivermectin kills anopheline mosquitoes and could be combined with an antimalarial drug during mass administration campaigns to decrease malaria transmission. We found no studies or clinical trials examining the effect of mass drug administration of ivermectin and dihydroartemisinin-piperaquine on malaria transmission.

**Added value of this study:** To our knowledge, this study is the first cluster-randomised trial designed to test the hypothesis that mass administration of ivermectin (a mosquitocidal drug) and dihydroartemisinin– piperaquine (an antimalarial treatment) can reduce malaria in settings where coverage of standard control interventions is high. Our findings show that mass drug administration of ivermectin and dihydroartemisinin–piperaquine significantly reduced malaria prevalence and incidence in The Gambia.

**Implications of all the available evidence:** This study provides useful insight into the potential of adding mass drug administration of dihydroartemisinin–piperaquine and ivermectin to the currently available malaria control interventions, which could further reduce malaria transmission and possibly accelerate malaria elimination in areas with high coverage of vector control interventions.

## 3.3 Introduction

Malaria control progress has recently stalled, after two decades of remarkable achievement,[1] and several high burden countries are losing ground.[2] Two of the goals of the Global Technical Strategy 2016-2030,[3] namely reduce malaria mortality rates and malaria case incidence, are off track. [2]

In The Gambia, the malaria burden has substantially declined.[4] Nevertheless, despite the high coverage of standard control interventions, malaria transmission persists in eastern Gambia were incidence of clinical malaria between 2013 and 2014, was 1·7/person-year while this was 0·2/person-year in central Gambia and 0·1/person-year in western Gambia. [5]

New interventions to further reduce transmission are needed.[6] Ivermectin is a broad-spectrum antiparasitic endectocidal drug able to kill mosquitoes feeding on treated humans.[7] Its safety profile is excellent.[8,9] When implemented as mass treatment, it may reduce vector survival and consequently malaria transmission.[10] The duration of this effect is dose-dependent.[9] Ivermectin is effective against insecticide-resistant mosquitoes,[7,11] and targets malaria vectors regardless of their biting patterns.[12]

Mass drug administration (MDA) with antimalarials can have a pronounced effect on malaria prevalence but is prone to malaria resurgence, especially with sub-optimal coverage.[13] Dihydroartemisinin-piperaquine (DP) is one of the most attractive drugs for MDA because of its high efficacy, simple dosing schedule and longer post-treatment prophylaxis.[14]

Combining ivermectin with antimalarials for MDA may have an additive effect to standard vector control interventions; because of repeated biting, the likelihood that anophelines encounter a lethal dose is high even with incomplete ivermectin coverage.[12] We assessed the impact of MDA with ivermectin plus DP on the prevalence of *falciparum* infection and the survival of malaria vectors in an area of moderate malaria transmission and high coverage of insecticide-treated nets (ITNs) and indoor residual spraying (IRS) in The Gambia.

## 3.4 Methods

### 3.4.1 Study design and participants

This was a two-arm, open-label cluster randomised controlled trial carried out in Upper River Region (URR), eastern Gambia, an area of highly seasonal malaria with peak transmission between September and November, and high vector survival[5].

Thirty-two villages with a baseline *Plasmodium falciparum* prevalence (all ages) by molecular methods ranging between 7-46% and separated from each other by at least 3 km to reduce vector spill-over between villages, were selected and randomised to either the intervention or

the control group.[15] A buffer zone of 2 km radius was created around each intervention village to limit spill-over from neighbouring villages. Community sensitization meetings on the study purpose and its procedures were held in all study villages. Additional meetings for optimal participation were held in intervention villages 2-5 days before implementation. The enumeration of the study population was carried out in November 2017. Written informed consent was obtained from all eligible residents willing to participate. Consent and enrolment procedures were carried out throughout the trial to include new residents and individuals absent during the first consenting and enrolment procedures.

The trial protocol has been published elsewhere.[16] Ethical approval was obtained from The Gambia Government/MRC Joint Ethics Committee and the London School of Hygiene and Tropical Medicine Ethics Committee. The trial is registered with Clinical Trials.gov NCT 03576313.

### 3.4.2 Randomisation and masking

Villages were randomly allocated in 1:1 ratio to one of the two groups using a computer-based randomization performed by the trial statistician. Restricted randomisation (difference in baseline prevalence ≤10% between groups) was used to ensure comparability between study groups. [17] Masking was not possible given the nature of the intervention; observer bias was reduced as laboratory staff were masked to the origin of the samples. Datasets were unmasked once data critical for the listed endpoints were locked.

### 3.4.3 Procedures

In intervention villages, three monthly rounds of MDA with ivermectin (Laboratorio Elea, Argentina) and DP (Guilin Pharmaceuticals, China) were conducted each year over two malaria transmission seasons, in 2018 (August, September, and October) and 2019 (July, August, and September). DP was administered orally by body weight according to the manufacturer's instructions. Ivermectin was administered orally at the dose of 300-400 µg/kg/day for three consecutive days. Eligibility was assessed at each MDA round. Inclusion criteria were: (1) age/anthropometry, for ivermectin: weight ≥15 kg; for DP: age >6 months, (2) willingness to comply with trial procedures, and (3) written informed consent. The exclusion criteria for both ivermectin and DP were known chronic illnesses. Additionally, for ivermectin, exclusion criteria were (1) pregnancy (any trimester) or breastfeeding, (2) hypersensitivity to ivermectin, and (3) travel to *Loa loa* endemic countries (Central Africa); for DP: (1) first-trimester pregnancy, (2) hypersensitivity to DP, and (3) taking drugs that influence cardiac function or prolong QTc interval. Both control and intervention clusters received standard control interventions implemented by the National Malaria Control Program (NMCP), namely

IRs, IRS with pirimiphos-methyl (Actellic 300CS), prompt diagnosis and treatment with artemether-lumefantrine, Seasonal Malaria Chemoprevention (SMC) with sulfadoxine-pyrimethamine plus amodiaquine, and intermittent preventive treatment during pregnancy (IPTp) with sulfadoxine-pyrimethamine.[16] In intervention villages, during the monthly MDA round, SMC was administered only to children aged 3-6 months as children >6-59 months old received DP. After the third MDA round and if SMC rounds were scheduled, 3-59 months children in intervention villages received SMC.

In each intervention village, daily treatment was administered under direct observation. Eligible individuals absent at the time of drug administration were followed up at home. Individuals' participation, demographic data and relevant medical history were electronically captured by tablet computers (Galaxy Tab 10.1 LTE Samsung Electronics, Korea). Eligible residents in buffer zones were also treated with DP and ivermectin but were not included in the evaluation of the intervention.

**Adverse events**

Information on adverse events (AE) during the first 2 days of treatment was actively collected by the study team at the time of drug administration. A structured questionnaire on AE, including their severity (mild, moderate, or severe), date of onset and duration, was administered to all treated individuals seven days after the first dose. The relation to the study drug was assessed based on known side-effects and timing to treatment. Any identified AE was actively monitored until resolution. Throughout the study period, study participants were encouraged to inform the study team of any AE.

**Malaria prevalence and incidence**

Cross-sectional surveys to estimate malaria prevalence were carried out in November 2018 and November 2019. In each village, participants were randomly selected from the census list. A blood sample was collected by finger prick for dried blood spot. Malaria prevalence was estimated as the proportion of individuals positive for malaria infection by molecular methods over the total number of individuals sampled.

Passive detection of clinical malaria was established at both community and health facility level from July 2019, immediately after the first MDA round, until the end of December 2019, the end of the malaria transmission season. A rapid diagnostic test (RDT; SD BIOLINE Malaria Ag Pf Standard Diagnostics) was performed in all suspected cases (patients with fever and/or history of fever in the last 24 hours without any other cause than malaria); positive individuals were treated with artemether-lumefantrine. A blood sample for thick blood film and for later

qPCR analysis (blood spot on Whatman 3 Corporation, Florham Park, USA) was collected from all RDT positive cases.

**Sample processing**

DNA was extracted from blood-spot samples using an automated QIAxtractor robot (Qiagen) and tested for *P. falciparum* by qPCR. [18]

**Entomology**

In all villages, adult mosquitoes were collected indoors with CDC light traps. Seven to 14 days after each MDA round, intensive sampling for four consecutive nights was carried out in six randomly selected houses per village in 16 intervention and eight randomly selected control villages. Similar collections were carried out in the remaining control villages but only for one night. Subsequently, monthly collections were carried out in all villages for one night per month in six randomly selected houses per village, until the end of the transmission season (December). *An*. *gambiae s.l*. head and thorax samples were used for the detection of *P*. *falciparum* circumsporozoite protein (CSP) by ELISA.[19] Moreover, monthly human landing catches (HLC) (indoor and outdoor) were carried out in three houses for two nights in four randomly selected villages per arm. Vector density was estimated with CDC light traps, while for vector parity CDC light traps and HLC were combined. The direct mosquitocidal efficacy of ivermectin was evaluated by randomly selecting from one intervention village: 40 adults (≥18 years old) and 40 children (4-10 years old) who had taken, besides DP, the full ivermectin dose; the same number of individuals was selected from one control village. Blood samples (3 ml) were collected at 7-, 14- and 21-day post-intervention and fed to insectary-reared *A. gambiae* s.s. mosquitoes whose mortality was monitored daily until 14 days after feeding.

**Outcomes**

Primary outcomes measures were malaria prevalence by qPCR (all ages) at the end of the second intervention year, and *A. gambiae s.l.* parous rate, 7-14 days after MDA, determined by dissection.[20] Secondary outcomes were incidence of clinical malaria, duration of increased mosquito mortality in membrane feeding assays, vector density, sporozoite rate, AEs and intervention coverage.[16]

**Statistical analysis**

Sample size calculations were done for both primary outcome measures. For malaria prevalence, assuming an average prevalence of 15% and a coefficient of variation of 0.5, 16

villages per group and 200 individuals per village would be able to detect an effect size of 50%, i.e., from 15% to 7.5%, at 90% power and 5% significance level. For the vector parous rate, assuming parity would decrease from 85% to 75%, and a coefficient of variation of 0.25, dissecting 50 mosquitoes per village would have 90% power to find a significant difference between groups.

Analysis was done according to a pre-defined plan, finalised before the datasets were locked. Random effect logistic regression was used to compare the primary outcomes in the intention-to-treat-population between intervention and control groups; a random effect for study village (cluster) was included to account for clustering. An analysis adjusting for age, ITN use, closed eaves, village level baseline prevalence was also done. For the secondary outcomes, incidence of clinical malaria was compared between groups using random effects Poisson regression. Mosquito density (the number of mosquitoes collected per trap per night) was compared between groups using random effects negative binomial regression. Sporozoite rate was estimated on mosquitoes collected by CDC light traps. Sporozoite rate was compared between groups by random effect logistic regression. Entomological inoculation rate (EIR), the number of infective bites received per person during the transmission season, was estimated in each study group as 1.605 x (no. of positive ELISAs/no. of catches) x 180.[21] The 95% CI for EIR were calculated assuming a negative binomial distribution for the mean number of *A. gambiae* s.l./light trap/night to account for over-dispersion and taking village as the unit of analysis. Survival time of laboratory-reared mosquitoes after feeding was analysed using Cox regression to calculate hazard ratios (HRs). We used shared frailty with a gamma distribution to account for mosquitoes being from the same assay. Kaplan-Meier (K-M) plots were also presented summarising survival probability by treatment group over follow-up time. Coverage for each treatment (DP and ivermectin) was defined as the proportion of eligible individuals who received at least one dose. The denominator in 2018 was the eligible population at the beginning of the implementation while in 2019 it was the eligible population at the beginning of each MDA round. Overall coverage was defined as the proportion of individuals who received at least one treatment dose over the total population (eligible and non-eligible). AEs were reported by MDA rounds. Analyses were performed with STATA version 15.

**Role of the funding source**

The funder of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author and the trial statisticians had full access to all the data in the study and the corresponding author had final responsibility for the decision to submit for publication.

## 3.5 Results

The study population in the 32 study villages was 10638, of which 4939 (46%) were in the 16 intervention villages (figure 1). At baseline, in November 2017, malaria prevalence was similar between study groups (table 1).

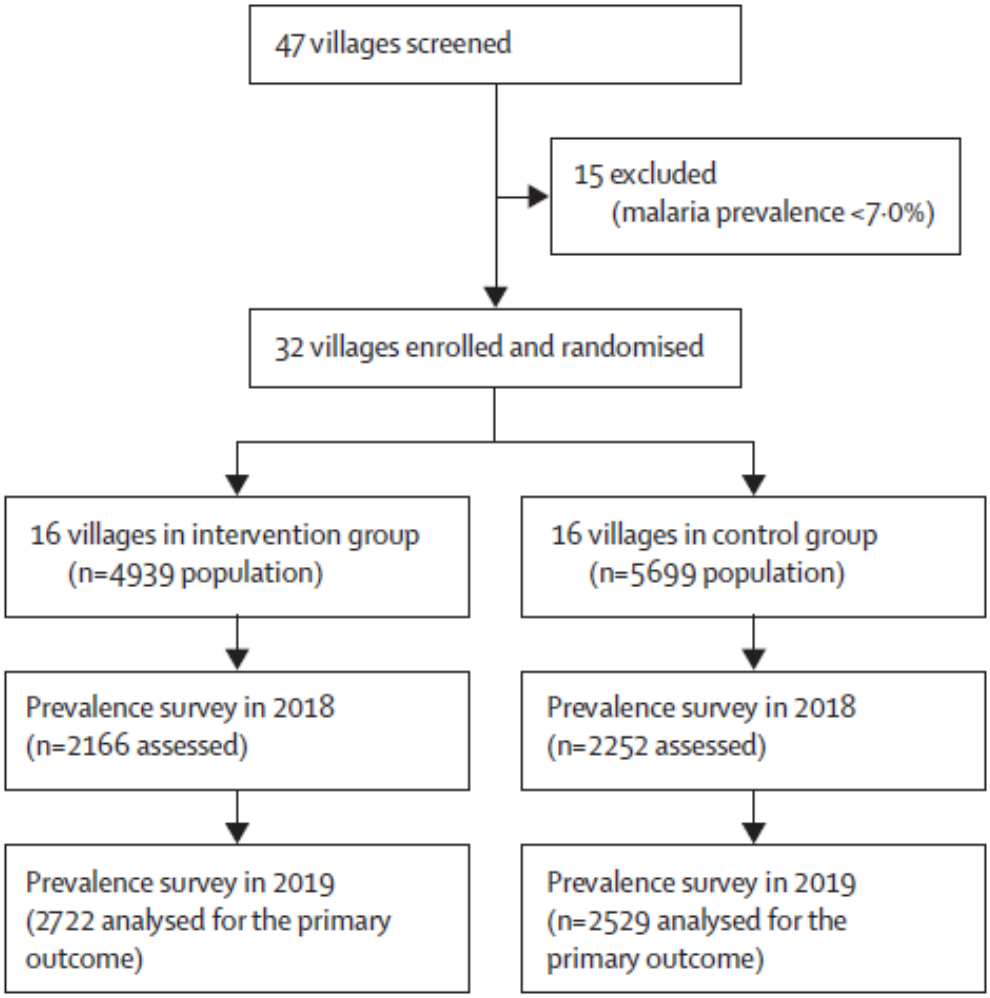


Figure 3.1 Trial profile

Table 3.1 Baseline characteristics in November 2017

| | Control group | Intervention group |
|---|---|---|
| Number of villages | 16 | 16 |
| Population | 5699/10 638 (54%) | 4939/10 638 (46%) |
| Baseline malariometric survey* | | |
| Female | 699/1217 (57%) | 828/1430 (58%) |
| Male | 518/1217 (43%) | 620/1430 (42%) |
| Age, years | | |
| Median | 13 (6–30) | 13 (6–31) |
| <5 | 198/1200 (16%) | 216/1420 (15%) |
| 5–14 | 458/1200 (38%) | 541/1420 (38%) |
| ≥15 | 544/1200 (45%) | 663/1420 (46%) |
| Insecticide-treated net use the night before the survey | 1023/1204 (85%) | 1233/1418 (87%) |
| Malaria prevalence | 211/1165 (18%) | 224/1392 (16%) |

Data are n, n (%), or median (IQR). *The baseline prevalence survey was done on a subset of the population.

Implementation of the trial was substantially delayed in 2018 as both ethical and regulatory approvals took longer than expected. This resulted in the implementation of the first MDA round at the end of August (instead beginning of July) and in several other logistical challenges that affected coverage, including late arrival of study drugs, and limited time for communities' engagement. In 2018, coverage for DP was 2552 (58·4%) of the 4370 eligible participants in the first round; 2246 (51·4%) of 4370 in the second round and 2143 (49·0%) of 4370 in the third round. Coverage for ivermectin was 1946 (52·2%) of the 3725 eligible participants, 1747 (46·9%) of 3725, 1771 (47·5%) of 3725 for rounds one, two and three, respectively. Activities carried out in 2018 and the challenges mentioned above were critically reviewed by the study team and corrective actions taken, e.g., restructuring of the field team, including posting one research staff in each study village, adequate planning, and time for community engagement. In 2019, the intervention was implemented as planned in July, August, and September, and coverage was substantially higher than in 2018. Coverage for DP was 3991 (86·0%) of 4640 eligible participants for the first round, 3750 (76·9%) of 4875 for the second round and 3752 (76·1%) of 4928 for the third round. Coverage for ivermectin was 3156 (82·9%) of 3805, 2952 (72·4%) of 4075 and 2979 (71·7%) 4155 for rounds one, two and three, respectively. Overall coverage for DP was between 51·4% and 58·4% in 2018 and between 76·0% and 85·5% in 2019; for ivermectin, overall coverage was between 40·0% and 44·5% in 2018 and between 60·3% and 65·5% in 2019 (appendices 1A and 1B).

In November 2019, malaria prevalence was 13% (324 of 2529) in the control group and 5% (140 of 2722) in the intervention group (OR 0·30, 95%CI 0·16-0·59; p<0·001) (table 2). The effect was similar after adjusting for age, ITN use, closed eaves, travel outside the village and baseline prevalence (OR 0·28, 95%CI 0·14-0·56, p<0·001) (appendix 2). The range of cluster level prevalence in the intervention group was from 0% to 18% compared to 5% to 51% in the control group (appendix 3). In 2018, malaria prevalence was similar between the two groups (appendix 4), with a range of cluster level prevalence between 1% to 46% (appendix 3).

Table 3.2 Malaria prevalence and incidence in November 2019

| | Control group | Intervention group | OR or incidence rate ratio (95% CI) | p value |
|---|---|---|---|---|
| **Malaria prevalence (primary outcome)** | | | | |
| Age group, years | | | | |
| <5 | 56/511 (11·0%) | 19/477 (4·0%) | OR 0·35 (0·13–0·93) | 0·035 |
| 5–14 | 109/883 (12·3%) | 46/948 (4·9%) | OR 0·31 (0·14–0·69) | 0·0040 |
| ≥15 | 159/1130 (14·1%) | 72/1200 (6·0%) | OR 0·34 (0·18–0·64) | 0·0010 |
| All ages | 324/2529 (12·8%) | 140/2722 (5·1%) | OR 0·30 (0·16–0·59) | <0·0001 |
| **Incidence of clinical malaria (secondary outcome)** | | | | |
| Age group, years | | | | |
| <5 | IR 0·32 (18/5700) | IR 0·20 (10/4940) | 0·58 (0·18–1·88) | 0·36 |
| 5–14 | IR 1·37 (144/10 507) | IR 0·30 (27/9106) | 0·22 (0·09–0·54) | 0·0010 |
| ≥15 | IR 0·98 (151/15 481) | IR 0·19 (25/13 417) | 0·18 (0·09–0·38) | <0·0001 |
| All ages | IR 1·10 (348/31 686) | IR 0·24 (65/27 460) | 0·21 (0·10–0·43) | <0·0001 |

Data are n/N (%), unless stated otherwise. IR=incidence rate, OR= odds ratio

Clinical malaria incidence was not determined in 2018. Between July and December 2019, 413 clinical malaria episodes were reported (65 in the intervention and 348 in the control group). The incidence of clinical malaria was 1·10/100 person-months (348/31686) in the control group and 0·24/100 person-months (65/27460) in the intervention group (incidence rate ratio 0·21, 95% CI 0·10-0·43; p<0·0001), (table 2). The effect of the intervention was particularly marked between September and November (figure 2). There was some evidence of overdispersion in the Poisson model but fitting a negative binomial regression to account for overdispersion provided similar results albeit with slightly wider confidence intervals.

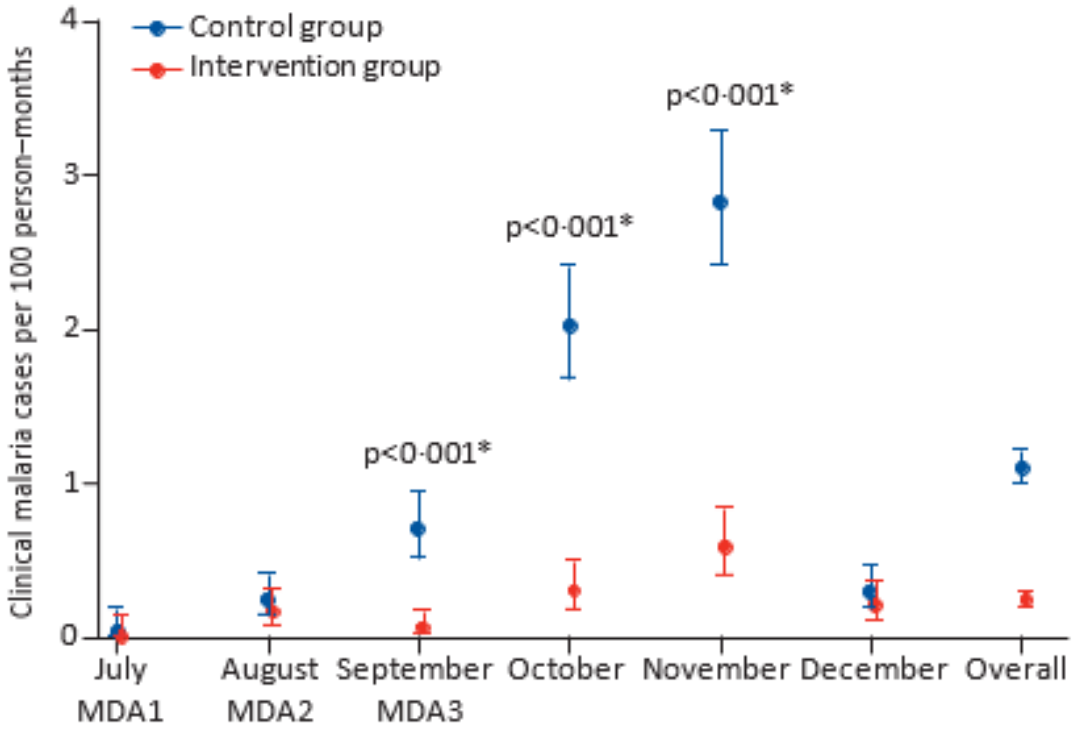


Figure 3.2 Clinical malaria incidence in 2019

(Bars represent 95% CI. MDA=mass drug administration. *STATA software output, specific p values cannot be given.)

Although there was a tendency for a lower vector parity in the intervention group, both in 2018 and 2019, the difference was not statistically significant (p=0·322 and p=0·537 in 2018 and 2019, respectively) (table 3).

Table 3.3 Vector parity, sporozoite rate, EIR, and vector density by study group and year

| | 2018 | | | | 2019 | | | |
|---|---|---|---|---|---|---|---|---|
| | Control group | Intervention group | Odds ratio (95% CI) | p value | Control group | Intervention group | Odds ratio (95% CI) | p value |
| **Vector parity (primary outcome)** | | | | | | | | |
| MDA round one (57·3%) | 297/518 | 190/364 (52·2%) | 0·98 (0·52–1·87) | 0·951 | 131/186 (70·4%) | 72/94 (76·6%) | 1·37 (0·77–2·42) | 0·284 |
| MDA round two (69·7%) | 442/634 | 262/391 (67·0%) | 1·00 (0·58–1·71) | 0·987 | 81/105 (77·1%) | 107/155 (69·0%) | 0·78 (0·43–1·41) | 0·405 |
| MDA round three (82·8%) | 130/157 | 52/80 (65·0%) | 0·31 (0·16–0·61) | <0·001* | 238/259 (91·9%) | 229/252 (90·9%) | 0·87 (0·47–1·62) | 0·661 |
| Survey 1 (73·2%) | 30/41 | 17/20 (85·0%) | 1·75 (0·23–3·47) | 0·591 | 83/95 (87·4%) | 28/32 (87·5%) | 0·59 (0·14–2·46) | 0·469 |
| Survey 2 (50·0%) | 1/2 | 0 | .. | .. | 19/19 (100·0%) | 5/7 (71·4%) | .. | .. |
| *Anopheles gambiae* (*s l*) parous rate (66·6%) | 900/1352 | 521/855 (60·9%) | 0·85 (0·62–1·17) | 0·322 | 552/664 (83·1%) | 441/540 (81·7%) | 0·90 (0·66–1·25) | 0·537 |
| **Secondary outcomes** | | | | | | | | |
| Vector density† (1572/1002) | 1·6 | 0·7 (562/858) | 0·49 (0·90–1·29) | 0·150 | 3·4 (3088/912) | 1·4 (1914/1344) | 0·36 (0·21–0·64) | <0·0001 |
| Sporozoite rate† (0·4%) | 2/456 | 4/202 (2·0%) | 4·58 (0·83–5·24) | 0·080 | 37/3047 (1·2%) | 14/1902 (0·7%) | 0·60 (0·32–1·12) | 0·109 |
| **Post-hoc analysis** | | | | | | | | |
| EIR (95% CI) (0·08–3·86) | 0·58 | 1·35 (0·61–·68) | 2·34 (0·41–2·42) | 0·23 | 11·7 (6·72–·91) | 3·00 (1·76–5·14) | 0·26‡ (0·13–0·51) | .. |

EIR=entomological inoculation rate. MDA=mass drug administration. *STATA software output, specific p values cannot be given. †Centers for Disease Control and Prevention light traps. ‡EIR ratio.

In 2018, 530 members of the *A. gambiae* complex were collected using CDC light traps, 151 (28·5%) from the intervention group. In 2019, 1780 *A. gambiae s.l*. mosquitoes were collected, 916 (51·5%) in the intervention group. Species composition was similar in both study groups (appendix 5).

In 2018, vector density tended to be lower in the intervention group than in the control group and was significantly lower in 2019 (RR 0·36, 95%CI 0·21-0·64; p<0·001) (table 3). Sporozoites rates were similar between the two study groups in both years (table 3). In 2018, EIR was similar between intervention and control villages. However, in 2019, EIR was significantly lower in intervention (3·00; 95%CI 1·76-5·14) than in control (11·7; 95%CI 6·72-16·91) villages (EIR ratio: 0·26, 95%CI 0·13-0·51; p<0·001) (table 3). Mortality among

mosquitoes fed on blood samples from participants treated with ivermectin at 7, 14 and 21 days post-treatment was higher than in the control group, with the highest effect observed seven days post-treatment (Hazard ratio [HR] 2·5, 95%CI 2·17-2·87; p<0·01) (figure 3, appendix 6). In the Cox-regression model, the effect on mosquito's mortality remained significant up to 21 days post-treatment across all time-points (appendix 6). In the sub-group analysis, mosquito mortality was more pronounced in individuals with body mass index (BMI) ≥ 22, with the greatest effect at day 14 days post-treatment (HR 6·32, 95% CI 4·04-10·08, p<0·0001) (appendix 6).

Table 3.4 Vector parity, sporozoite rate, EIR, and vector density by study group and year

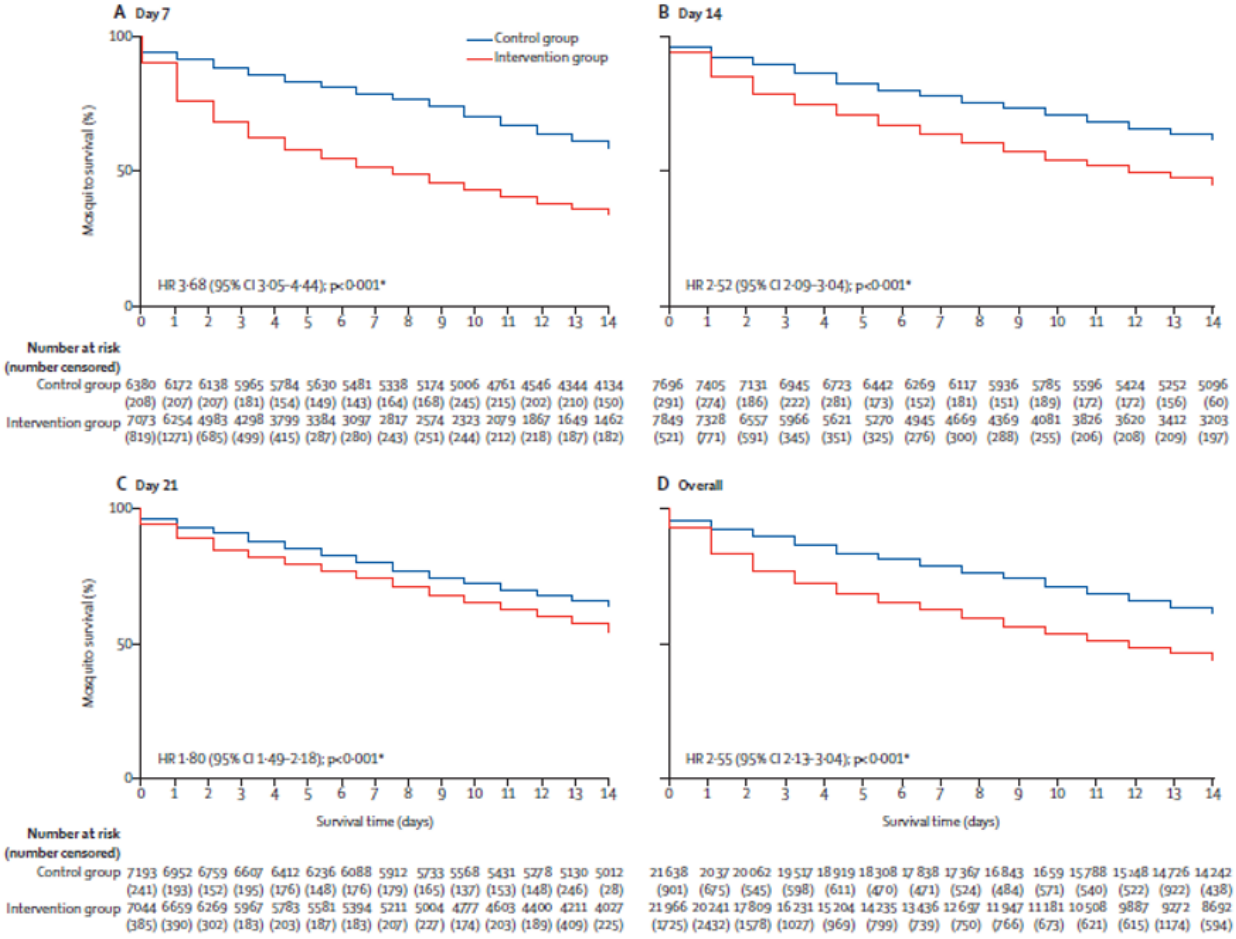


Figure 3.3 Mosquito survival post-treatment:

(Kaplan-Meier plots showing mosquito survival over time. HR=hazard ratio. *STATA software output, specific p values cannot be given)

In 2019, AEs were recorded in 386 (9·7%) of 3991 participants in round 1, 201 (5·4%) of 3750 in round 2 and 168 (4·5%) of 3752 in round 3. Most AEs were classified as Grade 1 severity, i.e. mild (table 4). All AEs resolved in a few days. Few cases of transient visual disturbance were reported (table 4). There were 11 serious adverse events (SAEs) (appendix 7), none of them related to the investigational products; three of them resulted in death, i.e., a road traffic accident with multiple injuries; a gastro-enteritis case secondary to HIV infection; and an undiagnosed illness. The latter occurred in a woman >70 years old who died at home after a short illness and without reporting to the health centre. She had received one daily dose of MDA during the first round, in July 2019, and death occurred in September 2019.

Table 3.5 Adverse events by MDA round and severity in 2019

| Adverse event | MDA round round one (n=3991) | MDA round two (n=3750) | MDA round three |
|---|---|---|---|
| Headache | 82 (2·0%) | 53 (1·4%) | 46 (1·2%) |
| Diarrhoea | 49 (1·2%) | 16 (0·4%) | 15 (0·4%) |
| Vomiting or nausea | 40 (1·0%) | 11 (0·3%) | 7 (0·2%) |
| Abdominal pain | 34 (0·9%) | 20 (0·5%) | 12 (0·3%) |
| Pyrexia | 34 (0·9%) | 25 (0·7%) | 29 (0·8%) |
| General body pain or join pain | 32 (0·8%) | 22 (0·6%) | 8 (0·2%) |
| Malaise | 18 (0·5%) | 1 (0·03%) | 8 (0·2%) |
| Cough | 10 (0·3%) | 6 (0·2%) | 12 (0·3%) |
| Transient visual disturbances | 7 (0·2%) | 11 (0·3%) | 15 (0·4%) |
| Itching | 4 (0·1%) | 1 (0·03%) | 0 |
| Other | 76 (2·0%) | 29 (0·8%) | 16 (0·4%) |
| Grading (severity)* | | | |
| 1 (mild) | 333 (86·3%) | 157 (78·1%) | 153 (91·1%) |
| 2 (moderate) | 40 (10·4%) | 35 (17·4%) | 13 (7·7%) |
| 3 (severe) | 5 (1·3%) | 3 (1·5%) | 1 (0·6%) |
| Not recorded | 8 (2·0%) | 6 (3·0%) | 1 (0·6%) |
| Total | 386 (100·0%) | 201 (100·0%) | 168 (100·0%) |

Individuals who received at least one dose of treatment and had an adverse event. MDA=mass drug administration. *Grading (severity) of the adverse events divided by the total number of events recorded.

## 3.6 Discussion

MDA with ivermectin and DP reduced malaria prevalence, the primary parasitological endpoint, by about 60% and malaria incidence by about 80% but not vector parity, the primary entomological endpoint measuring vector survival. This could indicate that the observed difference in malaria prevalence and incidence between study groups may be essentially due to DP. Although there was no difference in mosquito population survival in both study groups,

as shown by the similar parity, the intervention resulted in lower vector density. Such decline was insufficient to reduce the overall vector survival rate, perhaps because of spill-over of vector populations from control villages mixing with those from intervention villages, as already observed in The Gambia.[22] This may have occurred despite the implementation of MDA in all villages located within 2Km of each intervention village. Nonetheless, in 2019, when ivermectin coverage was above 70%, the impact of the intervention on vector density resulted in a 74% lower EIR in intervention villages, indicating decreased malaria transmission.

The vision of WHO and the global malaria community is a world free of malaria. All countries can accelerate efforts towards elimination through combinations of interventions tailored to local contexts.[3] However, currently available tools may not be sufficiently effective to interrupt malaria transmission.[23] One of the pillars of the current Global Technical Strategy is accelerating efforts towards elimination while research is one of the two supporting elements of this strategy.[3] The results of this trial fit within this context, particularly when considering The Gambia has recently set the goal of elimination by 2025.[24] Achieving such a goal may prove challenging with standard control tools since, despite high coverage, malaria transmission in the study area has not been interrupted. MDA with ivermectin and DP could provide an additional tool towards the goal of elimination.

The current trial design is unable to determine the individual effect of each component of the MDA. The trial assessed the combined effect of DP and ivermectin because, at the time of designing the trial, MDA with ivermectin alone was considered unlikely to be implemented; combining DP, an efficacious antimalarial, with ivermectin, a mosquitocidal agent, would have a synergistic effect as the former would reduce the population parasite biomass and provide post-treatment prophylaxis while the latter would reduce vector densities and thus the number of infectious bites during and after the intervention.[7,25] Eventually, ivermectin would reduce the minimum coverage required by MDA as mosquitoes, by feeding on several individuals over a short period, may also take a toxic dose of ivermectin. Recent mathematical models, however, predict that in highly seasonal transmission settings, such as our study site, ivermectin alone, either as a single dose of 400 µg/kg or 3 daily doses of 300 µg/kg implemented over 3 monthly rounds per season, would achieve a reduction of clinical incidence between 62% and 71%; by adding DP, the reduction would be between 91% and 94%. [12] The same model predicts that 3 monthly rounds of DP with ivermectin, the latter either as a single dose of 400 µg/kg or 3 daily doses of 300 µg/kg, would reduce malaria prevalence by 70% to 72%. [12] Notably, the model predicts that combining ivermectin with DP would prolong the overall effect of the MDA intervention. Our results are slightly lower than the model predictions, namely a reduction of

60% in malaria prevalence and 79% in clinical incidence, while the model predicts a reduction of 70% and 94%, respectively.

Our results differ from the two other cluster randomized controlled trials assessing MDA with DP alone carried out in sub-Saharan Africa. A reduction in the prevalence of infection and incidence of clinical malaria was observed in Zambia only in lower-transmission areas (prevalence <10%) while in Zanzibar the intervention had no effect on malaria prevalence or incidence of clinical malaria.[26,27] In Zambia, malaria prevalence was determined by RDT and microscopy, and only in children below six years of age, while in Zanzibar this was by molecular methods and in all age groups. Therefore, in Zambia, prevalence in lower-transmission areas (between 7% and 9%) may be comparable to The Gambia had molecular methods been employed. Results in Zanzibar suggest that at baseline prevalence of 1.6%, MDA with DP or any other antimalarial may not be indicated.

In 2018, DP coverage was below 60% while ivermectin coverage was 50% or less, underlining the challenges to achieve the required 70-80% MDA coverage of the eligible population. Such less-than-optimal coverage was the result of poor community sensitization and involvement of the study population due to the delay in obtaining the required approvals and the little time available, given the short transmission season, for MDA implementation. One of the main barriers to non-participation and non-adherence in MDA is short-term mobility.[28] Villagers may not be available during the enumeration, consent process, or MDA rounds, requiring the setup, throughout the trial implementation, of a complex system ensuring these individuals are registered, provide written informed consent, and are followed up at home for treatment. Perceived adverse drug reactions, inconveniences related to the logistics of MDA (e.g., waiting times) and the perceived lack of information about MDA are additional factors that require careful planning for continuous sensitization meetings to provide accurate information on procedures, drug regimens and expected adverse drug reactions. High uptake of the intervention is key for the success of MDA campaigns. [29] This may be challenging if MDA becomes part of the intervention package. Nevertheless, the SMC coverage achieved, on average above 80%,[2] suggests that reaching the required MDA coverage may be feasible.[30] In addition, MDA should not be implemented for an indefinite number of years but for the time necessary to reduce malaria prevalence to extremely low level, e.g. 1-2%, when surveillance of clinical cases or other targeted interventions would be more adequate than MDA. This is also supported by the lack of impact of MDA in Zanzibar.[27] Restricting MDA to a limited number of years and ensuring good adherence to treatment would also decrease the risk of selecting drug resistance parasites. Moreover, to decrease the risk of selecting drug resistance

parasites, we purposely choose for MDA a different antimalarial treatment than the first line treatment, which is artemether-lumefantrine in The Gambia.

Sporozoite rates were also similar in both study groups, which is surprising given the high coverage with DP and the decreased parasite reservoir in the intervention group. This finding provides further support for vector spill-over between intervention and control villages. Although the sporozoite rates were comparable between study groups, there were far fewer *A. gambiae* s.l. in the intervention villages in 2019, resulting in >70% lower EIR than in control villages. Our results suggest that using MDA with ivermectin over a much larger area, to reduce invasion of mosquitoes from untreated villages, could reduce vector population survival, resulting in even greater reductions in the EIR. *A. arabiensis* was the most abundant species in the study site, representing more than half of the malaria mosquitoes collected in 2018 and more than two-thirds in 2019. Given this species also feeds on animals, this may have diluted the impact of ivermectin on its vector population survival and suggests that ivermectin administered to both humans and cattle may provide improved mosquito killing.

Mosquito survival was reduced by about 60% after 7 days post treatment and by about 30% after 21 days post-treatment, indicating a robust and prolonged mosquitocidal effect, and confirming earlier results from Kenya and Thailand. [9,25] Adding DP to ivermectin increases the peak concentration and overall exposure to ivermectin, resulting in higher toxicity to mosquitoes and a prolonged effect because of the slow-release of ivermectin metabolites.[25] The mosquitocidal effect is markedly pronounced with higher BMI, as mosquito mortality increased significantly when fed on blood of participants with a body mass index ≥ 22. This phenomenon has been described previously and may be due to the accumulation of ivermectin in fat tissue which would then be slowly released, increasing its blood concentration over time and thus the mosquitocidal effect.[9]

Overall, the intervention was safe and well tolerated, confirming the high safety profile of repeated and high dose of ivermectin co-administered with DP. [8,9] Most AE were mild; few individuals had transient visual disturbances that resolved in a few hours. Nevertheless, no systematic monitoring of biochemistry parameters was carried out nor an electrocardiogram was performed. This would have been important to detect any liver or renal injury or any QTc (QT interval corrected for heart rate) prolongation when considering the co-administration of DP and ivermectin can result in higher concentration of ivermectin and piperaquine. [25]

Our study has some limitations. First, the study communities could not be blinded to the intervention. Second, we did not achieve the sample size required for measuring vector parity in both study groups. Third, although the villagers were separated by distances of 3 km and

the implementation of MDA in villages located within 2 km of each intervention village, there may have been spill-over between adjacent villages.

This is the first study to show that community administration of three-monthly MDA of DP and high dose ivermectin is safe and well-tolerated and reduces residual malaria transmission in an area of highly seasonal malaria with high coverage of control interventions. Adding MDA with ivermectin and DP to the currently available malaria control interventions could further reduce malaria transmission and possibly accelerate malaria elimination in areas with high coverage of vector control interventions.

**Contributors**

UDA conceived the study. EDD, HMS, FC, JB, BK, MRS, HS, KPG, HB, TB, CD, SWL, JA and UDA contributed to refinement of the protocol and approved the final version. JB was the trial statisticians. EDD, HMS and UDA had access to and verified the data. EDD, HMS, UDA and NM contributed to data analysis. EDD, HMS, BC, FC, and MON did the field work and data collection. EDD and UDA drafted the manuscript. All authors read and approved the final manuscript before submission.

**Declaration of interests**

We declare no competing interests.

**Data sharing**

After publication, trial data will be made available on reasonable request to the corresponding author. A proposal with a detailed description of study objectives and a statistical analysis plan will be needed for assessment of requests. Additional materials might also be required during the process of assessment. Deidentified participant data will be provided after approval by the sponsor and trial management group.

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# Chapter 4 Efficacy, Safety and Tolerability of Pyronaridine-artesunate in Asymptomatic Malaria-infected Individuals: a Randomized Controlled Trial

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EDD and SH contributed equally to this work; CM and JA contributed equally to this work.

**Key points**

This randomized study in The Gambia and Zambia evaluated pyronaridine-artesunate as full or incomplete treatment in individuals with asymptomatic *Plasmodium falciparum* infection. High efficacy and good tolerability suggest that pyronaridine-artesunate could be useful in mass drug administration campaigns in Africa.

**Short title.** Pyronaridine-artesunate in asymptomatic malaria

**Keywords.** pyronaridine-artesunate; malaria; asymptomatic; pediatric; randomized controlled clinical trial.

**Clinical Trials Registration.** ClinicalTrials.gov: NCT03814616.

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## 4.1 Abstract

***Background.*** Pyronaridine-artesunate (PA) is a registered artemisinin-based combination therapy, potentially useful for mass drug administration campaigns. However, further data are needed to evaluate its efficacy, safety and tolerability as full or incomplete treatment in asymptomatic *Plasmodium falciparum*-infected individuals.

***Methods.*** This phase II, multi-center, open label, randomized clinical trial was conducted in The Gambia and Zambia. Participants with microscopically confirmed asymptomatic *P. falciparum* infection were randomly assigned (1:1:1) to receive a 3-day, 2-day, or 1-day treatment regimen of PA (180:60 mg), dosed according to bodyweight. The primary efficacy outcome was PCR-adjusted adequate parasitological response (APR) at day 28 in the per-protocol population.

***Results.*** A total of 303 participants were randomized. Day 28 PCR-adjusted APR was 100% for both the 3-day (98/98) and 2-day regimens (96/96), and 96.8% (89/94) for the 1-day regimen. Efficacy was maintained at 100% until day 63 for the 3-day and 2-day regimens, but declined to 94.4% (84/89) with the 1-day regimen. Adverse event frequency was similar between the 3-day (51.5% [52/101]), 2-day (52.5% [52/99]), and 1-day (54.4% [56/103]) regimens; the majority of adverse events were of grade 1 or 2 severity (85% [136/160]). Asymptomatic, transient increases (>3xULN) in alanine transaminase/aspartate transaminase were observed for 6/301 (2.0%) participants.

***Conclusion:*** PA had high efficacy and good tolerability in asymptomatic *P. falciparum*-infected individuals, with similar efficacy for the full 3-day and incomplete 2-day regimens. Although good adherence to the 3-day regimen should be encouraged, these results support the further investigation of PA for mass drug administration campaigns.

## 4.2 Introduction

In 2015, the World Health Organization (WHO) Global Technical Strategy set ambitious goals for reducing malaria mortality and incidence rates by at least 90%, and achieving malaria elimination in at least 35 countries by 2030 [1]. Eleven countries worldwide, ten of them in sub-Saharan Africa, contribute about 70% of global malaria morbidity and mortality [2]. Even in areas with high coverage of control interventions, malaria transmission persists and has become increasingly heterogeneous [3-5]. Innovative tools and strategies are needed to reduce malaria transmission and promote elimination.

A major challenge for malaria elimination is transmission from asymptomatic malaria-infected individuals carrying low density infections [6-8]. Interventions targeting the human transmission reservoir, such as mass drug administration (MDA), can reduce malaria prevalence and transmission [9-16]. Effective MDA requires high coverage and good adherence to treatment [17-19], and there is a need for efficacious, well tolerated, and affordable treatment for this purpose.

Pyronaridine-artesunate (PA) is a fixed-dose artemisinin-based combination therapy (ACT) shown to be highly efficacious and well tolerated for the treatment of uncomplicated falciparum malaria [20-32]. This study is the first to evaluate PA efficacy, safety, and tolerability in individuals with asymptomatic *Plasmodium falciparum* infection. To assess the potential impact of sub-optimal adherence on parasitological efficacy, PA was administered at the full therapeutic dose (once daily for three days) and as incomplete treatment (once daily for 2-days or 1-day).

## 4.3 Methods

### 4.3.1 Ethics statement

The protocol was approved by the Gambian Government/MRC Joint Ethics Committee in The Gambia, the Tropical Diseases Research Centre (TDRC) Ethics Review Committee and the National Health Research Ethics Board in Zambia, and the Ethics Committee of the London School of Hygiene and Tropical Medicine. The study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice, and applicable national regulations. Written informed consent was obtained from all patients or their parents/guardians if aged under 18 years; documented assent was obtained from children aged 12–17 years.

### 4.3.2 Study design and participants

This phase II, multi-center, open label, randomized clinical trial was conducted in Basse (Upper River Region), Eastern Gambia, and Nchelenge (Luapula Province), Northern Zambia, between 2nd October 2018 and 16th May 2019. Trial sites were in areas of moderate-to-high malaria transmission. Potential study participants were identified by systematic pre-screening

for malaria infection in local communities and schools until the required sample size was reached.

Inclusion criteria were confirmed *P. falciparum* mono-infection with a parasite density between 20 and 50,000/µL, no clinical malaria signs or symptoms for the past 72 hours, age >5 years, body weight >20 kg, and the ability to swallow oral medication. Participants were excluded if they had a hemoglobin level <7 g/dL, evidence of severe malnutrition, known allergy to the study drugs. Complete eligibility criteria are described in Supplementary Methods 1.

### 4.3.3 Study drug

Pyronaridine-artesunate (180/60 mg) fixed-dose combination tablets (Shin Poong, Pharmaceutical, Co, Ltd) were given orally, once daily, according to body weight: 20 to <24 kg, 1 tablet; 24 to <45 kg, 2 tablets; 45 to <65 kg, 3 tablets; and ≥65 kg, 4 tablets. Treatment was administered for 3 days (3-day regimen), two days (2-day regimen), or one day (1-day regimen). All doses were directly supervised. Vomiting within 30 minutes prompted repeat dosing. Vomiting of the repeat dose resulted in participant withdrawal and rescue treatment as per local recommendations.

### 4.3.4 Randomization and masking

Participants were randomized (1:1:1) to receive the PA 3-day regimen, 2-day regimen, or 1-day regimen according to a computer-generated randomization list provided by the study sponsor. Treatment allocation was in sealed envelopes sequentially numbered with the study participant's unique code. Participants were allocated in enrolment order to the treatment in the next available envelope. Participants and clinical staff were not masked to treatment regimen; microscopists responsible for reading malaria smears remained blinded to treatment allocation throughout the study.

### 4.3.5 Procedures

Pre-screening for malaria infection was done using a standard rapid diagnostic test (RDT; SD Bioline Malaria Ag Pf, Standard Diagnostics Inc.) or hypersensitive (HS)-RDT (Alere Malaria Ag Pf, Standard Diagnostics, Inc.) in Zambia and HS-RDT in The Gambia, with confirmation by microscopy. Eligible participants received their first PA dose on day 0; a blood slide was collected 4–8 hours after the first dose. Participants returned on days 1, 2, 3, 7, 14, 21, 28, 35, 42, and 63, or at any time if they felt unwell. Insecticide-treated bed nets were provided to all participants on day 0. The assessment schedule is shown in Table 1.

Table 4.1 Assessment schedule.

| Assessment | Study day/ visit |
|---|---|

| | BL | D0[a] | D1 | D2 | D3 | D7 | D14 | D21 | D28 | D35 | D42 | D63 | EW/UV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Demographics, medical history | ● | | | | | | | | | | | | |
| Urine pregnancy test | ● | | | | | | | | ● | | | ● | ● |
| Physical examination[b] | ● | | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● |
| Thick/thin blood smears | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● |
| Blood spot (PCR genotyping)[c] | ● | | | | | ●[d] | ●[d] | ●[d] | ●[d] | ●[d] | ●[d] | ●[d] | ● |
| Hematology/biochemistry | ● | | ● | | | ● | | | ● | | | | ● |
| Adverse events | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● |
| Concomitant medication | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● |
| Study drug administration | ● | | ● | ● | | | | | | | | | |

[a]4–8 h, [b]Physical examination, malaria signs and symptoms, vital signs and body temperature; [c]Increases in aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total or conjugated bilirubin >3 times the upper limit of normal (xULN) prompted collection of an additional sample within 24 h and repeated sampling at 48-h intervals until values were ≤2xULN; [d]Assessment was only done in the event of recurrent infection; Abbreviations: BL, baseline; ET, early withdrawal; UV, unscheduled visit.

Giemsa-stained thick and thin blood smears for parasite identification and quantification were examined independently by two microscopists using standard methods [33]. Any discordant blood smears or those with >30% variance in parasite density were reviewed independently by a third microscopist, with external quality control on approximately 4% of slides. To distinguish between recrudescence and re-infection, blood spots were obtained for *P. falciparum* polymerase chain reaction (PCR) genotyping. Recrudescence was defined as at least one matching allelic band in all markers (*P. falciparum* genes *msp 1*, *msp 2*, and *glurp*) between samples from baseline and recurrence [34].

Demographic characteristics were recorded, and a medical history taken at screening. Physical examination, vital signs, malaria signs and symptoms and adverse events were assessed throughout the study and categorized using the Medical Dictionary for Regulatory Activities (version 22.1). Blood samples were collected for hematology and clinical chemistry.

**Outcomes**

The primary efficacy outcome was day 28 PCR-adjusted adequate parasitological response (APR), defined as a microscopically negative slide at Day 28, irrespective of axillary

temperature, in participants without previous treatment failure. Secondary efficacy endpoints were: i) PCR-adjusted APR at days 7, 14, 21, 35, 42, and 63; ii) PCR-unadjusted APR at days 7, 14, 21, 28, 35, 42, and 63; iii) recurrence, re-infection and recrudescence incidence rate until day 63; iv) the proportion of participants parasite-free by microscopy between 4–8 h post first PA dose and by day 1, 2, and 3 post-first dose; and v) gametocyte carriage up to Day 14, by microscopy.

Safety outcomes were adverse event frequency, and abnormal vital signs, hematological parameters, or clinical chemistry values. Serious adverse events were defined as death, life-threatening, requiring hospitalization or prolongation of hospitalization, congenital abnormalities, or birth defects, persistent or significant disability or incapacity, or Hy's law (alanine aminotransferase [ALT] or aspartate aminotransferase [AST] >3 times the upper limit of normal [xULN] plus a serum total bilirubin >2xULN [>35% direct bilirubin], in the absence of alkaline phosphatase ≥2xULN or biliary injury).

**Sample size**

The 3-day regimen was assumed to have similar efficacy against *P. falciparum* in asymptomatic carriers as in patients with uncomplicated malaria, i.e. ≥97% at day 28 [20, 31, 32]. With a sample size of 90 participants, assuming an efficacy of 97.8% for the 3-day regimen, the lower limit of the one-sided Clopper–Pearson 90% confidence interval (CI) was 94.2%. The efficacy of the 2-day and the 1-day regimen was assumed ≥94%, providing reasonable precision given that the minimal acceptable efficacy for an MDA treatment is >90% [15]. Assuming 10% loss to follow-up, 100 participants per arm were needed to demonstrate ≥90% efficacy with 90% power.

**Statistical analysis**

For this exploratory study no formal statistical testing was planned. The primary efficacy endpoint was evaluated in the per-protocol (PP) population (Figure 1), with one-sided (lower) 90% and 95% CI (Clopper–Pearson) calculated for each treatment arm. Two-sided exact 95% CI for the difference in day 28 APR between each pairwise comparison were calculated, i.e. 3-day regimen versus 1-day regimen, 3-day regime versus 2-day regimen, 2-day regimen versus 1-day regimen (Wilson method without continuity correction). Statistical analysis was performed using SAS Version 9.4 or higher. A supportive analysis was conducted for the microbiological intention-to-treat (m-ITT) population (Figure 1).

Recrudescence rate and re-infection rate over 63 days were evaluated using Kaplan–Meier analysis in the m-ITT population. Participants with no recurrence event were censored at the

last available parasite assessment date and those with major protocol deviations at the time of the protocol deviation. The proportion of parasite-free participants was determined for the PP population. Gametocyte carriage was determined as area under the gametocyte density–time curve (AUC) calculated according to the trapezoidal rule for all participants having at least one positive gametocyte count in the PP population.

## 4.4 Results

**Participants**

Overall, 303 participants with confirmed *P. falciparum* mono-infection were enrolled (Figure 1). Baseline characteristics were generally comparable across the treatment arms (Table 2). Geometric mean parasite density was 573.9 $\mu L^{-1}$, and 18.8% (55/292) of evaluable participants had baseline gametocytes detectable by microscopy.

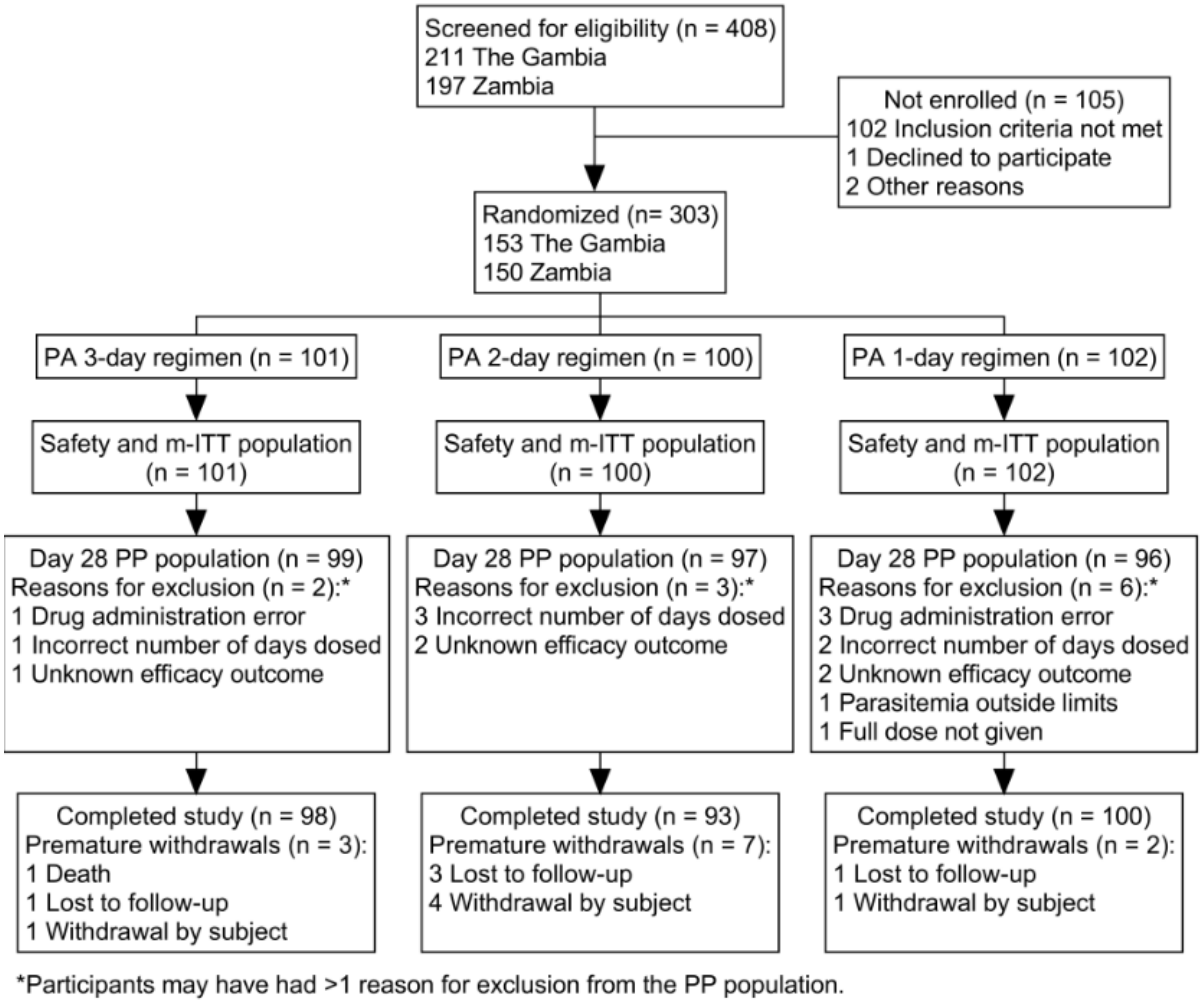



Figure 4.1 Participant disposition

Populations: safety population, all randomized participants who received at least 1 dose of study medication; m-ITT population, all randomized patients who received at least 1 treatment dose and who had confirmed positive parasitemia before treatment; PP population, all randomized patients who completed their treatment, had outcome data for the primary efficacy end point, and complied with the protocol. Abbreviations: mITT, microbiological-intention-to-treat; PA, pyronaridine-artesunate; PP, per-protocol.

Table 4.2 Baseline Characteristics

| Characteristics | Pyronaridine-artesunate treatment group | | | Overall (n = 303) |
|---|---|---|---|---|
| | 3-day regimen (n = 101) | 2-day regimen (n = 100) | 1-day regimen (n = 102) | |
| Country, n (%) | | | | |
| The Gambia | 51 (50.5) | 50 (50.0) | 52 (51.0) | 153 (50.5) |
| Zambia | 50 (49.5) | 50 (50.0) | 50 (49.0) | 150 (49.5) |
| Sex, n (%) | | | | |
| Male | 60 (59.4) | 39 (39.4) | 49 (47.6) | 148 (48.8) |
| Female | 41 (40.6) | 60 (60.6) | 54 (52.4) | 155 (51.2) |
| Age, years, mean (SD) [range] | 15.0 (8.3) [6–48] | 15.9 (9.9) [6–60] | 16.6 (11.5) [6–64] | 15.8 (10.0) [6–64] |
| Age group, n (%) | | | | |
| 5–≤12 years | 48 (47.5) | 49 (49.5) | 48 (46.6) | 145 (47.9) |
| >12–18 years | 32 (31.7) | 24 (24.2) | 25 (24.3) | 81 (26.7) |
| ≥18 years | 21 (20.8) | 26 (26.3) | 30 (29.1) | 77 (25.4) |
| Weight by age group, kg, mean (SD) [range] | | | | |

| | | | | |
|---|---|---|---|---|
| 5–≤12 years | 29.0 (8.2) [20.7–65.2] | 27.4 (6.3) [20.2–51.2] | 26.1 (4.4) [20.6–40.1] | 27.5 (6.6) [20.2–65.2] |
| >12–18 years | 42.8 (7.5) [33.2–62.4] | 46.5 (11.7) [27.8–72.0] | 40.2 (6.7) [29.3–56.1] | 43.1 (8.9) [27.8–72.0] |
| ≥18 years | 58.8 (11.6) [37.1–87.3] | 56.7 (10.5) [44.4–94.0] | 57.40 (10.7) [42.7–96.3] | 57.50 (10.8) [37.1–96.3] |
| Asexual parasites, µL-1, geometric mean (range) | 592.7 (20–38960) | 579.6 (24–47600) | 550.6 (16–33020) | 573.9 (16–47600) |
| Participants with gametocytes, n/N (%) | 17/99 (17.2) | 20/97 (20.6) | 18/96 (18.8) | 55/292 (18.8) |

**Efficacy**

For the primary outcome, day 28 PCR-adjusted APR in the PP population was 100% (98/98) for the 3-day regimen, 100% (96/96) for the 2-day regimen, and 96.8% (91/94) for 1-day regimen; the lower limit of the 95% CI exceeded 90% for all regimens (Table 3). There was no significant difference in day 28 PCR-adjusted APR across the three study arms (Figure 2). Efficacy was maintained until day 63 for the 3-day and 2-day regimens but declined for the 1-day regimen (Table 3). The m-ITT analysis supported the primary analysis (Supplementary Table 1, Supplementary Figure 1). In the Kaplan–Meier analysis, there were no recrudescence through day 63 for the 3-day and 2-day regimens (Figure 3A). Re-infections were more frequent in the shorter treatment regimens (Figure 3B).

Table 4.3 Parasitological Response in the Per-protocol Population

| APR, n/N (%) [one sided 95% CI] | Pyronaridine-artesunate treatment group | | |
|---|---|---|---|
| | 3-day regimen (n = 99) | 2-day regimen (n = 97) | 1-day regimen (n = 96)[a] |
| PCR-adjusted | | | |
| Day 7 | 99/99 (100) [97.0] | 97/97 (100) [97.0] | 95/96 (99.0) [95.2] |
| Day 14 | 99/99 (100) [97.0] | 96/96 (100) [96.9] | 94/95 (98.9) [95.1] |
| Day 21 | 98/98 (100) [97.0] | 96/96 (100) [96.9] | 92/95 (96.8) [92.0] |
| Day 28 | 98/98 (100) [97.0] | 96/96 (100) [96.9] | 91/94 (96.8) [92.0] |
| Day 35 | 96/96 (100) [96.9] | 93/93 (100) [96.8] | 89/92 (96.7) [91.8] |
| Day 42 | 96/96 (100) [96.9] | 92/92 (100) [96.8] | 88/91 (96.7) [91.7] |
| Day 63 | 93/93 (100) [96.8] | 86/86 (100) [96.6] | 84/89 (94.4) [88.6] |
| PCR-unadjusted | | | |
| Day 7 | 99/99 (100) [97.0] | 96/97 (99.0) [95.2] | 94/96 (97.9) [93.6] |
| Day 14 | 98/99 (99.0) [95.3] | 96/97 (99.0) [95.2] | 94/96 (97.9) [93.6] |
| Day 21 | 98/99 (99.0) [95.3] | 96/97 (99.0) [95.2] | 91/96 (94.8) [89.4] |
| Day 28 | 97/99 (98.0) [93.8] | 94/97 (96.9) [92.2] | 89/96 (92.7) [86.7] |

| | | | |
|---|---|---|---|
| Day 35 | 96/98 (98.0) [93.7] | 92/96 (95.8) [90.7] | 88/96 (91.7) [85.5] |
| Day 42 | 94/98 (95.9) [90.9] | 90/96 (93.8) [88.0] | 86/96 (89.6) [83.0] |
| Day 63 | 91/97 (93.8) [88.2] | 85/93 (91.4) [85.0] | 81/96 (84.4) [77.0] |

Abbreviations: PCR, polymerase chain reaction; APR, adequate parasitological response; [a]In the PCR-adjusted analysis, all treatment failures on or before day 42 and 4/5 on day 63 were late parasitological failures (parasitemia plus temperature <37°C), the remaining treatment failure on day 63 was a late clinical failure (parasitemia plus temperature ≥37°C).

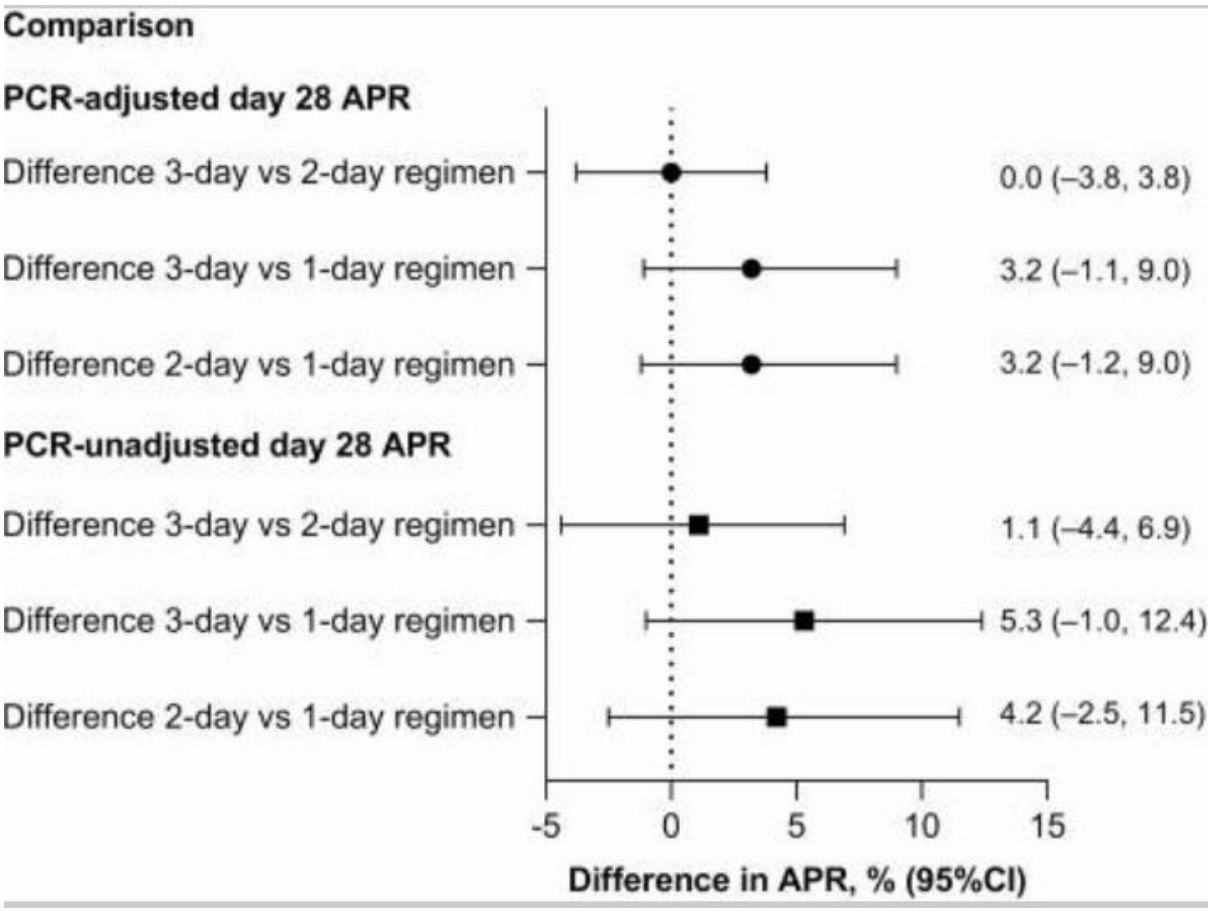
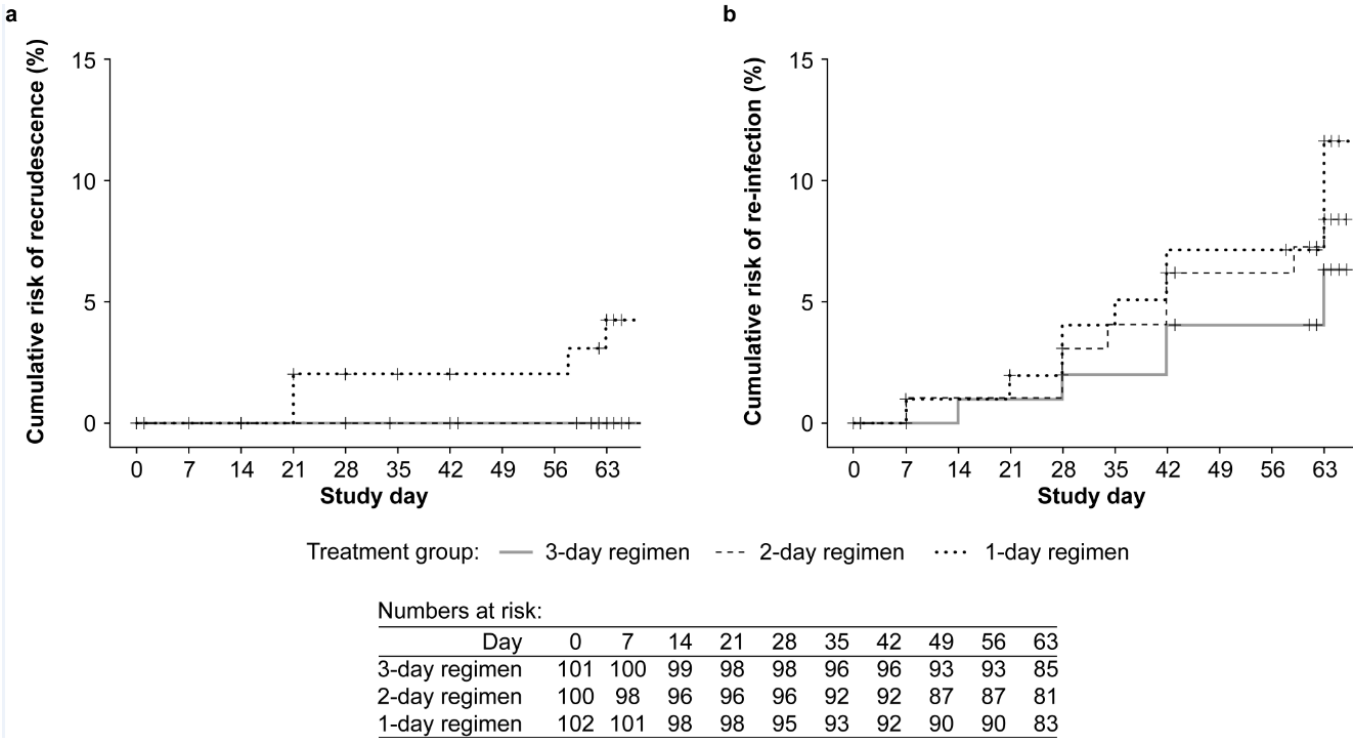



Figure 4.2 Adequate parasitological response at day 28 in the per-protocol population
Abbreviations: APR, adequate parasitological response; PCR, polymerase chain reaction.




Numbers at risk:

| Day | 0 | 7 | 14 | 21 | 28 | 35 | 42 | 49 | 56 | 63 |
|---|---|---|---|---|---|---|---|---|---|---|
| 3-day regimen | 101 | 100 | 99 | 98 | 98 | 96 | 96 | 93 | 93 | 85 |
| 2-day regimen | 100 | 98 | 96 | 96 | 96 | 92 | 92 | 87 | 87 | 81 |
| 1-day regimen | 102 | 101 | 98 | 98 | 95 | 93 | 92 | 90 | 90 | 83 |

Figure 4.3 Kaplan-Meier estimates

(a) recrudescence; and (b) reinfection in the microbiological intention-to-treat population

The proportion of participants without infection as determined by microscopy between 4–8h post first PA dose and day 3 was similar for the three treatment groups (Figure 4A). The mean

$log_{10}$ AUC gametocytes until day 14 was similar for all three regimens (Figure 4B). However, all baseline gametocytes were cleared by day 21 with the 3-day regimen but persisted until day 28 with the 2-day and 1-day regimens, re-appearing in one participant at day 63 with the day-1 regimen (Supplementary Table 2).

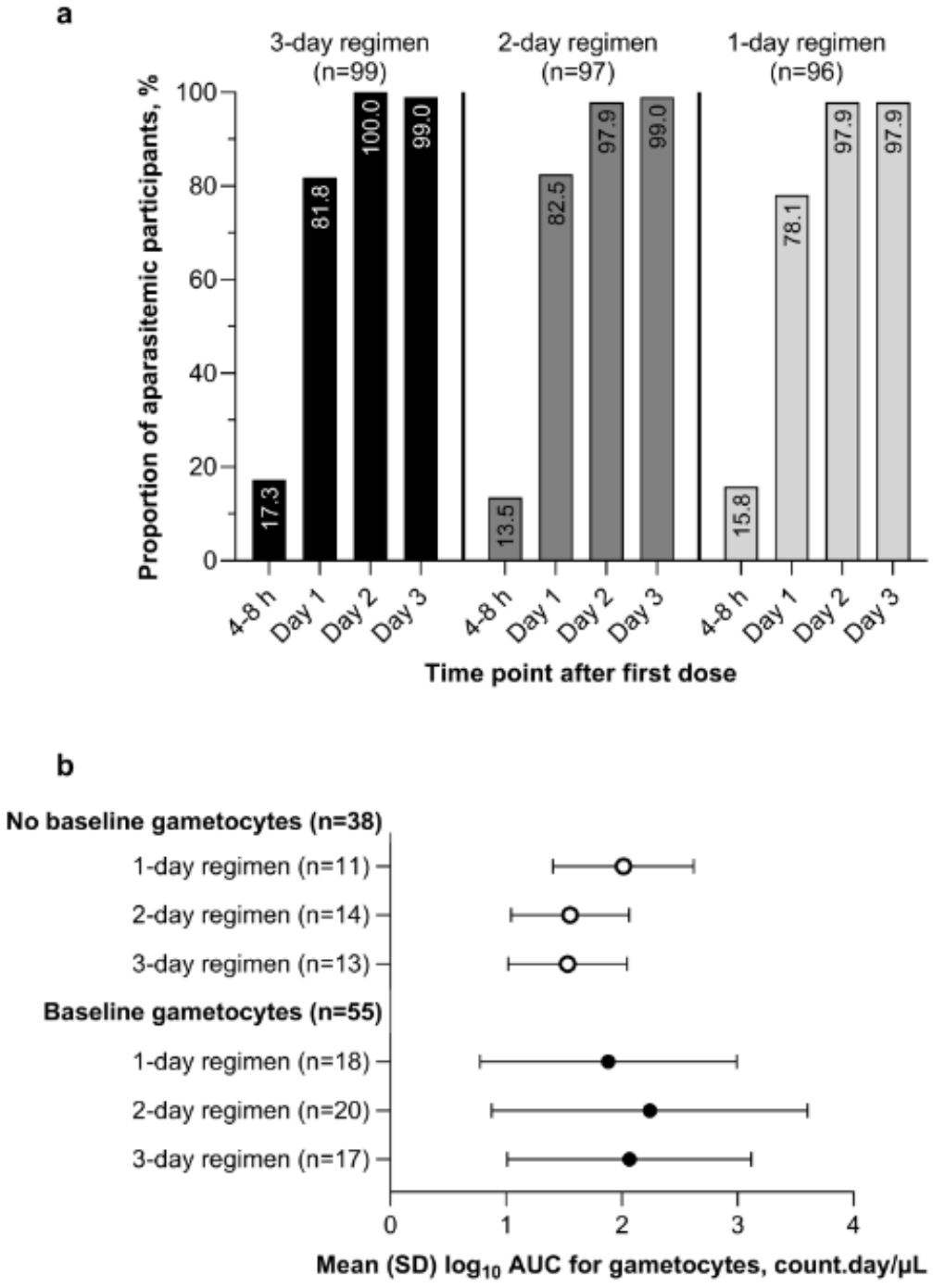



Figure 4.4 Parasite clearance in the per-protocol population:

(a) proportion of participants with asexual parasite clearance until day 3; and (b) mean (SD) log10 area under the curve for gametocytes up to day 14 in participants with or without baseline gametocytes. Abbreviation: AUC, area under the gametocyte density–time curve.

**Safety**

Adverse event frequency was similar between the 3-day (51.5% [52/101]), 2-day (52.5% [52/99]), and 1-day (54.4% [56/103]) regimens, though with some differences, i.e. a lower incidence of cough with the 2-day regimen, and a higher incidence of neutropenia and abdominal pain with the 2-day and 1-day regimens versus the 3-day regimen (Figure 5). Most adverse events were grade 1 or 2 in severity (85% [136/160]); grade 3+ adverse events were more common in the day-2 (8.1% [8/99]) and day-1 (12.6% [13/103]) regimens versus the day-3 regimen (2.0% [2/101]) (Supplementary Table 3). The frequency of treatment-related adverse events was lower for the 3-day regimen (6.9% [7/101]) versus the 2-day (12.1% [12/99]) and 1-day (12.6% [13/103]) regimens (Supplementary Table 4), as was the frequency of malaria-related adverse events (2.0% [2/101], 6.1% [6/99], and 6.8% [7/103]), respectively) (Supplementary Table 5). There were two serious adverse events, one death of a 12-year-old male by drowning at day 30 (day-3 regimen), and a missed abortion in a 35-year-old female at day 149 resolved by a vacuum aspiration at day 152 (2-day regimen); neither was considered treatment related.

| | | |
|---|---|---|
| 15.8 | 14.1 | 12.6 |
| 7.9 | 13.1 | 7.8 |
| 10.9 | 2.0 | 9.7 |
| 2.0 | 7.1 | 10.7 |
| 2.0 | 5.1 | 9.7 |
| 5.0 | 2.0 | 2.9 |
| 2.0 | 1.0 | 3.9 |
| 1.0 | 3.0 | 2.9 |
| 2.0 | 2.0 | 0 |
| 2.0 | 2.0 | 0 |
| 2.0 | 0 | 1.9 |
| 1.0 | 2.0 | 1.0 |
| 2.0 | 1.0 | 0 |
| 0 | 1.0 | 1.9 |
| 2.0 | 0 | 1.0 |
| 0 | 1.0 | 1.9 |
| 0 | 2.0 | 1.0 |
| 2.0 | 0 | 1.0 |
| 0 | 3.0 | 0 |
| 0 | 0 | 2.0 |
| 0 | 0 | 1.9 |

Figure 4.5 Most common treatment-emergent adverse events of any cause in the safety population.

Adverse events occurring in >1 participant in any one treatment group. Values are percentage frequency. Participants may have had more than one adverse event. Abbreviation: P. falciparum, Plasmodium falciparum.

Most laboratory abnormalities were grade 1 or 2 and resolved by day 28 (Supplementary Tables 6 and 7). Post-baseline hemoglobin declines >2 g/dL were observed in 3.7% (11/297) of participants, but hemoglobin levels were >8 g/dL in all participants by day 28 (Table 4). Asymptomatic, transient increases in ALT/AST >3xULN were observed in 6/301 (2.0%) participants, three of whom had increases >5xULN. All values had normalized by day 28 (Table 4). There were no Hy's Law cases.

Table 4.4 Changes in Hemoglobin, Alanine Aminotransferase and Aspartate Aminotransferase

| Parameter | Time point | Pyronaridine-artesunate treatment group | | |
|---|---|---|---|---|
| | | 3-day regimen (n=101) | 2-day regimen (n=99) | 1-day regimen (n=103) |
| Change in hemoglobin from baseline >2 g/dL, n/N (%) | Post-baseline | 3/99 (3.0) | 6/97 (6.2) | 2/101 (2.0) |
| | Day 1 | 2/98 (2.0) | 3/95 (3.2) | 1/98 (1.0) |
| | Day 7 | 2/97 (2.1) | 3/95 (3.2) | 2/98 (2.0) |
| | Day 28 | 1/97 (1.0) | 3/96 (3.1) | 0/95 |
| Mean hemoglobin (SD) [range], g/dL | Baseline | 11.9 (1.5) [7.6–16.1] | 12.1 (1.6) [7.3–17.4] | 11.8 (1.6) [7.1–16.9] |
| | Day 1 | 11.6 (1.8) [7.2–19.2] | 11.6 (1.5) [8.0–16.4] | 11.5 (1.7) [6.9–19.0] |
| | Day 7 | 11.3 (1.4) [7.8–15.0] | 11.6 (1.6) [8.5–19.9] | 11.4 (1.8) [7.7–21.8] |
| | Day 28 | 12.0 (1.2) [8.2, 15.6] | 12.2 (1.2) [9.1, 16.2] | 12.0 (1.3) [8.6, 15.6] |
| Post-baseline ALT or AST >3xULN, n/N (%) | Day 1 | 1/101 (1.0) | 4/97 (4.1) | 1/98 (1.0) |
| | Day 7 | 0/101 | 2/99 (2.0) | 0/100 |
| | Day 28 | 0/99 | 0/98 | 0/95 |
| Post-baseline ALT or AST >5xULN, n/N (%) | Day 1 | 0/101 | 2/97 (2.1) | 1/98 (1.0) |
| | Day 7 | 0/101 | 0/99 | 0/100 |
| | Day 28 | 0/99 | 0/98 | 0/95 |

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; xULN, times the upper limit of normal.

## 4.5 Discussion

This study evaluated PA efficacy in asymptomatic individuals infected with *P. falciparum.* In addition, the potential consequences of poor adherence to the full 3-day regimen during MDA campaigns were evaluated by administration of 2-day and 1-day regimens. It is important to stress that this study was not designed to support any change to the 3-day PA regimen for the treatment of uncomplicated malaria, nor does it support abbreviated dosing to clear parasitemia in asymptomatic individuals. The reason of investigating incomplete treatment regimens was to determine PA efficacy when given for community-based interventions aiming at reducing the human reservoir of malaria infection, e.g., mass drug administration or mass testing and treatment. In these circumstances, when treatment may not be directly supervised, treated individuals may take only one or 2 days of treatment. Therefore, it is reassuring the day-28 efficacy was similar across the three treatment regimens and that efficacy for the 3-day and 2-day regimens was maintained until day 63.

Single-dose PA had unexpectedly good efficacy in this population. In a murine blood-stage malaria model, single-dose pyronaridine was shown to reduce parasitemia more rapidly and completely than artesunate, chloroquine, or amodiaquine [35]. This potent effect may have been sufficient to suppress and/or clear parasites after only one dose in most individuals with low parasite density. Although there was no significant difference in PCR-adjusted day-28 APR, recrudescence occurred in the 1-day regimen group from day 7. Recrudescence drives resistance development [36]. Thus, there is a concern that the 1-day regimen would increase the risk or rapidity of resistance emergence to PA. In the Greater Mekong Sub-region, PA has been shown to be efficacious in regions where dihydroartemisinin-piperaquine and/or mefloquine-artesunate have been abandoned as first-line therapy for uncomplicated *P. falciparum* malaria owing to multi-drug resistance [21, 24-26]. Therefore, adherence to full treatment for PA is extremely important, given this combination might be an alternative option in case of emerging resistance to other ACTs [37]. With the 3-day and 2-day regimens, PCR-adjusted efficacy was maintained at 100% through day 63, with one-sided 95% CIs exceeding 96% in both arms. Such a high efficacy probably reflects the low baseline parasite density (geometric mean 573.9, $\mu L^{-1}$ blood); in contrast, African patients with uncomplicated malaria, have mean parasite densities typically above 15,000 $\mu L^{-1}$ blood [20, 22, 27, 28, 30, 32].

Re-infections were more frequent with the 2-day and 1-day versus the 3-day PA regimen and occurred earlier; from day 7 with the 1-day and 2-day regimens versus day 14 for the 3-day regimen. This was expected given that a larger dose of pyronaridine will result in a longer half-life for the pyronaridine component, providing an extended period of post-treatment protection [30, 38]. Although the half-life of pyronaridine is about 14–18 days, the effect of this early difference in re-infection could still be observed at day 63.

Parasite clearance by day 3 was 99.0% for both the 3-day and 2-day regimens and slightly lower (97.9%) for the 1-day regimen. Similar rapid parasite clearance has been previously demonstrated for 3-day PA in patients with uncomplicated *P. falciparum* malaria [22, 29, 30, 32]. Only a small proportion of patients were parasitemic at day 3 following the 1-day PA regimen. However, because the half-life of artesunate and its active metabolite dihydroartemisinin is short (up to 1.5 h) [39], these parasites will be exposed to pyronaridine monotherapy. As these parasites may be also those least susceptible to artesunate, any subsequent recrudescence increases the risk for the selection of artemisinin-resistant strains.

Clinical studies in patients with uncomplicated *P. falciparum* malaria indicate that ACTs have limited efficacy in clearing gametocytes, which is dependent primarily on the non-artemisinin component [40, 41]. Pyronaridine is thought to have limited efficacy against gametocytes, with conflicting *in vitro* data [42-45]. In Kenyan children with uncomplicated *P. falciparum* malaria treated with PA, quantitative reverse-transcription PCR indicated that 25.3% (20/79) of patients harbored gametocytes at day 14 [46]. In the current study, though the AUC values with all three regimens were similar, microscopically determined gametocytemia persisted to day 14 with the 3-day regimen, and to day 63 following the 1-day regimen. Thus, co-administration of PA and single low-dose primaquine may be needed if MDA is to rapidly clear gametocytes from asymptomatic individuals infected with falciparum malaria, as has been demonstrated with artemether-lumefantrine/primaquine and dihydroartemisinin-piperaquine/piperaquine [14, 47, 48].

PA was generally well tolerated, with adverse events consistent with previous studies of 3-day treatment of patients with uncomplicated malaria [20, 22, 24-32, 49]. There was a trend for fewer adverse events with the 3-day versus the 2-day and 1-day regimens. Although the study population was asymptomatic for malaria, falciparum infection is not necessarily benign, being associated with immune system dysregulation and inflammation [50]. The full therapeutic dose may have been more effective in resolving the more subtle health impacts of malaria infection, and emergent malaria symptoms were observed more frequently with the abbreviated regimens. Consistent with the known safety profile for PA [20, 31, 32], transient, asymptomatic increases in ALT and AST were observed for six participants (2.0%). Notably, post-baseline ALT or AST >5 xULN only occurred with the 2-day and 1-day regimen.

A limitation of this study was the selection of participants based on microscopy, whereas individuals with sub-patent infection are an important component of the transmission reservoir [6, 7]. Nevertheless, given the lower parasite densities, PA efficacy is likely to be similar, if not higher against sub-microscopic infections. Moreover, we could not exclude the possibility of low-level residual parasitemia in PA-treated participants. In Kenyan children with

uncomplicated *P. falciparum* malaria treated with either PA or artemether-lumefantrine, residual parasitemia at day 7 detected by quantitative PCR was not associated with parasite recurrence at day 28 or day 42 [51]. Given study participants were followed up until day 63 post-treatment in our study, it is unlikely that any recrudescence was missed. Nevertheless, it is possible that infections acquired during follow up may have had sub-patent densities at day 63 and may have been missed by microscopy. A further limitation of this study was the lack of an ACT comparator.

This study indicates the potential of PA for community-based malaria control interventions, in conjunction with other tools. The finding that the 2-day and 3-day regimens had similar efficacies in this population is reassuring given the challenges related to treatment adherence during MDA, as treatment is unlikely to be supervised for 3 days. However, this does not negate the importance of adherence to the 3-day regimen when used for acute malaria. This study supports further investigation of PA in comparative operational studies to examine adherence and outcomes in asymptomatic *P. falciparum* infection.

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**Conflicts of interest**

IBF, and SD are full time employees of Medicines for Malaria Venture and JS is employed by Shin Poong Pharmaceutical Company. RM consulted for Shin Poong during the study and is the Shin Poong qualified person for pharmacovigilance. All other authors declare no competing interests.

**Data sharing**

After publication, trial data will be made available on request to the corresponding author. De-identified participant data will be provided after approval by the sponsor and trial management group.

**Supplementary data**

Supplementary Methods 1. Study Eligibility Criteria

Supplementary Table 1. Adequate parasitological response in the microbiological intention-to-treat population.

Supplementary Figure 1. Adequate parasitological response at day 28 in the microbiological intention-to-treat population.

Supplementary Table 2. Participants with *Plasmodium falciparum* gametocytes (per-protocol population).

Supplementary Table 3. All treatment-emergent adverse events of any cause by severity (safety population).

Supplementary Table 4. Treatment-emergent adverse events considered to be study drug related (safety population).

Supplementary Table 5. Treatment-emergent adverse events considered to be related to malaria (safety population).

Supplementary Table 6. Hematology.

Supplementary Table 7. Clinical biochemistry.

**Abbreviations**

ACT, artemisinin-based combination therapy; APR, adequate parasitological response; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HS-RDT, hyper-sensitive rapid diagnostic test; MDA, mass drug administration; m-ITT, microbiological intention-to-treat; PA, pyronaridine-artesunate; PCR, polymerase chain reaction; PP, per-protocol; RDT, rapid diagnostic test; ULN, upper limit of normal; WHO, World Health Organization.

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# Chapter 5 Field performance of the highly sensitive rapid diagnostic test for detecting low-density *Plasmodium falciparum* infections in The Gambia

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Submitted for publication

## 5.1 Abstract

Background

The malaria burden in The Gambia has significantly decreased over the past two decades and the country is now targeting malaria elimination by 2030. Such efforts would require better rapid diagnostic tests. This study evaluated the performance of the highly sensitive rapid diagnostic test: Alere™/Abbott Malaria Ag P.f RDT under field conditions for detecting low-density infections.

Methods

A cross-sectional survey was conducted before peak transmission in 17 Gambian villages. Finger-prick blood samples were collected from healthy individuals to detect *Plasmodium falciparum* infections using the highly sensitive rapid diagnostic tests (HS-RDT) Alere™. HS-RDT sensitivity and specificity were estimated against PCR, which was used as reference test.

Results

*Plasmodium* falciparum prevalence by HS-RDT was 1.7% (56/3317). Its sensitivity was 14.0% (95% CI: 9.0%, 20.0%) and decreased with decreasing parasite density. HS-RDT specificity was 99.0 % (95% CI 99.0% - 99.0%) and remained high regardless of parasites density, level of malaria transmission and age group.

Conclusion

HS-RDT (Alere™/Abbott Malaria Ag P.f RDT) was unable to detect most low-density infections, suggesting its impact in elimination efforts would be limited.

Keywords

Highly sensitive rapid diagnostic test, Malaria, Mass screening and treatment, *Plasmodium falciparum,* The Gambia

## 5.2 Introduction

Between 2000 and 2015, the scale-up of malaria interventions, namely insecticide-treated bed nets (ITNs), indoor residual spraying (IRS), and prompt diagnosis and treatment with artemisinin-based combination therapy (ACT), resulted in a substantial reduction of malaria morbidity and mortality worldwide, including in sub-Saharan Africa (1). This achievement renewed interest in malaria elimination and eradication (2,3). Several countries are now targeting malaria elimination, including The Gambia, which aims for malaria elimination by 2030 (1,4,5).

In a low transmission setting such as The Gambia, the challenge to achieve elimination is to target the hidden parasite reservoir in the human host. Several strategies to target the human reservoir of infection have been suggested and these include mass screening and treatment or focal screening and treatment. However, current standard diagnostics tools, i.e., microscopy and Rapid Diagnostic Tests (RDTs), are unable to identify infected individuals with low-parasite densities. Sub-patent infections, which represent most of the human reservoir of infections, are detectable only by molecular methods (6,7). In The Gambia, 60% of malaria infections during the dry season and 30% during the transmission season were asymptomatic, and a third of them were sub-patent (8,9).

Molecular nucleic acid-based tests are highly sensitive, with a detection limit as low as 0.02–0.1 parasites/µl but require sophisticated laboratories facilities and skilled staff, making their field deployment as a screening tool operationally challenging (6,7). Therefore, for mass screening and treatment campaigns and for detecting, characterizing and monitoring malaria cases in the context of malaria surveillance an easy to use, cheap and field deployable diagnostic tool is needed. In 2017, Alere™/Abbott Malaria Ag P.f RDT, an RDT similar to the standard RDT but with a ten-fold greater sensitivity than standard tests, was launched (10). We determined its sensitivity and specificity in The Gambia.

## 5.3 Methods

### 5.3.1 Study design and participants

Between 9th June and 31th August 2021, a cross-sectional survey was carried out in 17 villages in Upper River Region, eastern Gambia. This region is characterized by a long dry season between October and June and a single short rainy season from July to September (11). Malaria transmission is highly seasonal with peak transmission between October and November (11).

Individuals aged at least 6 months, in good health condition (following a medical examination), and with an axillary temperature < 37.5°C as determined by digital thermometer were recruited; finger-prick blood sample was collected for HS-RDT (Alere™/Abbott Malaria Ag P.f

RDT). A dried blood spot was also collected onto filter paper (Whatman 3 MM filter paper; Whatman, Florham Park, NJ, USA) for later molecular analysis by var-ATS qPCR. Participants with a positive HS-RDT were treated with artemether-lumefantrine, the first line treatment in The Gambia.

### 5.3.2 Laboratory procedures

The HS-RDT was performed according to the manufacturer's instructions. Briefly, the blood sample was applied to the test port and then four drops of the assay diluent were added. Result was read by a study nurse within 20 min (12).

For the var-ATS qPCR assay, three 3-mm dried blood spots were punched into 96-well plates and digested in 20 µl of proteinase K and 180 µl of ATL tissue lysis buffer solution. The Plasmodium DNA was extracted using the QIAamp 96 DNA QIAcube HT kit (Qiagen, Germany) and Qiacube HT® robot. Extracted DNA was eluted into 80 µl of elution buffer and stored at −20°C untill further use. For the analysis, the var gene acidic terminal sequence (varATS, 59 copies/genome) of *P. falciparum* was amplified (13). All PCR reactions included 10 standards prepared from tenfold serially diluted samples containing known numbers of infected erythrocytes diluted in whole blood. The limit of detection of the PCR assay is approximately 0.2 parasites/µl of blood (13). The PCR output was analysed using the BioRad CFX Manager software.

### 5.3.3 Data management and statistical analysis

Field data were collected and managed using REDCap (Research Electronic Data Capture) data management software. Electronic data were exported to R software for analysis. Malaria prevalence was calculated for each diagnostic test (HS-RDT and qPCR). qPCR was the reference test to estimate the diagnostic accuracy of HS-RDT. Malaria prevalence was calculated by dividing the number of positive samples, either by HS-RDT or PCR, by the total number of tested samples. The sensitivity was estimated as follows: true positives/(true positives + false negatives); specificity as true negatives/(true negatives + false positives); positive predictive value as true positives/(true positive + false positives); negative predictive value as true negatives/(true negatives + false negatives).

## 5.4 Consent

The study received approval from the Scientific Coordinating Committee (SCC) of the Medical Research Council The Gambia Unit at London School of Hygiene and Tropical Medicine (MRCG at LSHTM) and The Gambia Government/Medical Research Council Joint Ethics Committee (Ref: 25499). Community verbal approval was followed by individual written consent for adults who also provided consent for children below 18 years of age. Children between 12 and 17 years gave assent.

## 5.5 Results

### 5.5.1 Baseline characteristics

Of the 3317 participants enrolled in the study, 57.6% (1912/3317) were females with a median age of 17 years (IQR: 9-35). All participants were apyretic with a mean axillary temperature of 36.2°C (SD: 0.5) (Table 1).

Table 5.1 Participants demographic characteristics

| Variables | N(%) |
|---|---|
| **Gender (n=3317)** | |
| Female | 1912 (57.6%) |
| Male | 1405 (42.4%) |
| | |
| **Age group (n=3310)** | |
| 0 to < 5 | 68 (2.0%) |
| 5 to < 20 | 1753 (53.0%) |
| 20 to <100 | 1489 (45.0%) |
| All age | 3310 (100%) |
| Age years, median (Q1, Q3) | 17 (9, 35) |
| **Temperature, mean (SD) (n=3317)** | 36.2 (0.5) |
| | |
| **Prevalence by diagnostic test** | |
| HS-RDT % (n) | 1.7% (3317) |
| qPCR % (n) | 5.0% (3317) |

Q1= first quartile, Q3=third quartile, SD= standard deviation, HS-RDT= highly sensitive diagnostic test, qPCR quantitative polymerase chain reaction

*Plasmodium falciparum* prevalence was 1.7% (56/3317) by HS-RDT and 5.0% (167/3317) by qPCR. The age group 5-20 years had the highest *Plasmodium falciparum* prevalence, 1.9% (33/1753) and 6.0% (105/1753) by HS-RDT and qPCR, respectively (Table 2). The highest *P.f* prevalence was detected in August, 1.5% (15/10035) for HS-RDT and 5.9% (59/1003) for qPCR (Table 2).

Table 5.2 : Plasmodium falciparum prevalence by diagnostic test

| Variables/parameters | HS-RDT+ | qPCR |
|---|---|---|
| **Age group** | | |
| 0 to < 5 | 1.5% (1/68) | 4.4% (3/68) |
| 5 to < 20 | 1.9% (33/1753) | 6.0% (105/1753) |
| 20+ | 1.5% (22/1489) | 4.0% (59/1489) |
| All ages | 2.0% (56/3310) | 5.0% (167/3310) |
| **Gender** | | |
| Female | 1.6% (30/1912) | 5.0% (96/1912) |
| Male | 1.9% (26/1405) | 5.1% (71/1405) |
| **Months** | | |
| June | 2.5% (32/1258) | 3.9% (49/1258) |
| July | 0.9% (9/1056) | 5.6% (59/1056) |
| August | 1.5% (15/1003) | 5.9% (59/1003) |
| All months | 1.7% (56/3261) | 5.0% (167/3150) |

HS-RDT= highly sensitive diagnostic test, qPCR quantitative polymerase chain reaction

### 5.5.2 Diagnostic accuracy of HS-RDT

Using qPCR as the reference test, 167/3317 participants were positive for malaria. Out of the 167 positive samples, HS-RDT identified 13.8% (23/167) of them as positives, corresponding to a sensitivity of 14.0% (95% CI: 9.0%, 20.0%) (Table 3).

Table 5.3 HS-RDT performance compared to qPCR (95%CI)

| | | | | | Prevalence by qPCR | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|---|---|---|---|---|
| | | qPCR | | | | | | | |
| | | Pos | Neg | Total | | | | | |
| HS-RDT | Pos | 23 | 33 | 56 | 5.0% (4.0%, 6%) | 14.0% (9.0% ,20.0 %) | 99.0% (99.0%, 99.0%) | 41.0% (28.0%, 5.0%) | 96.0% (96.0%, 96.0%) |
| | Neg | 144 | 3117 | 3261 | | | | | |
| Total | | 167 | 3150 | 3317 | | | | | |

PPV = positive predictive value, NPV = negative predictive value, Pos= positive, Neg =negative

The HS-RDT sensitivity decreased with decreasing parasite density. Among individuals with parasite density between 10 and 100 parasites/µl by qPCR, HS-RDT sensitivity was 47.0% (95%CI 24.0% - 71.0%) and dropped to 9.0% (95% CI: 5.0%-15.0%) among individuals with parasites density below 10 parasites/ µl (Table 4).

HS-RDT's sensitivity tended to decrease with decreasing prevalence. HS-RDT sensitivity in the 5 to 20 years old group (the group with the highest *P.f* prevalence) was 17.0% (95%CI:10.0%, 25.0%) while it was 8.0% (95%CI:3.0%, 19.0%) among adults. HS-RDT specificity was high, 99.0 % (95% CI 99.0% - 99.0%) and remained high regardless of parasites density, level of malaria transmission and age group (Tables 4, 5, and 6).

The HS-RDT positive and negative predictive values were 41.0% (95%CI:28.0%, 55.0%) and 96.0% (95%CI: 96.0%, 96.0%), respectively. In individuals with very low parasite densities (1-<10 parasites /µl), positive and negative predictive values were 30.0 % (95% CI: 17.0%-45.0%) and 96.0% (95% CI: 95.0% - 97.0%), respectively; for those with parasite densities of 10-100 parasites /µl, these values were 21.0% (95% CI: 10.0% - 37.0%) and 100% (95% CI: 99.0% - 100%) (Tables 4, 5, and 6).

Table 5.4 : HS-RDT performance stratified by parasite density, compared to qPCR (95%CI)

| Parasite density (parasite/µl) | | | qPCR | | | Prevalence by qPCR | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | pos | Neg | Total | | | | | |
| 1 to < 10 | HS-RDT | Pos | 14 | 33 | 47 | 4.0% (4.0%, 5.0%) | 9.0% (5.0%, 15.0%) | 99.0% (99.0%, 99.0%) | 30.0% (17.0%, 45.0%) | 96.0% (95.0%, 97.0%) |
| | | Neg | 134 | 3117 | 3251 | | | | | |
| | | | Pos | Neg | Total | | | | | |
| 10 to 100 | HS-RDT | Pos | 9 | 33 | 42 | 1.0% (0.0%, 1.0%) | 47% (24.0%,71.0%) | 99.0% (99.0%, 99.0%) | 21% (10.0%, 37.0%) | 100% (99.0%, 100.0%) |
| | | Neg | 10 | 3117 | 3127 | | | | | |
| | | Total | 19 | 3150 | 3169 | | | | | |

PPV = positive predictive value, NPV = negative predictive value, Pos= positive, Neg =negative

Table 5.5 HS-RDT performance by village level prevalence (95%CI)

| Village level prevalence | | | qPCR | | Total | Prevalence by qPCR | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pos | Neg | | | | | | |
| Very low transmission <5% | HS-RDT | Pos | 8 | 20 | 28 | 3.0% (2.0%, 4.0%) | 13.0% (6.0%,24.0%) | 99.0% (98.0%, 99.0%) | 29.0% (13.0%, 49.0%) | 97.0% (97.0%, 98.0%) |
| | | Neg | 53 | 1938 | 1992 | | | | | |
| | Total | | 61 | 1958 | 2019 | | | | | |
| | | | Pos | Neg | | | | | | |
| Low-to moderate transmission (5- <15%) | HS-RDT | Pos | 15 | 7 | 22 | 9.0% (7.0%, 10%) | 16.0% (9.0%,24.0%) | 99.0% (99.0%, 100.0%) | 68.0% (45.0%, 86.0%) | 93.0% (91.0%, 94.0%) |
| | | Neg | 81 | 1016 | 1097 | | | | | |
| | Total | | 96 | 1023 | 1119 | | | | | |

PPV = positive predictive value, NPV = negative predictive value, Pos= positive, Neg =negative

Table 5.6 HS-RDT performance by age group (95%CI)

| Age group | | | qPCR | | Total | Prevalence by qPCR | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pos | Neg | | | | | | |
| 5- <20 years | HS-RDT | Pos | 18 | 16 | 34 | 9.0% (7.0%, 1.0%) | 17.0% (10.0%,25.0%) | 99.0% (98.0%, 99.0%) | 53.0% (35.0%,70.0%) | 92.0% (91.0%, 94.0%) |
| | | Neg | 90 | 1097 | 1187 | | | | | |
| | Total | | 108 | 1113 | 1221 | | | | | |
| | | | Pos | Neg | | | | | | |
| 20 - <100 years | HS-RDT | Pos | 5 | 17 | 22 | 4.0% (3.0%, 5.0%) | 8.0% (3.0%,19.0%) | 99.0% (98.0%, 99.0%) | 23.0% (8.0%, 45.0%) | 96.0% (95.0%, 97.0%) |
| | | Neg | 54 | 1413 | 1467 | | | | | |
| | Total | | 59 | 1430 | 1489 | | | | | |

PPV = positive predictive value, NPV = negative predictive value, Pos= positive, Neg =negative

## 5.6 Discussion

The performance of HS-RDT was evaluated under field conditions in asymptomatic malaria-infected individuals with low parasite density. Sensitivity was low and the test missed most of the malaria infected individuals. Although sensitivity was much lower, these results confirm the findings of previous studies carried out in The Gambia (8). The difference in the sensitivity reported may be due to the timing of the survey and the exclusion of symptomatic individuals. Indeed, the previous study by Mwesigwa and colleagues was implemented at peak transmission (November-December), and included symptomatic individuals. Instead, the study reported in this paper was carried out before the peak transmission season. Indeed, prevalence of infection by molecular methods was 5% while for the previous study this was 13.1%. The poor performance of HS-RDT was comparable with that reported in low transmission settings such as Myanmar where HS-RDT sensitivity was 36.6% (14); Papua New Guinea, 51% (15) and Ethiopia, 33.9% (16).

The HS-RDT (Alere™/Abbott Malaria Ag P.f RDT) was specifically developed to improve the detection of low-density infections with a reported tenfold higher limit of detection than conventional RDTs (10,12). Nonetheless, a key factor of RDT performance is the overall HRP2 carriage in the population, which itself is determined by *P. falciparum* infection density (17–19). Indeed, sensitivity varied with malaria prevalence, with the highest values in low to moderate transmission villages and in the 5 to < 20 years age group, which has the highest prevalence of P.f. infections. Such findings are probably determined by the repeated exposures to malaria infections, resulting in higher parasite density and associated levels of HRP2 antigen reaching the detection limits. Additionally, false negatives, i.e., negative by HS-RDT and positive by PCR, were more common in individuals with very low parasite density (< 10 parasite/µl) for which the higher HS-RDT limit of detection was not sufficient to detect the low level of circulating HRP2 antigen. In a previous study done in The Gambia, about 10% of children carried infections with extremely low densities, detectable only by molecular tests, while both microscopy and RDT identified less than 10% of these infections (9). Nevertheless, low-density infections may still be infectious to the vector or may progress towards clinical disease (19).

Overall, the disappointing low sensitivity of HS-RDT raises the question of the added valued of this test for the elimination efforts. HS-RDT has been developed to improve the detection of low-density infection and used in low-transmission settings for mass screening and treatment (MSAT) or active or reactive testing and treatment. The impact of these interventions was limited in the

trials that used conventional RDTs (14,20–22). Therefore, these approaches are not recommended by WHO (23). It was argued that MSAT with HS-RDT would have a higher impact because they would be able to identify most infected individuals. This does not seem the case as the poor performance of HS-RDT will probably result in a limited impact of MSAT using these tests. With the decline of malaria burden, it is important to understand the potential use of HS-RDT in low-transmission and near-eliminating areas where MSAT and MDA strategies are likely to be applied (1,3,23). However, the poor performance of the highly sensitive RDT would potentially limit its use for malaria surveillance and MSAT. Additionally, mathematical modelling predicted a greater impact of MDA as compared with MSAT(8); in low transmission settings, MSAT with HS-RDT at 85% coverage would achieve similar impact than that of MDA at 65% coverage (8). Achieving effective coverage up to 85% is operationally challenging, requiring active community engagement, good organization and coordination (24). Currently, the WHO recommends that highly sensitive techniques able to detect low-density infections (below 100 parasites/µl) should be used only for research purposes until sufficient evidence on their impact on transmission is available (21). Indeed, several research questions on the relative importance and contribution of low-density infections to transmission and on the public health impact of strategies incorporating HS-RDT in different epidemiological settings remain unanswered. For example, what is the proportion of infections to be detected and treated to accelerate the reduction of transmission towards malaria elimination? What is the cost– benefit for health systems using HS-RDT for specific target groups and in elimination settings? What are the most cost–effective deployment strategies for HS-RDT?

This study has several limitations. The diagnostic performance was conducted using dried blood spot which might have not provided a sufficient volume of blood to perform the molecular analysis. Additionally, HS-RDT results could have been affected by storage conditions. The HS-RDT were kept at room temperature and may have been exposed at temperatures above 30°C (recommended maximum). Parasite factors such as HRP2 deletions may have occurred, although this is unlikely as it has not been reported from The Gambia.

## 5.7 Conclusion

HS-RDTs were developed to detect low-density infections, to treat them and thus contribute to malaria elimination efforts. However, given their poor performance, their impact on transmission would be limited if used in interventions such as MSAT.

## 5.8 Acknowledgements

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## 5.9 Authors' contributions

EDD participated in the design and implemented the study, performed statistical analyses, interpreted the data, wrote the first draft. HN and BB performed the molecular analyses and interpreted the laboratory data. AB provided support in data collection and obtaining approvals. AJ and AJ performed the laboratory sample collection in the field. LS provided oversight of the data management. JPV interpreted the data, reviewed the draft. UDA and AE designed the study, interpreted the data and reviewed the draft. All authors read and approved the final manuscript.

## 5.10 Competing interests

The authors declare that they have no competing interests.

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# Chapter 6 Perceptions and acceptability of the Controlled Human Malaria Infection (CHMI) model in The Gambia: a qualitative study

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## 6.1 Abstract

Controlled human malaria infection (CHMI) studies, i.e. the deliberate infection of healthy volunteers with malaria parasites to study immune response and/or test drug or vaccine efficacy, are increasingly being conducted in malaria endemic countries, including in sub-Saharan Africa. However, there have been few studies on the perceptions and acceptability of CHMI by the local communities. This qualitative study assessed the perception and acceptability of such studies in The Gambia following the first CHMI study conducted in the country in March-May 2018. Data were collected through non-participant observation, in-depth interviews and focus group discussions and analyzed using NVivo 12 software with an inductive-deductive approach. Sixty-seven participants were involved, including volunteers enrolled in the CHMI, community stakeholders and members of the Gambian Ethics Committee. Respondents expressed a positive view about CHMI. Key motivating factors for participation were the financial compensation, comprehensive health checks, and willingness to support malaria research. Risks associated with participation were considered low. Concerns raised included the frequency of bleeding and the blood volume collected.

## 6.2 Introduction

Progress towards the vision of a malaria-free world has recently stalled, with 2 of the 4 goals of the Global technical strategy (GTS) for malaria off track [1, 2]. Indeed, the expected reduction by at least 40% of both malaria mortality and morbidity by 2020 as compared to the 2015 levels has not been achieved. To reverse this trend, novel and innovative interventions are needed. These may include safe and effective vaccines and drug products with the potential of interrupting malaria transmission to ultimately achieve elimination [3, 4]. However, vaccines and drug products development is a lengthy, complex process requiring substantial resources and time [5, 6]. For example, the timeframe for vaccine development can be up to 18 years, with a cost between USD200 million to USD900 million and an overall probability of success of approximately 11% [5, 7].

Controlled human malaria infection (CHMI) studies consist of deliberate infection of healthy volunteers with malaria parasites, either by mosquito bites or direct injection of sporozoites or parasitized erythrocytes. These well-controlled proof of concept studies allow to both understand the development of the immune response against malaria infection, and to rapidly screen for potential vaccine and drug candidates [8–12]. They are substantially smaller (only tens of participants), shorter (can be completed in a few weeks), and less expensive than large clinical trials, and allow for the selection of candidate vaccines and drug products worthy of further investigation in larger field trials [5, 13]. Unlike large field trials, the CHMI enables the investigators to entirely control the exposure to malaria parasites both in terms of strain and dose [6, 14]. Thus, CHMI studies are valuable tools to accelerate vaccines and drugs products development.

Since the first well-documented CHMI with laboratory-reared infectious mosquitoes carried out in 1986 at the US Walter Reed Army Institute of Research (WRAIR), the number of CHMI studies have increased in the United States and Europe, and are increasingly being conducted in malaria-endemic countries, including in sub-Sahara Africa [6, 15, 16]. Because of population differences related to naturally acquired immunity, genetics, nutrition, etc., it is important to conduct CHMI studies in malaria-endemic countries rather than ‘northern’ malaria-naïve countries to allow early assessment of vaccine and drug efficacy in a population with pre-existing malaria immunity [17, 18]. Conducting CHMI studies is also important in building the capacity and infrastructures of research institutions in endemic countries and enables African researchers to become involved in the earlier stages of vaccine or drug development [9, 10]. However, the deliberate infection of healthy volunteers with malaria parasites violates the fundamental principle in medicine of “*primum non nocere”* (“first, do not harm”) and raises multiple ethical concerns. In CHMI studies,

volunteers have little or no direct benefit from their participation. Instead, CHMI studies aim at advancing scientific knowledge for public health gains. Selecting a specific category of volunteers from the same area raises the concern that relatively privileged populations may be the primary beneficiaries of research conducted in underprivileged populations. Time commitments, discomfort of being infected, and study procedures can be burdensome. In addition, volunteers are often confined (which can prevent them engaging in their daily activities) for close monitoring and to prevent inadvertent transmission to a third-party [14]. Financial payment for compensating time lost may lead to undue inducement, especially in populations of low socio-economic status [19]. Therefore, careful, and rigorous ethical reviews are vital to ensure the safety of volunteers while maximizing scientific gains. In addition to ethical concerns, it is crucial to understand the perception and acceptability of CHMI studies in communities where they are done as this is key for their success [20]. However, until now, few qualitative studies have evaluated perceptions and acceptability of volunteers and communities' stakeholders. As an ancillary study of the first CHMI study conducted in The Gambia in 2018, we assessed perceptions and acceptability of a CHMI study among volunteers' and malaria endemic rural communities.

## 6.3 Controlled Human Malaria Infection study in The Gambia

The CHMI study was implemented between March and May 2018 at the Clinical Services Department of the Medical Research Council Unit The Gambia at the London School of Hygiene and Tropical Medicine (MRCG at LSHTM), situated in Kanifing, an urban area near the capital city Banjul [21]. Its aim was to assess the parasite kinetics and functional immunity in Gambian adults following PfSPZ Challenge administration. Briefly, this was an open-label, non-randomized clinical trial. The study screened healthy male volunteers aged 18–35 years from tertiary learning institutions; and a total of 19 individuals were enrolled in the study. All participants were administered an intravenous dose of 3.2 x $10^3$PfSPZ Challenge and were closely followed for 28 days. As financial compensation for the time lost by participating in the study, each volunteer received USD 160 over the period of follow-up.

## 6.4 Methods

### 6.4.1 Study settings

The qualitative study was implemented in two very different settings. The first was Kanifing, West Coast Region, where the volunteers for the CHMI study were recruited. Kanifing is an urban environment and part of the greater Banjul (capital city) area. The second setting was Basse, Upper River Region, where most malaria research projects are conducted. It is a rural setting,

approximately 375 km from Banjul, with moderate but highly seasonal malaria transmission, mainly between September and December [22, 23].

### 6.4.2 Data collection

Data collection was carried out in two stages. During the first stage, non-participant observation, informal conversations, and exit questionnaires with the CHMI trial participants were done concurrently to the trial, between April and May 2018. The second stage, carried out between January and December 2019, consisted of in-depth interviews (IDIs) and focus group discussions (FGDs) with CHMI trial participants (volunteers), members of the Gambian Ethics Committee (EC), and community stakeholders from both study areas. Respondents enrolled in the CHMI study were contacted through a phone call by the research team while respondents from EC and the community stakeholders were identified through a professional network of MRCG at LSHTM field assistants. Sampling was purposive to ensure maximum variation in personal viewpoints, age, sex, education and professional background, and to reflect differences in residence (urban *versus* rural setting). Interviews lasted 20 to 60 min and were conducted face to face and audio-recorded. The respondents chose a convenient time and location for the interviews. An interview guide with open-ended questions was used to facilitate reflection and dialogue with the respondents. The interviews were led by the two lead co-investigators (EDD and NB) and covered several broad domains, including benefits and perceived risks, reasons for participation, selection of volunteers, decision-making process, and financial compensation. Participants' recruitment continued until theoretical saturation was reached. The data collection team were all staff from MRCG at LSHTM.

### 6.4.3 Data analysis

All interviews were transcribed, translated into English where indicated (interviews conducted in local languages) and managed using NVivo 12 software. Data were analyzed using thematic analysis with an iterative process. The themes were developed both deductively (from literature) and inductively (from the emerging themes in the transcripts)[24]. Emerging themes were compared to identify similarities and differences based on respondents' characteristics. Data processing and analysis were processed and analyzed by the two lead co-investigators (EDD and NB) with the support of the research team in an iterative process.

### 6.4.4 Ethics clearance

The study was reviewed and approved by the Gambia Government MRC joint Ethics Committee (SCC 1615). Written informed consent was sought from all participants for the interviews (IDIs

and FGD) and for audio recording. All methods were carried out in accordance with relevant guidelines and regulations.

## 6.5 Results

**Participants characteristics**

A total of 67 participants were recruited; 43 males and 24 females whose age ranged from 18 and 70 years. We conducted a total of 31 IDIs (n=31) and 6 FGDs (n=36). In Kanifing, IDIs included 8 respondents enrolled in the CHMI study, 3 members of the EC and 8 respondents from the community while the two FGDs included 12 respondents. In Basse, IDIs included 12 respondents while the 4 FGDs 24 respondents. Respondents were farmers, religious leaders (Imam), head of households, students and self-employed (Table 1).

Table 6.1 Characteristics of the respondents in the CHMI qualitative study

| Characteristics | Volunteers enrolled in the CHMI study n=8 | Gambia Ethics Committee members n=3 | Community stakeholders n=20 | Community stakeholders n=36 |
|---|---|---|---|---|
| | In-depth interviews | | | Focus group discussions |
| Age range | | | | |
| 18-35 | 8 | - | 5 | 20 |
| 36-45 | - | - | 5 | 10 |
| 46-65 | - | 3 | 5 | 6 |
| 65-70 | - | - | 5 | - |

| | | | | |
|---|---|---|---|---|
| Gender | | | | |
| Male | 8 | 3 | 12 | 20 |
| Female | - | - | 8 | 16 |
| Education level | | | | |
| None | - | - | 4 | 6 |
| Primary education | - | - | 6 | 10 |
| Secondary education | - | - | 8 | 16 |
| Tertiary education | 8 | 3 | 2 | 4 |
| Occupation | | | | |
| None | - | - | - | 6 |
| Student | 8 | - | 5 | 8 |
| Subsistence farmer | - | - | 8 | 12 |
| Religious leader(Iman) | - | - | 2 | 2 |
| Self-employed/business | - | 1 | 3 | 4 |
| Employed/Civil Servant | - | 2 | 2 | 4 |
| Location | | | | |
| Kanifing (West Coast Region) | 8 | 3 | 8 | 12 |
| | - | - | 12 | 24 |

| | | |
|---|---|---|
| Basse (Upper River Region) | | |
| | Total in-depth interviews | Total focus group discussions |
| Kanifing (West Coast Region) | 19 | 2 |
| Basse (Upper River Region) | 12 | 4 |

**Perceived benefits**

Overall, the eight respondents previously enrolled in the CHMI study considered their participation as a positive experience. There were several points that were considered extremely positive, including the detailed information sheet provided prior to enrolment and the opportunity to ask questions for clarification; the extensive health check, including laboratory tests, that confirmed they were healthy prior to enrolment; the accommodation in a residence near the study clinic throughout the follow up period, with free access to internet; the professionalism of the research staff; and the financial compensation. Being accommodated together with other study participants was particularly appreciated for the opportunity to meet other study participants with whom they could establish friendly links as stated by one of the respondents *"I really enjoyed the CHMI study, I made new friends and had access to internet all throughout, that was great for me as a student to learn more through internet… The research team was so nice and friendly. I also made some money which definitely helps me a lot because as a student I have no income…."* (CHMI study participant 1).

Respondents were also happy to contribute improving knowledge on malaria by participating in this research project and appreciated that the CHMI study was conducted in The Gambia where malaria is a major public health problem. A student stated: *"There is no malaria in the developed world, CHMI study should be conducted where malaria is a problem like in The Gambia, so it is important and useful for us to volunteer and help scientists to know more about malaria and how to treat it"* (CHMI study participant 2).

Similarly, respondents from the EC commented on the impact of CHMI studies, arguing of the public health benefits. They felt that the ethical acceptability of the CHMI studies should be related to generating scientific knowledge that is particularly relevant to the local communities. They argued that if scientifically sound, a CHMI study should be conducted in countries like The Gambia where malaria is a public health problem. As stated by a member of EC "*Yes…! It makes sense to conduct such studies in The Gambia. Our people suffer a lot from malaria"* (EC member 1). Although they acknowledged the expertise and experience of MRCG at LSHTM in malaria research, they stated that the capacities of other stakeholders involved in granting permission for the conduct of the CHMI study, such as National Regulatory Authority and the EC, should be strengthened by specific trainings. Such trainings should build scientific, ethical review, and regulatory capacity for CHMI studies in The Gambia to ensure that these studies are conducted according to the highest standards as mentioned by an EC member: *"Although these studies have been conducted in the western world for a long time, they [CHMI studies] are new in The Gambia. As members of Ethics committee we need to understand the implications of such studies through specific trainings so that we can ensure that the studies are conducted to high standard for the safety of the study participants"* (EC member 2).

Most respondents from the wider community, regardless of provenance and socio-economic status, indicated that it would be acceptable to conduct CHMI studies in The Gambia though the majority had never heard of such studies as indicated by a respondent *"I have never heard such studies conducted within our community, I know that mosquito bites cause malaria but to infect a healthy person with malaria, this is my first time of hearing such…."*(Male, community stakeholder 1, Basse). However, most respondents argued that malaria is highly prevalent and such studies should be welcomed as they would improve scientific knowledge which would ultimately help defeat malaria in The Gambia. However, these views were influenced by the technical expertise and trust in the long-standing collaboration between MRCG at LSHTM and the local communities as mentioned by a stakeholder in Basse *"MRC has been working in The Gambia for a long time, they [MRCG at LSHTM] help a lot our communities. I remembered I beneficiated from MRCG at*

*LSHTM treatment, and I know they do a good job, so I feel like supporting MRCG at LSHTM work which in return would contribute to advance malaria research and this would be beneficial to our communities in the future.* (Male, community stakeholder 2, Basse).

**Perceived risks**

All respondents enrolled in the CHMI studies indicated that they had experienced minor malaria symptoms during the follow-up period but without consequence on their daily activities. Most interviewees from the wider community and the members of EC thought the risk of participating in the CHMI study was low. The perceived risks in relation to safety were mitigated by several factors, including previous exposure to malaria, malaria as a curable disease, and trust in the Research Institution (MRCG at LSHTM) as indicated by a student *"I had malaria several times in my life, and I know if you get malaria there are good medicines even when you have severe malaria doctors can cure you…. So, for me I did not see any problem or a major risk of taking part of this study conducted by MRCG at LSHTM"* (CHMI study participant 3). On the other hand, a few respondents expressed some concerns about the frequency and volume of blood collections. A student said: *"You know…. we do not have enough blood…. and you want to collect blood again and again.… how would that person feel at the end?"* (CHMI study participant 4). Additionally, a few respondents raised concerns about the study schedule which had interfered with their own university lectures schedule. They admitted that the information was provided in the information sheet. However, they did not anticipate these potential disruptions as mentioned by a student "*At the start, I did not realize it would be difficult to concurrently attending classes and the study schedule, I missed few classes especially during the first week post challenge where we had frequent medical checks…. I was in the study clinic in the morning and in the afternoon and we were also asked to stay for observation …. So, I ended up missing classes …"* (CHMI study participant 5).

**Motivation for participation and participation in future studies**

Financial payment as a compensation for time lost was the first motivating factor when interviewing the respondents enrolled in the CHMI study. They mentioned that the money was used for their daily expenses, including school fees, restaurant bills, mobile phone credit cards, data for internet, books, and stationery. A student stated: *"The compensation was great, it helped me a lot… I paid part of my school fees and used some of the money to buy personal stuffs and for transport fare"* (CHMI study participant 6). Similarly, respondents from the wider community valued the monetary compensation. However, there were mixed answers when asked what a fair

compensation would be. While elderly respondents felt that they should rely on what the research institution offers, young respondents regardless of the provenance expressed that the amount should be higher than what was given to the volunteers in the CHMI study. Nonetheless, respondents from the EC stated that the amount of financial payment to the study participants as compensation for their time lost was fair given the number of days volunteers were confined in a residence and the discomfort and burden of the study procedures. However, one respondent from the EC stated that the amount provided is higher than what a student would expect if involved in a professional activity and raised a concern for potential inducement. He mentioned that the amount of cash should be indexed on a stipend for a student if this category was to be recruited "*I felt the financial compensation is quite high given the Gambian context. A good approach could be to align the compensation with local wage or students' stipend"* (EC member 3).

While respondents from the EC viewed the free comprehensive medical check as intrinsic to the CHMI study, most respondents enrolled in the CHMI study considered it as the second motivating factor for participation. They stated that a comprehensive medical review would not have been possible otherwise as commented by a student *"For a long time, I wanted to go to hospital for check-up, I wanted a doctor to check my heart…. I know you guys are doing electrocardiogram (ECG) tests…., in town this exam is quite expensive, and I cannot afford it…. You know, we students we have no money to check our health condition."* (CHMI study participant 7).

Another motivating factor was the willingness to contribute to malaria research. Irrespective of provenance, age, education and professional background, respondents felt their participation was important as a way to contribute to malaria research which would ultimately benefit their community. Nonetheless, the expertise, trust, and longstanding relationship of MRCG with the local communities influenced their willingness to participate.

When asked if they will be happy to participate in future CHMI studies, most respondents from the CHMI study and community stakeholders reported their willingness to participate in future CHMI studies. *"I did not encounter any problems while participating in the study, I know there is no problem in participating…. So, I would take part in another CHMI study"* (CHMI study participant 2). Some mentioned that they will recommend their friends and family to join the study as mentioned by a stakeholder in the community "*I will encourage my family members to participate in future CHMI studies*" (Male, stakeholder 3, Kanifing).

**Decision making process**

All respondents enrolled in the CHMI study consulted their family before taking the decision to participate. Opinions of the family members were key in the decision-making process. The decision-making process involves informing parents and respected community members, discussing and balancing inconvenience and advantage, and obtaining permission or favorable opinion from the family members. While most young respondents stated they will first inform and discuss with their mother to obtain their permission, some elderly respondents argued that they will seek advice from religious leaders (Imam) and the elderly in the community. Parents' opinions and approval was key and was considered as a norm and societal value. Respondents felt it is important to receive a "blessing" from their parents before taking part to a study. A student said: *"That is how it should be as a human being you need to have parents or guardians who look after you so whatever you want to engage yourself into inform them and seek for advice and blessings"* (Male, Community stakeholder 4, Basse).

**Selection of participants**

Respondents from the wider community indicated that the selection of participants should include all community members, regardless of the level of education. Most respondents from Basse, where most MRCG at LSHTM malaria projects are implemented, stated that community members can understand what the study is about provided the right information is given in local languages as stated by a community stakeholder: *"Many people in our communities are illiterate…. but when they are involved in MRCG at LSHTM work, they understand… they sign (thumbprint) the informed consent, they follow the study procedures… and I never heard that a participant has been excluded because he failed to understand the study"* (Community stakeholder 5, Basse). Some added that visual aids (videos and pictorials) could facilitate comprehension of this new concept.

## 6.6 Discussion

This study reports on the perceptions and acceptability among participants and community stakeholders of the first CHMI study implemented in The Gambia. Overall, most respondents expressed a positive view about this type of study, with all prior CHMI volunteers showing enthusiasm on their participation. Similar positive experiences were also reported by Njue et al in Kenya [25]. The financial compensation offered in exchange of the time lost was a key motivation as volunteers were asked to stay at a hotel near the clinical services for safety reasons and to facilitate their follow up. The agreement of the volunteers' parents for their participation was

extremely important as well as the long term and well-established expertise of the MRCG at LSHTM and its longstanding relationship with the local communities. Given the nature of the CHMI, namely the infection of healthy volunteers with malaria, its implementation would have been extremely difficult, if not impossible, without the trust of the local communities have in the implementing institution.

Despite these positive perceptions and attitudes, some concerns for study procedures were expressed and were mainly related to both the frequency and volume of blood sampling. This is not surprising as similar concerns are usually expressed for studies with a less intense blood sampling schedule [26]. In Kenya, CHMI volunteers were also concerned about blood sampling which was found to be burdensome [25]. Collecting blood samples from individuals recruited into clinical research projects in sub-Saharan Africa can be challenging and often related to rumours of “blood stealing” or “blood selling” [26, 27]. Such rumours represent a social diagnosis and a logical attempt to make sense of the clinical trial in today’s world that should be countered by communicating with research participants in culturally appropriate ways and by addressing their concerns. CHMI volunteers received exhaustive explanations about the study and its procedures and none of them expressed any fear about the improper use of the blood samples collected. Nevertheless, they probably underestimated the inconvenience related to the daily visits, blood draws and confinement, as happened also in Kenya and in the USA [25, 28].

Because CHMI studies are complex and logistically challenging, it is generally thought that recruiting study participants with higher level of education would facilitate the informed consent process as these participants would be in better position to understand the study aim and procedures than uneducated ones [14, 29]. This is the reason why most CHMI studies included volunteers with at least a tertiary education level [9, 10]. Nevertheless, many stakeholders in our qualitative study expressed strong support for the recruitment of volunteers with a lower education level or even illiterate as they would be able to comprehend study procedures if explained in local languages and with visual aids. A qualitative study carried out in Kenya reported that less educated individuals are able to provide adequate informed consent, especially with well-designed community engagement and multiple opportunities to discuss and clarify the study procedures [25]. Moreover, selecting only individuals of a certain level of education may be unethical and would raise several concerns. Indeed, highly educated individuals do not fully represent the communities to be targeted by an intervention such as a new vaccine. Less educated individuals are probably at higher risk for malaria and excluding them would be unfair, resulting in their exclusion from the benefit of study. Therefore, fairness in the selection of CHMI

volunteers is key as well as evaluating the specific vulnerabilities of the potential study population as it would reduce the risks of burden and harms [16].

There has been considerable debate in the scientific community regarding monetary payment of study participants involved in clinical research [30]. It has been argued that the perceived risk and level of burden of CHMI justified higher compensation for study participants [20, 31]. However, this may unduly induce study participation by impairing decision-making, with participants potentially accepting more risks than they would usually accept and thus "invalidating" the informed consent process [14]. In our study, the financial payment was a key motivating factor for accepting to be part of the CHMI, similar to Njue *et al* in Kenya and Kraft *et al* in the USA [25, 28]. However, many volunteers acknowledged that the comprehensive medical checks, trust in the research institution were important determinants of their participations. They further indicated their willingness to participate in future CHMI studies.

Because monetary payment had positive effects on respondents' willingness to participate in research [20, 30], it is vital that the Institutional Review Board (IRB) and Ethics Committees cautiously determine the appropriateness of the level of financial payment. A study in Kenya where volunteers received USD250-500 [10, 25] resulted in a short-lived controversy in the local media [32, 33]. Indeed, an article in a local newspaper titled "Want Cash? Volunteer for a dose of malaria parasite" suggested this was a quick and easy economic activity for participants. In response to the article, the research Institution issued a statement detailing the rationale of the study, its procedures, and the reason for the level of payment. In The Gambia, most members of the Ethics Committees indicated the level of compensation was fair after extensive discussions before the study received ethical approval. However, young respondents in the wider community suggested CHMI volunteers should be compensated with a larger amount given the level of burden, raising the question on how to determine an acceptable amount. Dickert and Grady recommend the adoption of the wage payment model by which payment is based solely on standard wage payment for unskilled labour, with additional payments being made for uncomfortable procedures (34). This model reduces undue inducement concerns, standardizes payment schedules, and establishes a system in which payment is based on the contribution subjects make, consistent with the principle of equal pay for equal work [34].

**Limitations**

The method of selection of the respondents using a professional network of MRCG at LSHTM's fieldworkers may have resulted in selection bias. However, to minimize it, we ensured that

respondents varied in terms of age, education and professional background, and provenance. Another bias is that this qualitative study was an ancillary study of the CHMI trial and data collection was done by MRCG at LSHTM's staff involved in both studies. This may have resulted in respondent bias as some respondents may not have felt at ease in discussing or revealing some negative views about the study. Nevertheless, the study provides an insight into the perception and acceptability of the CHMI model in The Gambia.

## Conclusion

There have been recent calls for more CHMI studies in malaria endemic settings to accelerate vaccine development and test new interventions in communities with the highest disease burden (8,16). The impact of CHMI studies on the communities justifies their conduct in malaria endemic countries such as The Gambia. Weighing the potential benefits and burdens associated with CHMI studies requires a careful and rigorous ethical and scientific review of the study protocol and should also consider local communities' perception and acceptability. Findings from our study indicate that CHMI studies are acceptable for Gambian communities but are greatly influenced by the longstanding trust and relationship between local communities and MRCG at LSHTM. Nonetheless, all stakeholders involved in CHMI studies (investigators, IRB, EC, and local communities) need to adopt policies and guidelines to adapt CHMI studies to the local context and ensure risks are appropriately minimized during their implementation.

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## Author contributions

EDD designed and planned the study. UDA, JA and MMA reviewed the study proposal. EDD and NB conducted the interviews and data analysis. EDD wrote the first manuscript. AF, NB, JA, JPVG, AE, MMA and UDA critically reviewed the manuscript. All authors have reviewed and approved the final the manuscript.

## Data availability

Data are available from the corresponding author on a reasonable request.

**Competing interests**

Authors declare no competing interest.

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# Chapter 7 : Discussion

After the substantial reduction of the malaria burden that occurred over the past 2 decades, progress has recently stalled, with 2 of the 4 goals of the Global Technical Strategy (GTS) for malaria off track (1,2). In 2017, WHO reported that the number of malaria cases had levelled off; for a second consecutive year, no new gains were made (3). It is widely acknowledged that with the current tools, malaria elimination may not be achieved, underlining the need for new tools and interventions to achieve elimination. The GTS, adopted by the World Health Assembly in 2015, promotes 3 strategies, namely universal access to prevention and treatment, acceleration of efforts towards elimination; and malaria surveillance as a core intervention, and 2 supporting elements (research and a conducive environment) to guide global malaria elimination efforts (4).

This thesis explores in the context of The Gambia some of the potential interventions to support malaria elimination efforts. It focuses on mass treatment strategies to accelerate transmission reduction and on the performance of highly sensitive antigen-based diagnostic tests for improved surveillance and mass testing and treatment (MTaT). It also explores the local communities' acceptance of research using Controlled Human Malaria Infection (CHMI) models as these can be used to evaluate new treatments and vaccines.

## 7.1 Mass treatment strategies for accelerating reduction of malaria transmission

The Gambia has witnessed a substantial reduction in the malaria burden and the country is now targeting elimination by 2030 (5). Nevertheless, despite the high coverage of standard control interventions, malaria transmission, which is markedly seasonal, has not been interrupted and has become increasingly heterogenous (6,7). In a low transmission setting such as The Gambia, the challenge to achieve elimination is to target the hidden human reservoir of infection.

The two main strategies to directly target the human reservoir of infection are mass drug administration (MDA) and MTaT. MDA typically consists of treating all members of a community, regardless of their infection status, with a full course of an antimalarial treatment while MTaT consists of screening and treating only positive cases (8). The WHO, in countries such as The Gambia where coverage of vector control interventions is already high, recommends MDA with an artemisinin-based combination treatment (ACT) as a strategy to accelerate reduction of local transmission (2,9). The aim of this intervention is to reduce transmission by quickly reducing the parasite biomass in a community and to prevent new infections for a certain period. Repeated rounds of MDA can clear parasites in asymptomatic infected individuals and prevent new

infections. To quickly reduce and potentially interrupt transmission and avoid resurgence, several rounds are required, in combination with other existing malaria control interventions such as effective vector control, access to prompt diagnosis and treatment, and intensified surveillance.

MDAs strategies have been modified to address the clustering of malaria infections in high-risk locations (hotspots) and populations (hot-pops). These approaches are the 'targeted' and 'reactive' strategies (9). The targeted strategies consist of administering mass treatment to groups at higher risk of infection within a population while reactive strategies are triggered by confirmed malaria cases.

In addition to high coverage, adherence to treatment is key to achieve the desired impact of an MDA campaign. Artemether-lumefantrine and dihydroartemisinin-piperaquine are the ACTs used for treating uncomplicated malaria in The Gambia; as first and a second line treatment, respectively. Dihydroartemisinin-piperaquine is often used for MDA because of its simple dosing schedule, long post-treatment prophylactic period [13–15], and good safety profile [16]. However, ACTs are primarily employed for the treatment of uncomplicated malaria and ideally those used for the treatment of malaria patients should not be employed for MDA campaigns because of the potential risk of selecting resistant parasites.

Pyronaridine-artesunate (PA), a registered fixed-dose of ACT, is highly efficacious and well tolerated and could be used for MDAs. Despite its simple dosing schedule, one dose per day for three days, some malaria infected individuals may not complete the 3-day treatment course during an MDA campaign as most of them would be asymptomatic. Nevertheless, parasite density in asymptomatic malaria-infected individuals is usually lower than in clinical cases and an incomplete treatment may be sufficient to clear the infection. As part of the doctoral work, we implemented a clinical trial to assess the efficacy and safety of PA at different dosages (full or incomplete treatment) in asymptomatic *P. falciparum*-infected individuals. A total of 303 participants were included and randomized to the 3-day, 2-day or 1-day regimen. Day 28 PCR-adjusted Adequate Parasitological Response was 100% for both the 3-day (98/98) and 2-day regimens (96/96), and 96.8% (89/94) for the 1-day regimen. There was no difference in adverse events between the three study groups; most adverse events were of grade 1 or 2 severity (85% [136/160]). These results are encouraging and suggest PA could be used for MDA as it is safe and efficacious, even when treatment is incomplete.

In The Gambia, MDA is probably most effective if implemented repeatedly during the dry season when vector and parasite densities are at their lowest, to reduce the prevalence of infection before

transmission surges (10). Combining an antimalarial treatment with a gametocytocidal drug may further reduce transmission. PA administered during MDA campaigns could be complemented by a single low-dose of an 8-aminoquinoline such as primaquine to clear mature *P. falciparum* gametocytes. However, there are concerns about the safety of these drugs when used for MDA in populations where glucose-6-phosphate-dehydrogenase deficiency (G6PDd) is common. Nonetheless, the World Health Organization (WHO) recommends a single low dose of primaquine (0.25 mg/kg) as a gametocytocidal treatment in combination with an ACT for *Plasmodium falciparum* malaria for elimination and artemisinin resistance containment scenarios (9). Administration of the single low dose is safe and effective, even in G6PDd individuals, and can be given without G6PD testing. A phase 3 clinical trial to assess the transmission-reducing effect of combined PA and primaquine resulted in an important reduction of *P. falciparum* transmission to mosquitoes indicating the potential high impact of such an intervention when deployed at community level (11).

MDA with an ACT usually have a short-term impact and only a few studies showed sustained impact beyond six months post-MDA (12). In The Gambia, MDA with dihydroartemisinin-piperaquine decreased malaria prevalence and incidence of clinical disease during the first three months after the intervention. Such reduction was maintained in low-transmission areas, but not in eastern Gambia, where transmission is moderate (13). This is largely due to residual transmission whereby transmission persists despite universal coverage with ITNs and/or IRS.

In eastern Gambia, some malaria vectors tend to bite and rest preferentially outdoors, thus escaping the standard vector control interventions. Therefore, to sustain gains and accelerate elimination efforts, innovative vector control tools are needed. Systemic insecticides such as ivermectin target mosquitoes regardless of their biting patterns and could contribute to decrease residual transmission. Given the limited duration of protection post-MDA with an ACT alone, combining an ACT with a mosquitocidal agent could be a new approach for malaria control. Such a combination would have a synergistic effect with the ACT, reducing the population parasite biomass and providing post-treatment prophylaxis while ivermectin would reduce vector densities and thus the number of infectious bites during and after the intervention. Furthermore, ivermectin would likely reduce the minimal coverage required by MDA as mosquitoes, by feeding on several individuals over a short period, may take a toxic dose of ivermectin from any one of them. Therefore, as an objective of this doctoral work, we determined the impact of mass drug administration of dihydroartemisinin-piperaquine and ivermectin on malaria transmission. The intervention was evaluated by implementing a community-based cluster-randomized trial that

included 32 villages randomized to either the intervention or control group (n=16 in each group). These villages were identified after screening 47 villages for eligibility. The intervention decreased malaria prevalence by about 60% (odds ratio [OR] 0.30, 95% CI 0.16–0.59; p<0.001) and vector density by 58% (OR: 0.39, 95% CI 0.20- 0.74, p<0.005); although it did not affect vector parity (OR: 0.90, 0.66–1.25; p=0.537), a proxy of mosquito survival. The intervention was safe and well-tolerated and could potentially complement other malaria control measures.

MDA with ivermectin is expected to have important synergies with current core vector control tools, including mitigation of insecticide resistance (14,15). Ivermectin belongs to a different chemical class with a different mode of action than the insecticides used for malaria control. With raising concerns of insecticides resistance, ivermectin could play an important role in the management of insecticides resistance. Nonetheless, the optimal mode of delivery of mass administration of ivermectin should be considered. One option would be to integrate ivermectin delivery in the current SMC programme. Although the target populations of SMC and ivermectin MDA differ, the latter could benefit from the door-to-door delivery strategy for SMC to children, to also deliver ivermectin to the rest of the eligible population. Additionally, there may also exist the opportunity to create synergies with the National Control Programme of Neglected Tropical Diseases. Nonetheless, if ivermectin MDA is recommended by WHO, additional activities for its large-scale deployment would be required. These include the incorporation of ivermectin MDA as an intervention in the national strategic plans; specific delivery modes, metrics for entomological and epidemiological monitoring and evaluation strategies; engagement with key stakeholders and communities.

MDA, or any other chemoprevention intervention, could be associated with the administration of a malaria vaccine. To date, RTS,S is the only malaria vaccine recommended and prequalified by the WHO and it is to be used as an additional tool for the prevention of *P. falciparum* malaria in children living in areas of moderate to high malaria transmission. In Mali and Burkina Faso, children who received both RTS,S and SMC before the beginning of the transmission season had a significantly lower incidence of clinical malaria than those who received just one of the two interventions, suggesting that chemoprevention and vaccination may have a synergistic effect (16). The development of a malaria vaccine has focused on preventing malaria morbidity and mortality in children <5 years of age. Nevertheless, mathematical modelling shows that mass vaccination, when combined with MDA in a seasonally targeted manner, may substantially reduce malaria prevalence and, in some settings, interrupt transmission (17). There is currently shortage of RTS,S vaccine, with GSK able to provide 18 million doses for the next 3 years. However, a

new malaria vaccine, R21, similar to RTS,S was recently approved by WHO. If this vaccine is pre-qualified by WHO, its manufacturer could produce at least 100 million doses per year. This would probably open the possibility of using the vaccine in older age groups or for mass vaccination. Results of mathematical modelling should be confirmed by well-designed epidemiological studies that will investigate the impact of mass vaccination on malaria transmission.

While PA and ivermectin show promise for use in MDA, some challenges should be considered. Implementing MDA campaign is a complex, logistically challenging operation, requiring substantial investments of resources (human, financial and logistic) and careful organization, planning and coordination. Successful MDA campaigns depend on high coverage and adherence (i.e., > 80%) of the target population, thus requiring a high level of community engagement and participation. Implementation strategies should therefore ensure the highest possible level of community mobilization. Door-to-door distribution is generally preferred to centralized distribution at a fixed site, and directly observed treatment (DOT), where feasible, is the best way to ensure adherence to treatment as poor adherence could compromise the impact of the intervention and leads to selection of resistant parasites. Factors related to poor adherence include absence of symptoms, forgetting to take the tablets, side- effects (19,20). These factors should be addressed through community engagement. Additionally, concerns remain that MDA campaigns will increase drug pressure, resulting in the emergence and spread of drug resistance. Although there is no evidence that MDA with an ACT at therapeutic doses is related to the emergence of resistance, monitoring of resistance should be an essential component of an MDA campaigns (4).

MTaT, an alternative approach to MDA, consists of the treatment of infected individuals identified by active detection. WHO recommends MTaT as a strategy to target asymptomatic malaria infections to reduce its prevalence. MTaT allows minimizing antimalarial drug exposure in those who do not need them, thus reducing the risk of antimalarial drug resistance and enabling better use of resources. However, RDTs, the diagnostic test usually used for MTaT, has well-documented limitations, including limited sensitivity for low-density infections (i.e., <100 parasites/µL). Recent studies using sensitive molecular techniques have shown that conventional microscopy and RDTs miss many asymptomatic carriers that contribute to maintain residual transmission. This underlines the need for more sensitive tests, able to detect low-density parasitemia, and easily deployable at community level. Such highly sensitive diagnostic tests could be deployed for MTaT campaigns, malaria surveillance and for monitoring the impact of elimination strategies (21).

## 7.2 Highly sensitive diagnostic tests and elimination efforts

Field-deployable antigen-based RDTs are available, with promising results in their ability to detect low density *P. falciparum* infections (22,23). The highly sensitive RDTs (Alere™) has a 10-fold lower limit of detection than routine RDTs but evidence supporting their use in MTaT campaigns is limited. As part of the doctoral work, we assessed the field performance of Alere™ in asymptomatic malaria-infected individuals with low density parasitaemia. Our findings show a poor performance of the test, detecting only a few individuals with low-parasites density as compared to qPCR. These results confirm a previous study carried out in The Gambia in which the sensitivity of Alere™ was about 38% (24). In other low transmission settings such Myanmar, Papua New Guinea, Haiti and Ethiopia, the field performance of the highly sensitive RDT was poor (25–28). Such poor performance is probably related to the low concentration of HRP2, which is directly related to the parasite density.

In low transmission settings, including The Gambia, most asymptomatic malaria infections are of low density and largely detectable only by ultra-sensitive molecular tests (29). The poor performance of the highly sensitive RDT would potentially limit its use for malaria surveillance and MDA strategies. However, additional evidence is required. Currently, the WHO recommends that highly sensitive techniques capable of detecting low-density infections (below 100 parasites/µl) are used only for research purposes until there is sufficient evidence they can have a significant impact on transmission (21). Indeed, several research questions on the relative importance and contribution of low-density infections to transmission and on the public health impact of strategies incorporating highly sensitive diagnostic tests in different epidemiological settings remain unanswered. For example, what is the proportion of infections to be detected and treated to accelerate the reduction of transmission towards malaria elimination? What is the cost–benefit for health systems using highly sensitive diagnostics for specific target groups and in elimination settings? What are the most cost–effective deployment strategies for highly sensitive diagnostics tests?

A surveillance tool that may be useful in low transmission settings such as The Gambia is malaria serology. Serology can be used to determine transmission trends over time. The choice of the specific antigens targeted for serological monitoring will depend on their immunogenicity, seroconversion rates and the persistence of specific antibodies. Serological testing could provide useful population-level data to measure progress. Additionally, field PCR techniques such as loop-mediated isothermal amplification (LAMP) and real-time PCR methods have been developed to detect malaria infections in field working conditions and could play a role in elimination settings.

A study carried out in The Gambia reported that the LAMP is a field-friendly, sensitive diagnostic test that could be useful for MTaT campaigns. Similar results were also found in Zanzibar (30,31), indicating its potential for malaria elimination efforts. However, its acceptability and cost-effectiveness would need further evaluations.

Elimination efforts would require an enhanced surveillance system to detect, characterize and monitor all malaria cases. Surveillance would enable stratification and deployment of tailored interventions packages based on the epidemiological profiles at subnational level. Stratification is a WHO-recommended strategy to optimize malaria responses within a country with heterogeneous transmission. This approach emphasizes the need of country-led, data-driven approaches developing a national malaria risk stratification and shift away from a “one size fits all” to a more tailored malaria control approach, with packages of interventions deployed most efficiently.

## 7.3 Community acceptance of CHMI model for developing new interventions

To date, RTS,S and R21/Matrix M are the only malaria vaccine recommended by the WHO. Meanwhile, novel malaria vaccine candidates are under development. This is a lengthy and complex process, requiring substantial resources and time (32,33).

CHMI is powerful tool to accelerate the development of vaccine candidates and drug products. It consists of deliberately infecting with malaria parasites healthy volunteers, either by mosquito bites or direct injection of sporozoites or parasitized erythrocytes. These studies allow to both understand the development of the immune response against malaria infection, and to rapidly screen for potential vaccine and drug candidates (34–38). They are substantially smaller, shorter, and less expensive than large clinical trials, and allow for the selection of candidate vaccines and drug products worthy of further investigation in larger field trials (32,39). However, until recently few CHMI studies have been conducted in Low-and Middle-Income (LMIC) countries because of technical, clinical, ethical and regulatory issues, as well as cultural norms (34). The first CHMI study in The Gambia was conducted in 2018, with the aim to assess its feasibility and to determine parasite kinetics in naturally exposed Gambian adults after PfSPZ Challenge (40). Following the first CHMI study, we conducted a qualitative study to evaluate community perceptions and acceptability. Communities have a positive view about CHMI, and CHMI studies are acceptable for Gambian communities. Similar results were found in Kenya and Uganda, indicating the willingness of the communities to take part of the CHMI studies (41–43). Therefore, conducting such studies in malaria endemic areas offer several benefits, including building and reinforcing

local capacities in term of scientific expertise, clinical facilities, laboratory diagnostic, governance and regulatory; and offer the opportunities to accelerate or streamlining the development of vaccine and treatment for sub-Saharan Africa. Nonetheless, careful attention is required to ensure that international collaborations are conducted fairly and transparently, with a fair distribution of benefits and responsibilities, and appropriate oversight and governance.

## 7.4 Perspectives and conclusion

Malaria control in The Gambia has considerably progressed over the past 2 decades thanks to the scale-up of effective control measures, including vector control interventions and treatment with efficacious antimalarial medicines. The country is progressing towards elimination and set the goal of achieving elimination by 2030. However, although this might be feasible at sub-national or district level, country-wide elimination seems more difficult to achieve. There is the need of optimizing the use of existing control interventions and deploying new tools and interventions.

Mass drug administration with ivermectin has shown promising results in eastern Gambia, a region where coverage of vector interventions is high, but transmission persists. This intervention could be combined with a gametocytocidal drug to increase the impact of the intervention and accelerate interruption of transmission. Moreover, PA, the newly available ACT showed high efficacy and a good safety profile in asymptomatic, malaria infected individuals, even at the incomplete dosing, indicating its potential for MDA campaigns. These tools and strategies would play a key role in the elimination efforts and should be evaluated by the National Malaria Control Programme (NMCP) and rapidly integrated within the existing interventions and implemented at community level.

The drive towards elimination requires active community engagement as the effective delivery of malaria interventions, and coverage of community-based interventions, depends on their acceptance by all members of the local communities. Therefore, the capacities of existing community structures such as villages health workers, health sub committees, women and youth groups should be strengthened through training and sensitization to support the delivery of malaria interventions and the promotion of the malaria elimination agenda. Additionally, private sector involvement in planning and programme implementation should also be enhanced through training and routine monitoring and supervision.

Additionally, a point of care diagnostic test with high sensitivity is needed. The highly sensitive RDT diagnostic performance determined in eastern Gambia is low, limiting its contribution for malaria surveillance and MTaT campaigns. Nonetheless, research for additional tools, including diagnostics tools, vaccines candidates and drugs products are under development to support elimination efforts. CHMI studies are important for developing such tools and are increasingly carried out in sub-Saharan Africa. In The Gambia, our findings show that such studies are acceptable and communities are willing to participate, which is key for recruiting and retaining study participants.

Despite renewed interest for elimination, efforts are threatened by several challenges, namely resistance to first-line treatments for *Plasmodium falciparum* malaria and to the insecticides used for vector control, insufficient funding, and weak health systems. In The Gambia, while no evidence of resistance to the first line treatment has been documented, there is a rising resistance to pyrethroids, the insecticide used on bed nets, which warrants extensive monitoring. In addition, continued monitoring of molecular markers and therapeutic efficacy studies would help to identify and track the prevalence of molecular mutations associated with drug resistance.

Moving towards malaria elimination requires more personnel, commodities, and thus more financial resources to sustain the gains already achieved and scale up novel interventions, and progress towards elimination. This may be possible through multisectoral collaboration that will include other government departments (Agriculture, Water Resources, etc.), research institutions (MRCG at LSHTM), NGOs and international funding organisms. Nonetheless, for a more sustainable resourcing of the elimination push, the NMCP should prioritize efforts aimed at domestic financing.

Malaria elimination in The Gambia would require relentless commitment of multiple stakeholders at national and regional levels. In addition to political and financial commitment, strong partnerships with Senegal, the neighbouring country, is essential for integrated activities and cross-border initiatives, which would be key for achieving elimination.

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