IMPACT OF ARTHEMETHER LUMEFANTRINE TREATMENT, CIRCADIAN RHYTHM AND SERUM REPLACEMENT ON THE MOSQUITO INFECTIOUSNESS OF WILD *P. FALCIPARUM* GAMETOCYTES TO *ANOPHELES GAMBIAE SENSU STRICTO* MOSQUITOES

Dorin Joachim Mmasi

A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science in Public Health Research of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

February, 2026

ABSTRACT

Asymptomatic gametocytemic carriers serve potential harbour for *Plasmodium falciparum* gametocyte stage V, and a threat for malaria transmission from infected humans to *Anopheles* mosquitoes. Therefore, it is important to optimise direct membrane feeding assays (DMFAs) to evaluate transmission blocking interventions (TBIs) on the infectiousness of wild *P. falciparum* gametocytes to *Anopheles* mosquitoes. This study assessed the impact of artemether-lumefantrine (AL) treatment, mosquito feeding time (day vs. night), and serum replacement on the infectiousness of wild *P. falciparum* gametocytes to *Anopheles gambiae sensu stricto (s.s) mosquitoes*. To conduct this experiment, 6 gametocytemic carriers were found to be eligible and donated 4 mL of venous blood. This blood was offered to female *Anopheles gambiae s.s* mosquitoes via DMFA under controlled conditions. Oocyst prevalence and intensity were determined on fed mosquitoes: 1) 9 days post AL treatment, 2) day versus night feeds, and 3) with and without serum replacement. Mosquito infection rates declined post-AL treatment, with significantly fewer mosquitoes infected (odds ratio (OR) = 0.20, 95% confidence interval (CI) = 0.13-0.31, p=0.001) compared to day 0. Feeding during the mosquito night-time did not significantly affect mosquito infection rates (OR=0.77, 95% CI: 0.53-1.12, p=0.175). Lastly, serum replacement increased infection rates (OR = 1.73, 95% CI: 1.33-2.25, p = 0.001) compared to whole blood. In conclusion, we confirm that DMFA should be conducted using blood from gametocytemic participants without a recent history of AL treatment, using serum replacement to enhance infection success. In this setting, assays could be conducted during the daytime without affecting results.

Keywords: DMFA, AL, TBIs, *Anopheles gambiae ss*, gametocytes, oocysts, asymptomatic.

DECLARATION

I, Dorin Joachim Mmasi do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this dissertation titled *"Impact of arthemether lumefantrine treatment, circadian rhythm and serum replacement on the mosquito infectiousness of wild p. falciparum gametocytes to anopheles gambiae sensu stricto mosquitoes"* is my original work and has never been or intending to be submitted for a degree award in any other institution

Dorin Joachim Mmasi 

Candidate name	**Signature**	**Date**

The declaration is confirmed by:

Dr. Mgeni Tambwe 

Supervisor	**Signature**	**Date**

Prof. Sarah Moore 

Supervisor	**Signature**	**Date**

COPYRIGHT

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Senate of the Nelson Mandela African Institution of Science and Technology, a dissertation entitled "Impact of artemether-lumefantrine treatment, circadian rhythm and serum replacement on the infectiousness of wild *P. falciparum* gametocytes to *An. gambiae* sensu stricto mosquitoes in partial fulfilment of the requirements for the award of the Degree of Master of Science in Public Health Research of the Nelson Mandela African Institution of Science and Technology.

Dr. Mgeni Tambwe

Supervisor	**Signature**	**Date**

Prof. Sarah Moore

Supervisor	**Signature**	**Date**

ACKNOWLEDGEMENT

First and foremost, I am very thankful to the Almighty God for giving me the strength, health and perseverance to complete this work. I would like to express my deepest and sincere gratitude to my supervisors, Dr. Mgeni Tambwe and Prof. Sarah Moore, for their invaluable guidance, support, and encouragement throughout the entire process of this dissertation. Their expertise, patience, and insightful feedback have been instrumental in shaping this research and are highly appreciated.

I am grateful to the Nelson Mandela African Institute of Science and Technology (NM-AIST) and the Ifakara Health Institute (IHI) for providing the resources and facilities necessary for conducting this study. My sincere thanks to the Vector Product Testing Unit, especially the insectary and laboratory team, for maintaining the rearing of *An. gambiae* s.s colony.

A special thanks to Dr. Kafuruki Shubis, Dr. Dickson Lwetoijera, and Ms. Cecilia Fransis for their encouragement, valuable advice and constructive feedback that helped to shape my school life.

I extend my appreciation to the participants in this study from Wamimkoko, Kibindu, Chamgoi and Miono villages for their willingness to contribute their time and insights, without which this research would not have been possible.

I am indebted to my friends and colleagues, Ms. Prisca Kweyamba, Ms. Fatuma Matwewe, Transmission Zero team, Mr. Masudi Suleiman, Mr. Nolvin John, Mr. Dismas Kamande, Ms. Jane Machange, Ms. Aidi Lugenge and Ms. Jilly Mseti for their encouragement, discussions, and assistance during various stages of this research. Last but not least, exceptional thanks go to my family, who always had faith in my ability throughout my studies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABREVIATIONS AND ACRONYMS

AL	Artemether-Lumefantrine
CDC	Centre for Disease Control and Prevention
CI	Confidence Interval
DMFAs	Direct Membrane Feeding Assays
DSF	Direct Skin Feeding
IHI	Ifakara Health Institute
IRB	Institutional Review Board
IRR	Incidence Risk Rate
LM	Light Microscopy
mRDT	Malaria Rapid Diagnostic Test
MTRL	Malaria Transmission Research Laboratory
NIMR	National Institute for Medical Research
OR	Odds Ratio
pLDH	Parasite Lactate Dehydrogenase
RBCs	Red Blood Cells
SMFA	Standard Membrane Feeding Assay
TBDs	Transmission-Blocking Drugs
TBVs	Transmission-Blocking Vaccines
WBCs	White Blood Cells
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background of the Problem

Despite substantial progress in reducing malaria-related morbidity and mortality, the disease continues to affect millions of people annually, 249 million cases and 608 000 deaths, the majority from the WHO Africa Region (WHO, 2023). Although partial emergence resistance to artemether–lumefantrine (AL) has emerged in regions such as South-Eastern Asia, Uganda, and Rwanda, primarily due to the slow clearance of lumefantrine, which suppresses drug-sensitive parasites while allowing resistant parasites to survive and continue transmission (Witmer *et al*., 2020). Artemether-lumefantrine (AL) is recommended as a first-line malaria treatment in endemic areas due to its safety profile across different age groups and efficacy against *P. falciparum* asexual (merozoites, trophozoites, and schizonts) and immature gametocyte stages I-IV. However, it has little effect on mature gametocytes, rendering them sterile and thereby reducing the transmission of the parasite to the mosquitoes (Makanga, 2014; Makanga & Krudsood, 2009; Ngasala *et al*., 2024). This action prevents further development of gametocytes, the sexual forms that are infectious to mosquitoes.

The global push toward malaria elimination has intensified efforts to explore novel strategies for reducing human-to-mosquito transmission. Among these strategies are transmission-blocking interventions (TBIs), such as transmission-blocking drugs (TBD), transmission-blocking vaccines (TBVs), and genetically modified mosquitoes (Yu *et al*., 2022), which target different stages of the malaria parasite, including sporozoites and ookinete and the gametocyte stage responsible for infecting mosquitoes, thereby breaking the transmission cycle. To evaluate the efficacy of these tools, a well-established facility staffed with skilled microscopists and equipped with the necessary instruments for effective evaluation of transmission, ensuring robust results. Unfortunately, malaria transmission is gradually decreasing, necessitating the need to optimise membrane feeding assays to utilise the gametocytemic carriers in evaluating TBIs.

Evaluation of TBI's in low malaria endemic countries is difficult and gives contradicting results. For example, Goncalves *et al.* showed that only one participant transmitted infection to mosquitoes 7 days post-treatment, compared to the participants who received AL and

primaquine drug were not transmitted (Gonçalves *et al*., 2016). Results from a more recent trial in Mali showed that by day 5, no gametocytemic carriers infected mosquitoes post-treatment with AL, similar to participants administered with AL and primaquine (Mahamar *et al*., 2024).

Parasites have developed complex strategies to manipulate their hosts, enhancing transmission and ensuring survival. For instance, *Wuchereria bancrofti,* the causative agent of lymphatic filariasis, increases its presence in human peripheral circulation at dusk, aligning with the peak feeding time of *Culex* mosquitoes, thereby increasing the chances of transmission (Hawking, 1967). Similarly, previous studies found that mosquitoes fed with gametocytes at dusk had higher oocyst intensity than those fed at dawn, as this period coincides with increased mosquito susceptibility to malaria parasites (Coulibaly *et al*., 2017; Habtewold *et al*., 2019). These adaptive mechanisms are governed by interactions between parasites and their hosts, including mosquito metabolism and immunity (Bento *et al*., 2025; Murdock *et al*., 2013; Rund *et al*., 2016; Reece, 2017). It is possible that feeding mosquitoes during the day, as is commonly done for DMFA, could affect the results of DMFA, as mosquito metabolism and immunity are governed by circadian rhythms (Murdock *et al*., 2013; Rund *et al*., 2016; Reece, 2017).

In cultured Plasmodium malaria parasites, it is known that dark feeding improves the likelihood of mosquito infectivity (Bento *et al*., 2025; Habtewold, 2022; Habtewold *et al*., 2019). Also, it has been reported that the majority of DMFA procedures are typically conducted during the day (Gonçalves *et al*., 2017; Mahamar *et al*., 2024; Omondi *et al*., 2019; Stone *et al*., 2013) and have proven to be effective in malaria transmission, although mosquitoes are most active in the late evening and night. However, the effect of nighttime feeding on the infectivity of wild *P. falciparum* gametocytes using laboratory DMFAs during the low malaria transmission season is still unknown.

Direct membrane feeding assay (DMFA), direct skin feeding (DSF), and standard membrane feeding assay (SMFA) are assays used for the evaluation of transmission-blocking, where mosquito infectiousness is assessed. Of these, DMFA is the only procedure where serum replacement is done. This is a procedure where donor serum is replaced with naïve AB serum. Their potentiality is based on the removal of transmission-blocking attributes that may hinder infectivity by replacing them with malaria naïve AB serum repeated assays with a large number

of mosquitoes being infected. More than 212 membrane-feeding experiments have been conducted and have proven that mosquito infectivity is enhanced (Bousema *et al*., 2012).

As well as mosquito immunity, DMFA is affected by human immune factors. Serum replacement is performed as part of DMFA to remove potential host malaria-immune factors, such as complement proteins, pro-inflammatory cytokines such as tumour necrosis factor-a and interleukin (Bousema *et al*., 2012; Naotunne *et al*., 1993) and antimalarial metabolites. These factors may decrease gametocyte viability or disrupt gamete fertilisation and ookinete development in the mosquito midgut. However, malaria-naïve serum in malaria-endemic countries is extremely expensive to conduct serum replacement. It is known that immunity is likely to wane following a decrease in malaria endemicity, thereby increasing the likelihood of mosquitoes' infectivity.

This study explored potential sources of variation in DMFAs and ways to make them more cost-effective in an area of falling malaria transmission. Specifically, the study assessed 1) whether participants who were initially infectious to mosquitoes remained infectious post-treatment with AL, 2) whether the timing of DMFA conduct could be optimised to maximise mosquito infectiousness, and 3) whether will be effect of serum replacement would be effective in areas that have changed from moderate to low endemicity at low transmission season

1.2 Statement of the Problem

For a mosquito to become infectious, it must feed on blood containing the mature stage of the malaria parasite, known as gametocytes (Bennink *et al*., 2016; Hillyer *et al*., 2007a; Smith *et al*., 2014) which then develop within the mosquito's body to sporozoites. Various control strategies are designed to interfere with this process to produce *Plasmodium*-free mosquitoes. These strategies are collectively referred to as transmission-blocking interventions (TBI). Currently, TBIs under development include drugs (Kone *et al*., 2010; Mahamar *et al*., 2024; Stone *et al*., 2022), vaccines (Alkema *et al*., 2024), insecticides (Kristan *et al*., 2016; Kweyamba *et al*., 2023) and the use of genetically modified mosquitoes (Dong *et al*., 2011; Kyrou *et al*., 2018) that are resistant to malaria parasite infection.

To assess the efficacy of TBIs, it is crucial to have a standard platform capable of conducting these evaluations and producing reliable findings. These evaluations involve experimentally feeding mosquitoes with blood containing gametocytes and observing their development over

a period of 8 days, and confirm that parasites have the potential to infect mosquitoes. A well-developed laboratory evaluation of these tools can be conducted using laboratory-cultured malaria parasites in a procedure known as the standard membrane feeding assay (SMFA). However, this may not accurately represent what occurs in real-world situations, where malaria parasites can exhibit different genetic variability. In Bagamoyo, a Category II transmission facility was established to conduct evaluations using cultured or naturally occurring malaria parasites through a procedure known as direct membrane feeding assay (DMFA). However, eligible gametocytemic carriers carrying stage V *P. falciparum* gametocytes that can be recruited for evaluation of TBIs are scarce in the field. This is because asymptomatic carriers do not show any symptoms, which leads them not to seek health care. Thus, mass screening is required to obtain asymptomatic gametocytemic individuals who are eligible for evaluation of TBI. Also, there is limited evidence on the impact of day and night feeding and serum replacement on the infectiousness of wild *P. falciparum* gametocytes to *An. gambiae* sensu stricto mosquitoes in a moderate to low malaria transmission setting during the low transmission season.

Therefore, this study aims at optimising DMFAs by evaluating the impact of AL, circadian rhythm and serum replacement on the infectiousness of wild *P. falciparum* gametocytes to *An. gambiae* s.s mosquitoes.

1.3 Rationale of the Study

Malaria elimination requires innovative approaches to reduce human-to-mosquito transmission if elimination of the disease is to be realised. particularly in endemic settings. The refinement of laboratory assays to evaluate factors that may affect infectiousness, such as the effect of AL treatment, circadian rhythm and serum replacement, is essential for developing evidence-based strategies. This study contributes to the growing body of knowledge on effective evaluation of TBIs by addressing critical issues in optimising assay techniques and improving their relevance for malaria control programs.

1.4 Research Objectives

1.4.1 General Objective

To assess the impact of artemether-lumefantrine treatment, circadian rhythm and serum replacement on the infectiousness of wild *P. falciparum* gametocytes to *An. gambiae* s.s mosquitoes

1.4.2 Specific Objectives

This study was pursued to achieve the following specific objectives:

(i) To assess the effect of AL on the infectiousness of wild *P. falciparum* gametocytes to *An. gambiae s.s* mosquitoes.

(ii) To assess the effect of day and night-time feeding on the infectiousness of wild *P. falciparum* gametocytes to *An. gambiae s.s* mosquitoes.

(iii) To assess the effect of serum replacement on the infectiousness of wild *P. falciparum* gametocytes to *An. gambiae s.s* mosquitoes.

1.5 Research Questions

(i) What is the proportion of mosquitoes infected on day 9 after AL administration relative to day 0?

(ii) What is the proportion of mosquitoes infected when fed blood at night compared to those fed in the daytime?

(iii) What is the proportion of infected mosquitoes fed on blood with serum replacement technique compared to whole blood?

1.6 Significance of the Study

The study addresses the need to explore factors influencing mosquito infection rates, the study provides critical insights for optimising existing membrane feeding assays on the evaluation of mosquito infectiousness.

1.7 Delineation of the Study

This study is crucial as it addresses the need to optimise the existing mosquito infection assay to evaluate the efficacy of TBI. Recent publications focus on determining the persistence of sub-microscopic densities post-treatment, which have limited evidence with mixed results on mosquito infectiousness. Additionally, no studies have been done to explain whether mosquito infectiousness varies depending on the timing of ingesting gametocytemic blood. This research aims to contribute vital insights into how best we can optimise the existing membrane feeding assays for the evaluation of mosquito infectiousness. Further researches are required to enhance the generalisation of the findings.

CHAPTER TWO

LITERATURE REVIEW

2.1 Epidemiology of Malaria

Malaria remains a major public concern globally. It accounts for 246 million cases of malaria in 2023, resulting in an estimated 569 000 deaths. Notably, in Africa, around 94% of all malaria-related deaths occur in the WHO African Region, with vulnerable groups, particularly children under five and pregnant women, accounting for 76% of the deaths in this region (WHO, 2024). Moreover, Tanzania, as one of the Sub-Saharan African countries, contributes 3.1% of the global burden (Coalson *et al*., 2016), where 8% of cases are among children aged 6-59 months (National Bureau of Statistics [NBS] *et al*., 2023). Underlying risks include poor housing conditions, the presence of heavy rainfall accompanied by high humidity and hot temperatures, and poor economic status (NBS *et al*., 2023; Mukabana *et al*., 2025), mobility of people from one country to another, and demographic factors such as the presence of school-aged children (Mlugu *et al*., 2020; Yimam *et al*., 2021).

2.2 Malaria Vectors and Parasite Life Cycle

The disease is caused by the *Plasmodium ssp* parasite and transmitted by *Anopheles* mosquitoes from one infected human to another. The most efficient malaria vectors in Africa are the *An. gambiae* s.l. and *An. funestus* groups (Sinka *et al*., 2012). In Africa, within the *An. gambiae* s.l. group, the key sibling species are *An. gambiae* s.s., *An. coluzzii*, and *An. arabiensis* (Odero *et al*., 2023), while in the *An. funestus* group, the most efficient sibling species is *An. funestus* s.s. (Kaindoa *et al*., 2017; Matowo *et al*., 2021).

Anopheles mosquito bites the infected individual and ingests the infective stage of the malaria parasite known as a gametocyte (Christian., 2016; Smalley & Sinden, 1977). The ingested gametocyte travels to the mosquito midgut, where male and female gametocytes fuse to form a zygote. A zygote then becomes an elongated ookinete, which is motile and penetrates the mosquito's midgut wall, eventually forming a round, bulky ball called an oocyst. The oocyst ruptures and releases sporozoites, which make their way to the salivary glands of the mosquito. At this stage, the mosquito is considered to be infective, and during the next blood feeding on a human, these *Plasmodium* sporozoites are injected into the human skin and initiate another

cycle. In humans, these parasites travel to the liver, undergo asexual replication (sporozoites to chizont) in 10-14 days. Within the liver cells, schizonts rupture and differentiate into merozoite daughter cells, which travel through the bloodstream and infect the red blood cells (RBCs). Thus, a proportion of asexually replicating parasites undergoes a developmental switch that diverts them from the asexual parasites to sexual parasites. Sexual differentiation leads to the development of gametocytes, the exclusive transmission stages enabling parasite transfer to the mosquito vector (Bennink *et al*., 2016; Hillyer *et al*., 2007b; Smith *et al*., 2014).

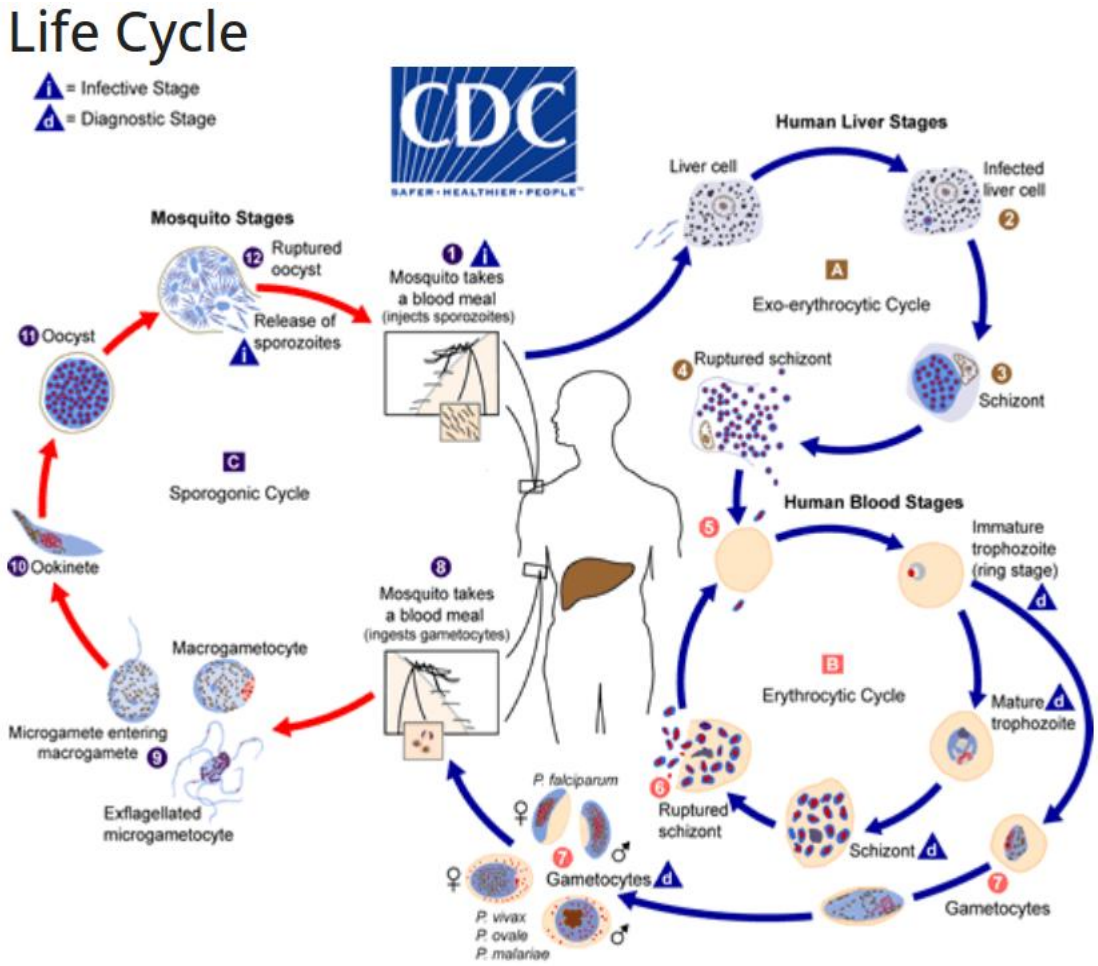


Figure 1: Life of a *Plasmodium.* Source: (CDC, 2023)

2.3 Transmission Blocking Interventions

The parasite completes its life cycle between humans and female mosquitoes; in each of these hosts, different forms exist. *Anopheles* mosquitoes can only be infected if they ingest an

adequate number of mature gametocytes present in the blood. To block transmission, it is necessary to reduce the number of mosquitoes that become infected with the parasite. A TBI is a collective term that refers to any of the control approaches that result in halting the transmission of malaria parasites from humans to mosquitoes. This includes TBDs such as tafenoquine and primaquine, which kill gametocytes (Bradley *et al*., 2019; Gonçalves *et al*., 2016; Stone *et al*., 2022). Secondly, TBVs which operate inside the mosquito midgut after the mosquito feeds on the blood of a vaccinated individual. The TBVs stimulate the body to produce antibodies which work inside mosquito body by preventing fertilization of the gametocytes or midgut invasion (Carter *et al*., 2016; Goodman & Draper, 2010; Richie & Saul, 2002). Third, genetically modified mosquitoes that block the development of malaria parasite oocyst in the mosquito midgut (Yu *et al*., 2022).

2.4 Factors Influencing the Mosquito's Ability to Transmit Malaria

The infectiousness of gametocytes to mosquitoes depends on several factors, including gametocyte density (Churcher *et al*., 2013) maturity (Lensen *et al*., 1999), the presence of antimalarial drugs (Bousema *et al*., 2010; Butcher, 1997; Kone *et al*., 2010), additional blood meal (Hofer *et al*., 2024), patient immunity against gametocytes (Bousema *et al*., 2012) and feeding time (Schneider *et al*., 2018). For example, a study done in Burkina Faso proved that the higher count of gametocyte density in peripheral blood and skin tissue leads to an increase in mosquito infection rate (Meibalan *et al*., 2019). Normally, in a laboratory infection experiment, mosquitoes are fed with infectious blood once. In Tanzania, an additional blood meal where mosquitoes were given another blood meal containing *P. falciparum* gametocyte stage V three days later increased the development of oocyst and sporozoites and appeared larger in size compared to mosquitoes infected with a single blood meal (Hofer *et al*., 2024). It is therefore assumed that the nutrients from the additional blood increase the parasite's extrinsic incubation period.

According to the WHO, AL is recommended as the first-line treatment for malaria in endemic settings (WHO, 2010). Previous studies have proven that AL is efficacious in clearing asexual and immature gametocytes (Chawla, 2021; Ngasala *et al*., 2024; Ngotho *et al*., 2019; Smith *et al*., 2014; Sutherland, 2005). A recent trial done in Mali demonstrated that two participants administered with AL alone were infectious on day 2 post treatment, but none were infectious on day 7. In contrast, some of the participants treated with a combination of Sulfadoxine-

pyrimethamine and amodiaquine remained infectious to mosquitoes up to day 7 (Mahamar *et al*., 2024). Similarly, a study done in Kenya proved that AL significantly reduced gametocyte prevalence 85.1% (126/148) at day 0 to 7.04% (5/71) at day 42 post-treatment in comparison to the baseline (Omondi *et al*., 2019). However, there is limited evidence regarding the persistence of sub microscopic gametocyte densities post-treatment that can lead to mosquito infectiousness. Thus, upon optimising existing assays towards re-using gametocytemic carriers in mosquito infectiousness experiments, further evaluations are recommended.

Circadian rhythm refers to the behavioural or physiological patterns in mosquitoes that arise from the circadian clock and are often entrained by environmental cues such as light–dark cycles, i.e., nocturnal biting activity, time-of-day variation in susceptibility to parasite infection and rhythmic changes in immune defence and gut permeability (Schneider *et al*., 2018). Whilst the circadian clock refers to the internal biological time-keeping system in organisms (Rijo-Ferreira *et al*., 2017; Rijo-Ferreira & Takahashi, 2019). Moreover, infection intensity is at the peak during the night, a time that corresponds to the active feeding period of *A. gambiae* mosquitoes. Nevertheless, a circadian clock study demonstrated the effect of the circadian clock on the lab-reared *An. gambiae* mosquito susceptible strain, using *P. falciparum* cultured gametocyte yielded higher infection at night compared to daytime (Habtewold, 2022). Similarly, a study done in showed a peak count of sporozoites at night compared to daytime in mice infected with blood containing *P. bergehei* cultured gametocyte (Bento *et al*., 2025). Contrary to the study done in Mali, which demonstrated that through DSF wild-caught *A. gambie* mosquitoes and fed on blood *P. falciparum* wild gametocytes, yielded higher infection on dusk than at dawn because feeds for dusk feeding was done on the baseline, while dusk feeding was done 2 days later (Coulibaly *et al*., 2017) led to such variability in infectivity. Although the efficacy of the laboratory-controlled experiments is potential towards assessing the effect of circadian rhythm. However, the use of DMFA in assessing the effect of nighttime feeding using wild *P. falciparum* gametocyte stage V is unknown. Additionally, host immunity decreases the infectiousness of *Plasmodium* parasites to mosquitoes, thereby reducing transmission potential. Mosquitoes that feed on immune, gametocytemic carriers are less likely to acquire and transmit malaria, thereby reducing the overall transmission cycle. This was experimentally demonstrated in DMFAs, where the participant's serum containing antibodies was removed. More than 212 membrane-feeding experiments have shown that serum obtained from the blood contains. *P. falciparum* gametocyte stage V and replaced with malaria naïve

serum to an increase in the number of infected mosquitoes (Bousema *et al*., 2012). However, the effect of serum replacement in a moderate to low malaria transmission setting during the low malaria transmission season is unknown.

2.5 Mosquito Strains for Testing Transmission-Blocking Interventions

Laboratory-reared mosquitoes that are well adapted to membrane feeding and demonstrate reasonable survival by day 16 are particularly important for the feeding assay. These mosquitoes allow researchers to study various aspects of mosquito biology, behaviour, and their role in disease transmission. For the mosquito feeding assay, these mosquitoes are preferred for several compelling reasons. Firstly, they are specifically adapted to membrane-feeding assays (Bousema *et al*., 2012), which are essential for studying mosquito and malaria parasite interactions. Secondly, laboratory-reared specimens are much more accessible than wild mosquitoes (Leite *et al*., 2024). Researchers can easily obtain these mosquitoes in sufficient quantities for experimentation, facilitating larger sample sizes and more replicable studies (Spitzen & Takken, 2005). This accessibility also allows for better standardisation across different research projects, enhancing the overall quality and comparability of the findings. Additionally, laboratory-reared mosquitoes are disease-free, eliminating the risk of introducing pathogens that could confound research outcomes. This is particularly important for studies focused on DSF, as it ensures a controlled environment where variables can be accurately measured.

2.6 Method used for Evaluation of TBI

The efficacy of TBI in blocking parasite multiplication in mosquitoes is assessed by measuring the reduction in infection rate (the proportion of mosquitoes carrying at least one oocyst) and infection intensity (the number of oocysts per mosquito) from feeding assays. Mosquito feeding assays through DMFAs, SMFAs and DSF are among the methods used for this evaluation.

In SMFA, lab-cultured malaria parasites are used as a source of infection (van der Kolk *et al*., 2005a, 2006). This mixture is then placed in a feeding cup covered with an artificial membrane. In DMFA, venipuncture blood obtained from gametocytemic carriers is fed to laboratory-reared mosquitoes. And, DSF refers to a method by which mosquitoes feed on blood directly from the skin of a gametocytemic carrier.

Direct membrane feeding assay has the following advantages: a) assesses the infectiousness of naturally infected individuals, b) repeated assays with a large number of mosquitoes, c) allows the removal of patient transmission blocking immunity and d) practical, acceptable methods across different age groups. Potential confounders, such as the effect of drugs and transmission-blocking components, can be removed. However, the disadvantages of DMFA are: a) in case the feeding temperature is not maintained at 37 °C, gametocytes may exflagellate before being administered to mosquitoes, and b) it is very expensive and time-consuming.

The SMFA has the following advantages: a) Ideal for testing transmission-blocking vaccines, monoclonal antibodies, drugs, or sera, b) highly standardised and reproducible (van der Kolk *et al*., 2005b). Direct skin feeding (DSF) has the following advantages: a) mimics the natural feeding, b) no waiting time between drawing the sample from gametocytemic carriers and feeding it to mosquitoes, c) there is no need to draw blood from volunteers using venipuncture. The disadvantages of DSF are a) not ethically and less practically acceptable to young age individuals (Bousema *et al*., 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Mosquito Rearing

Anopheles gambiae ss (Ifakara strain) colony is reared at the Bagamoyo branch insectary, Kingani. These mosquitoes were maintained under standard conditions at 12:12 h dark: Light cycle, 70±20% humidity and 27±2 °C. Larvae are fed on TetraMin® fish flakes (Tetra Ltd., UK) and kept at a density of 200 larvae (from L2 larval stage) per 1 L of distilled water. Adult mosquitoes are kept in 30×30×30 cm containers and allowed to feed on 10% sucrose solution (Hofer *et al*., 2023). For egg production, adult mosquitoes were given blood through the membrane (Siria *et al*., 2018). Adult mosquitoes were routinely checked for microsporidia infections and strict hygiene measures are maintained in the insectary.

3.2 Study Site, Participants and Ethics

This laboratory-controlled study for these assays was conducted at the Malaria Transmission Research Laboratory (MTRL) located at the Ifakara Health Institute (IHI) in Bagamoyo district, Tanzania, between May to August 2024. The target population included males and females aged 6–45 years from the villages of Wamimkoko, Miono, Chamgoi, and Kibindu. Participants included in the study met the following criteria (i.e. asymptomatic, consented, aged 6–45 years, with detectable *P. falciparum* gametocytes stage V only were enrolled for DMFA. Exclusion criteria included: Not consented, (primaquine use within four weeks before this study), fever or history of fever and evidence of severe illness. Gametocytes were quantified by counting against 500 white blood cells in thick blood smears. Gametocyte density was calculated based on an assumed leukocyte count of 8000 cells/μL of blood, as indicated in other studies (Kweyamba *et al*., 2023; Mahamar *et al*., 2024; Ouattara *et al*., 2024).

Figure 2: Malaria Transmission Research Laboratory

3.3 Screening Procedures

A mass test-and-treat (MTAT) approach was used to screen asymptomatic participants. Village workers informed the community members of the date, location, and time of sensitisation meetings, where consenting and screening would take place. Screening rounds were performed on participants who consented to participate in the study using the malaria Rapid Diagnostic test (mRDT) (SD BIOLONE Malaria Ag P.f/Pan (HRPII/pLDH), Standard Diagnostic, South Korea). Briefly, finger-prick blood samples were collected, and one drop of 50µl of capillary blood was applied to the test and run with four drops of standard buffer solution. The test was placed on a flat surface for 15 minutes, after which a laboratory technician read the results. For participants who tested mRDT positive, a small amount of finger-prick blood was also taken to make thick smear slides to confirm the presence of malaria parasites. To do this, slides were taken to the laboratories at IHI and stained for 45 minutes with 10% Giemsa stain. The slides were then examined for the presence of asexual and sexual stages (gametocyte stage V) of *P. falciparum* only using light microscopy (LM) (100×magnification, oil immersion). Asexual parasites (ring stages) were counted per 200 white blood cells (WBCs) and gametocytes per 500 WBCs. The slides that had no *P. falciparum* parasites were considered negative.

3.4 Blood Collection, Mosquito Feeding Assay and Dissection of Mosquitoes

The next day, participants confirmed to have gametocytes on the slides were invited to the IHI laboratory, where they donated 4ml of venous blood into Lithium-Heparin-coated vacutainers (Vacuette®, Greiner, Kremsmünster, Austria). The blood was then split into two aliquots, and one aliquot was transferred into pre-warmed 1.5 mL Eppendorf tubes (Eppendorf, Germany) and centrifuged for 3 minutes at 300 Relative Centrifugal Force and 37 °C (5702 RH, Eppendorf, Germany). The same amount of autologous serum was replaced with pre-warmed malaria-naïve AB serum set at 37 °C (Blood Transfusion Service, Basel, Switzerland). The malaria naïve AB serum it was obtained from European donors with blood type AB who have no prior exposure to malaria (Blood Transfusion Service in Basel, Switzerland). The other portion of the whole blood was directly dispensed to mosquitoes.

Mosquitoes were starved for 6 hours before feeding and were infected using DMFAs as described elsewhere (Hofer *et al*., 2024), in which blood was drawn from a participant and offered to mosquitoes through a pre-heated glass feeder. In this assay, water-jacketed glass feeders (14 mm Ø, Chemglass, New Jersey, USA) were covered with Parafilm® and connected to a circulating water bath (ELMI, Switzerland) set at 39 °C. The water bath was preheated for 15 minutes before the experiment began.

Eight cups, each with 50 mosquitoes, were used per participant, including four cups for serum replacement and an additional whole blood cup. Each feeder was filled with 300 µL of blood, and mosquitoes were allowed to feed for 15 minutes. During feeding, the cups were covered with a dark cloth, and the lights in the room were switched off to enhance feeding success. Post feeding, the cups were placed in Bug Dorm plastic cages (30×30×30 cm, Megaview Science Co., LTD, Taiwan) overnight, maintained at 75±2% relative humidity and 27±1 °C under a 12:12-hour dark-light cycle in a climatic chamber (S600PLH, AraLab, Lisbon, PortugalThe following morning, unfed mosquitoes were removed using a mouth aspirator, and the remaining mosquitoes were provided with cotton soaked in a 10% sucrose solution. The sugar cotton balls were replaced daily. AL treatment was administered to participants within 24 hours of diagnosis under parental supervision, following Tanzania's national guidelines (MoHSW, 2006).

Eight-day post-infection mosquitoes were dissected and midguts were stained for 8 min using 0.1% mercurochrome solution (Sigma, Switzerland) pre-examination under a compound microscope at 40×magnifcation for the presence of oocysts. The flow of the procedure is shown in Fig. 3.

3.5 Study Procedures

Experiment 1: To determine the effects of antimalarial treatment on the infectivity of gametocytes from naturally infected individuals to *Anopheles gambiae* s.s mosquitoes

A total of 4850 female mosquitoes were fed through DMFA on day 0 (3700) and day 9 (1150). The DMFA with serum replacement was done on day 0, pre-administration of AL treatment. Surviving mosquitoes were dissected for oocysts on day 8. The Second DMFA of 9 days post-treatment was repeated with participants who infected more than 20 mosquitoes from the first DMFA using a different batch of mosquitoes (Fig. 3). We proposed this cutoff point because the gametocyte density for these participants was approximately 48gam/µl on day 0. Consequently, we anticipated that there would be no infection during the second feeding.

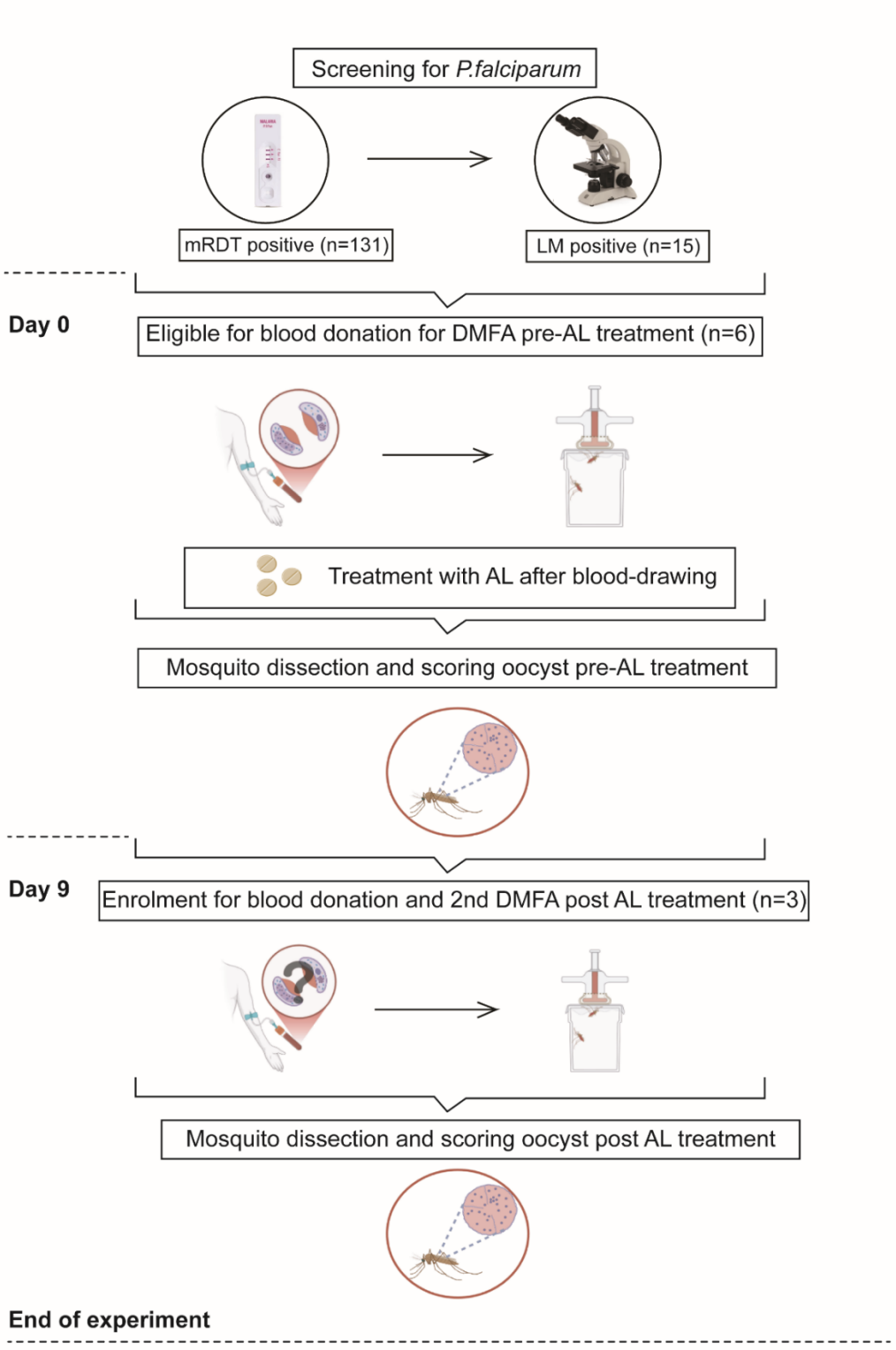


Figure 3: Evaluation of wild *P. falciparum* gametocyte infectiousness by collecting blood and subsequent DMFA feeding on day 0 and day 9 post-treatment

Experiment 2: To determine the effect of the time of DMFA conduct feeding on the infectivity of gametocytes from naturally infected individuals to *Anopheles gambiae* s.s mosquitoes

A total of 3300 female mosquitoes were fed through DMFA with serum replacement with blood obtained from the same participants who were infected during the day, also infected during the night. In total, 1750 were fed during the day (13:00-16:00) hours and 1550 at night (19:00-22:00) hours (Fig. 4).

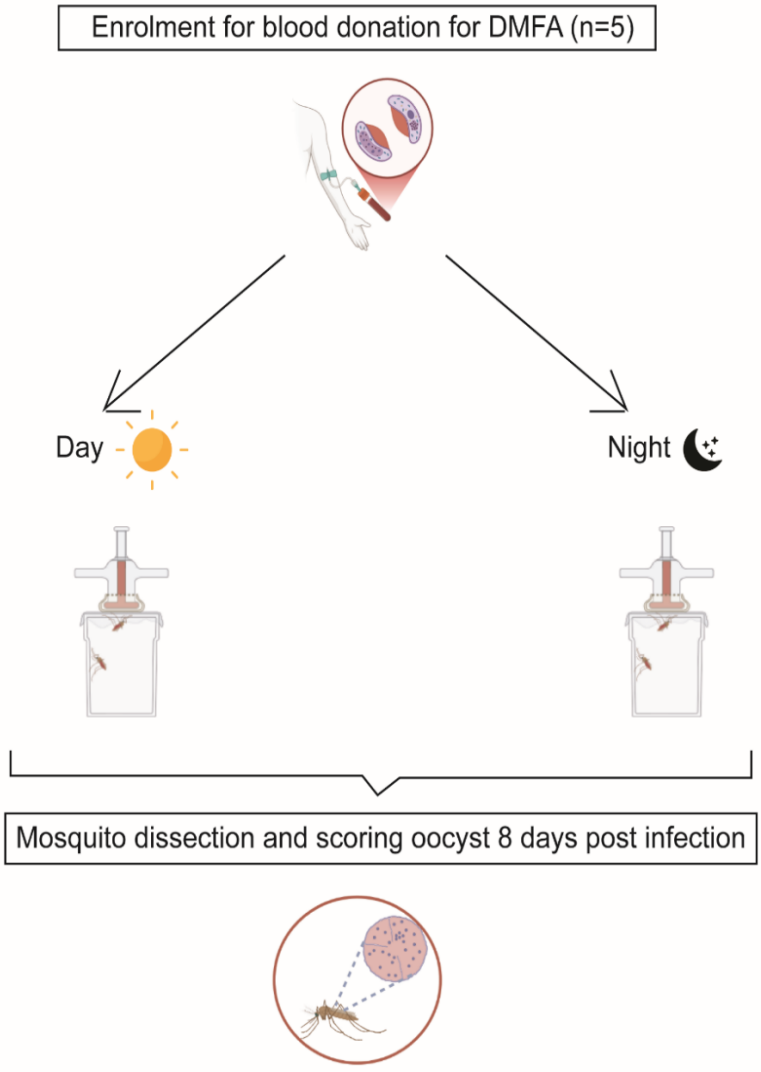


Figure 4: Evaluation of wild *P. falciparum* gametocyte infectiousness by collecting blood and subsequent DMFA feeding on day 0 (day and night)

Experiment 3: To determine the effect of serum replacement on the infectivity of gametocytes from naturally infected individuals to *Anopheles gambiae* s.s mosquitoes

A total of 4850 female mosquitoes were fed through DMFA with blood obtained from the same participants. In total, 3250 were fed on blood with serum replacement, and 1600 were fed on whole blood without serum replacement.

3.6 Statistical Analysis

Data was entered in Excel (Microsoft 2016) and analysed using STATA 17 software (StataCorp, College Station TX, USA). Descriptive statistics were used, whereby the arithmetic mean proportion of infected mosquitoes with a 95% confidence interval (CI) was reported. For oocyst intensity, median with minimum and maximum values were presented. Logistic regression was used to determine the prevalence of infected mosquitoes adjusting for days (fixed effect) and participants (random effect): 1) effect of AL, day 9 compared to day 0 (fixed effect); 2) time of DMFA conduct, night compared to day (fixed effect); and 3) effect of serum replacement compared to whole blood (fixed effect). Negative binomial regression with a log link was conducted for oocyst intensity for all experiments with the same fixed and random effects outlined above.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Malaria Prevalence Among Study Participants

A total of 309 participants aged 6-45 years were screened for *P. falciparum* infection in Chamgoi, Wamimkoko, Miono, and Kibindu villages. The gametocyte prevalence ranges from 0% (0/17) in Miono village, 1.27% (1/79) in Wamimkoko village, 3.54% (4/113) in a low malaria transmission setting and 9% (100/100) in Kibindu village in a moderate transmission setting. Out of 309, 131 (42%) were found to be positive for *P. falciparum* asexual parasites using mRDT. Of these, 15/131 (11.5%) were found to be positive for *P. falciparum* gametocytes using LM. Only 40% (6/15) each with counts exceeding 3 gametocytes/500 red blood cells, density equivalent to 48 gametocytes per µl of blood. found to be eligible and enrolled for DMFAs. The mean gametocytemic density among the participants at day 0 was 125 gam/µl.

4.1.2 Effects of AL on Individual Infectiousness to Mosquitoes

On day 0, all six participants (100%) infected at least one mosquito. Three participants were invited for the second DMFA as they infected more than 20 mosquitoes from the initial DMFA. In the second DMFA, only 1 out of 3 participants (33.3%) remained infectious. A total of 1,612 mosquitoes were dissected across two time points: 1125 on day 0 pre-treatment and 487 on day 9 post-treatment. The mean proportion of oocyst-infected mosquitoes was lower on day 9 with 0.06 (95% CI 0.04-0.09), compared to 0.17 (95% CI 0.15-0.19) on day 0 (OR = 0.20, [95% CI: 0.13-0.30], P=0.001) (Table 1). There was a significant difference in median oocyst intensity per mosquito between day 9 with 1 (1-7) compared to day 0 with 2 (1-16) (IRR= 0.46, [95% CI: 0.31-0.68], P=0.001) (Figs. 5A and 5B; Table 1).

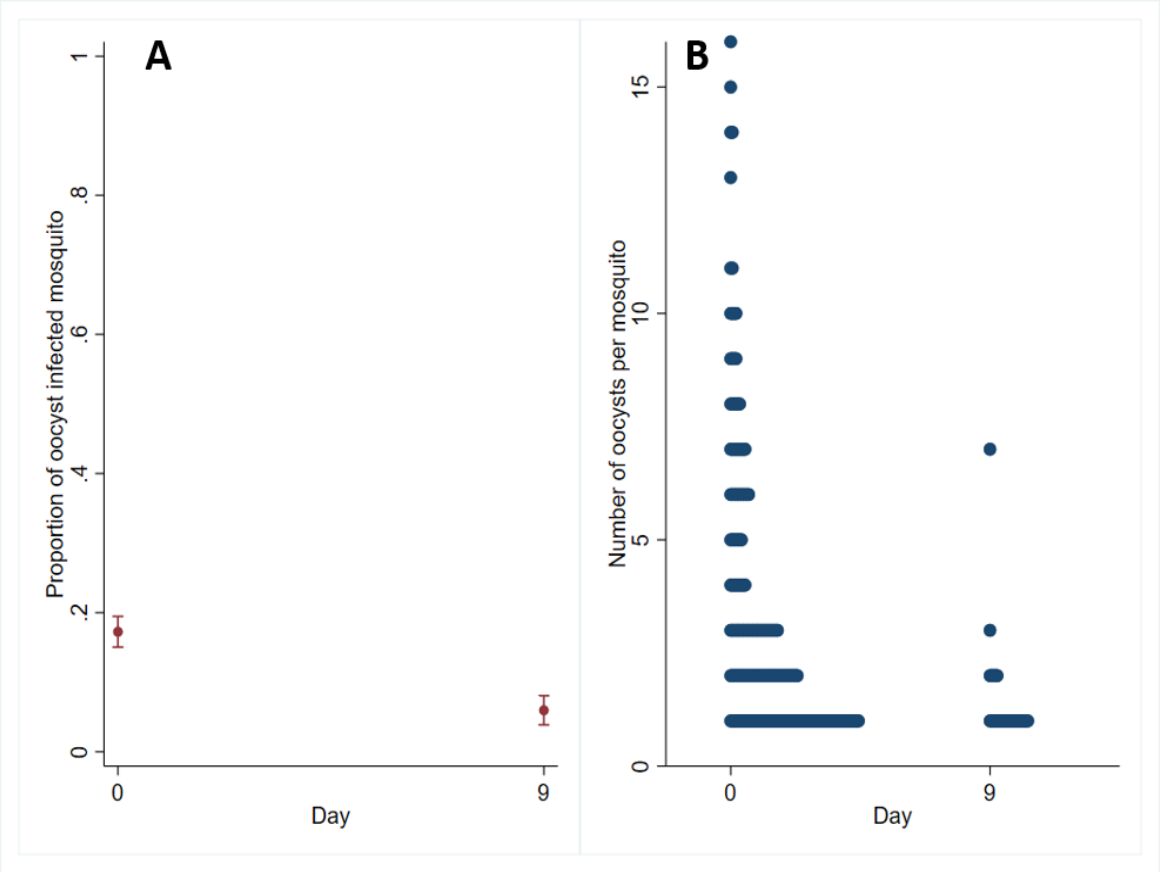


Figure 5: (A, B) Effect of AL post-treatment on the proportion of infected mosquito and oocyst intensity on *An. gambiae s.s* mosquito infectiousness from naturally infected participants on day 9 compared to day 0.

4.1.3 Effect of time of Feeding on the Proportion of Infected Anopheles Gambiae s.s Mosquitoes

A total of 867 mosquitoes were dissected during the day (13:00-16:00 hrs) and 915 during the night (19:00-22:00 hrs). The mean proportion of oocyst-infected mosquitoes was very similar: 0.08 (95% CI: 0.06-0.09) from day-feeds and 0.06 (95% CI: 0.05-0.07) from night-feeds, with a median of 2 oocysts per mosquito ranging from 1-16 in both cases. No evidence of a statistical difference was found for either the prevalence of oocyst-infected mosquitoes (OR=0.77, [95%CI:0.53-1.12], p=0.175) or oocyst intensity within mosquitoes (IRR=0.98, [95%CI:0.72-1.34], p=0.887) between the two groups (Figs. 6A and 6B; Table 1).

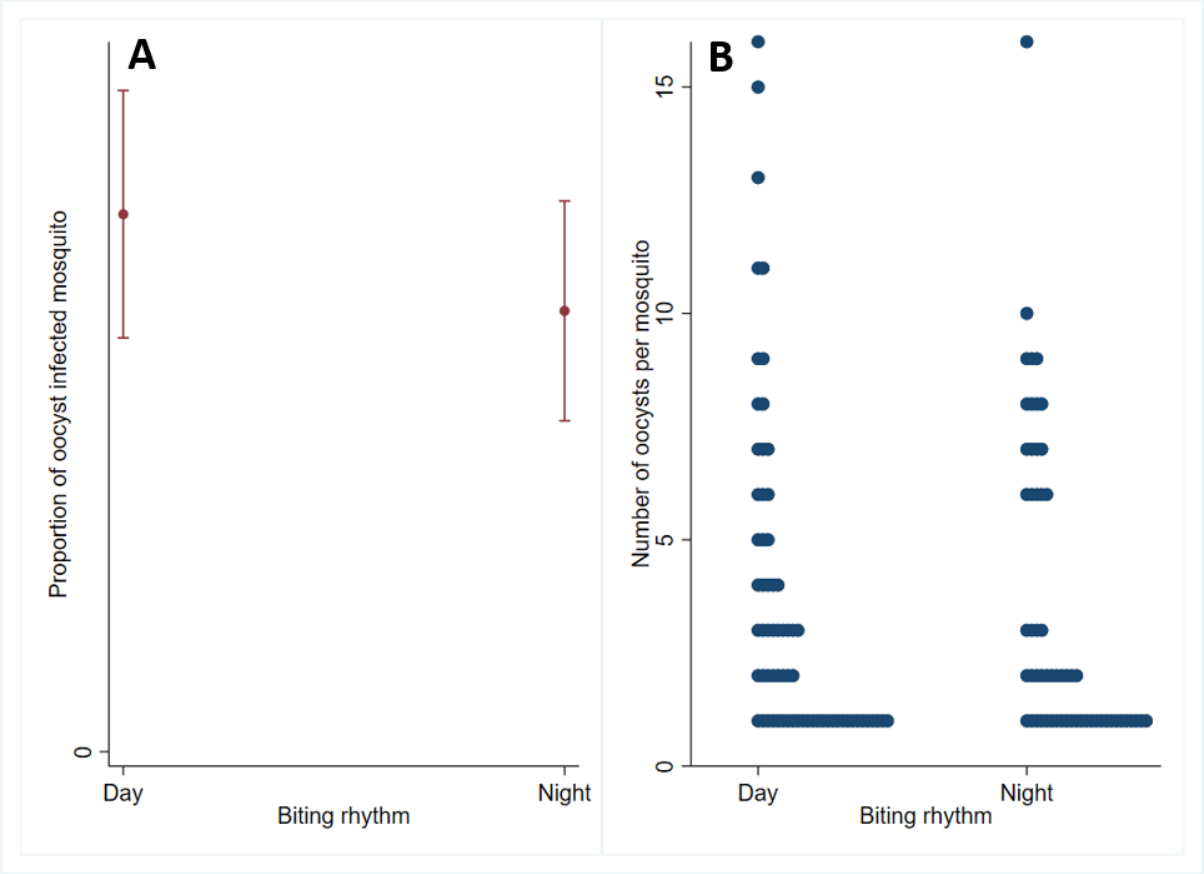


Figure 6: (A, B) Effect of circadian rhythm on the proportion of infected mosquitoes and oocyst intensity on *An. gambiae s.s* mosquito infectiousness from naturally infected participants infected during the day and night

4.1.4 Effects of Serum Replacement on Infection Rate

A total of 3394 mosquitoes were dissected, with 2160 from the serum replacement group and 1234 from the whole blood group. The mean proportion of oocyst-infected mosquitoes was higher in the serum replacement group at 0.12 (95% CI: 0.11-0.13) compared to mosquitoes fed with whole blood at 0.07 (95% CI: 0.06-0.08). There was sufficient evidence to support the claim that serum replacement increases the proportion of infected mosquitoes (OR=1.73, [95% CI:1.33-2.25] p=0.001). but not infection intensity (IRR=1.02, [95% CI:0.81-1.28], P=0.887) (Figs. 7A and7B; Table 1).

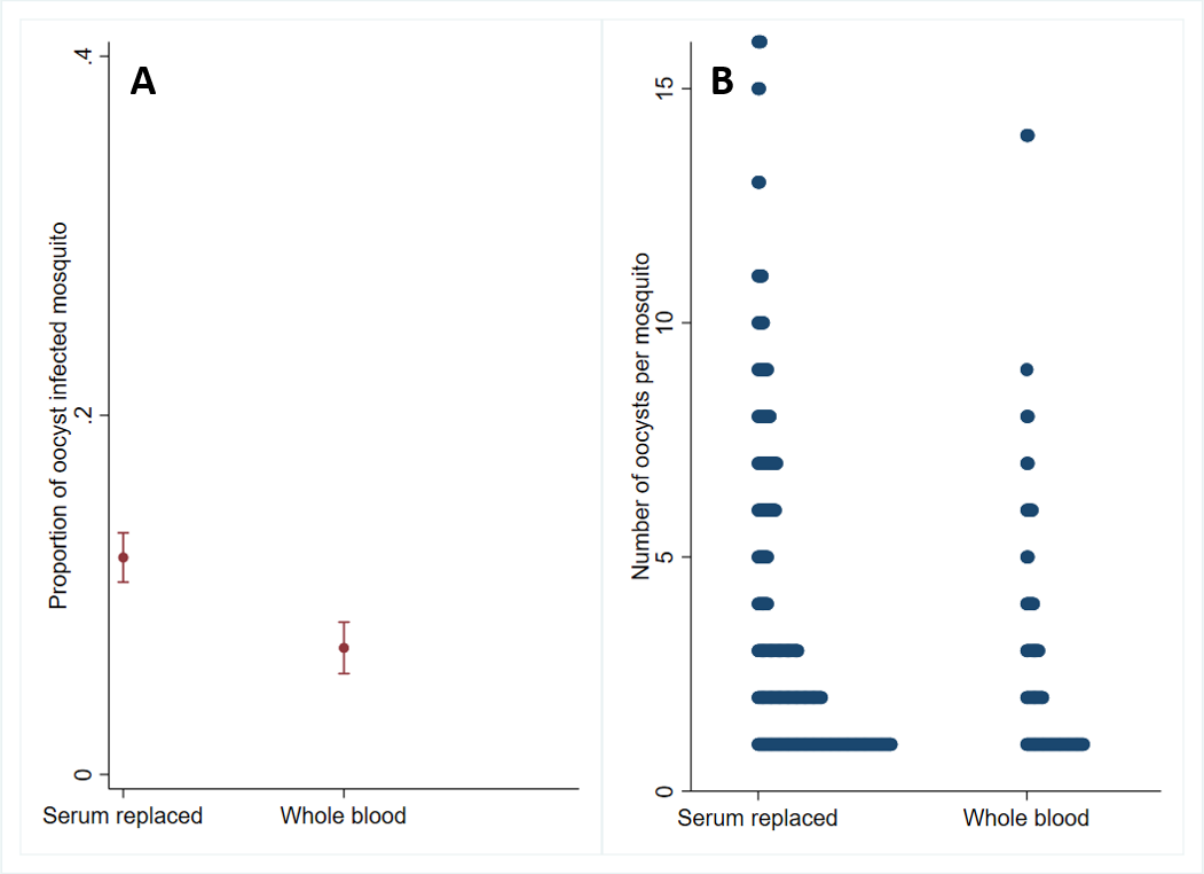


Figure 7: (A, B) Effect of serum replacement on the proportion of infected mosquitoes and oocyst intensity on *An. gambiae s.s* mosquito infectiousness from naturally infected participants

Table 1: Oocyst infection prevalence and infection intensity of *An. gambiae* s.s Day 0, day 9, circadian rhythm, and serum replacement

	*Infectious Participants	Infected mosquitoes (n/N) (%)	Prevalence		Oocyst Intensity	
			OR (95% CI)	P- value	IRR (95% CI)	P-value
Day 0 (Baseline)	6/6 (100%)	194/1125 (17.24%)	1			
Day 9	1/3 (33.3%)	29/487 (5.95%)	0.20 (0.13-0.31)	0.001	0.46 (0.31-0.68)	0.001
Day	5/5 (100%)	67/867 (7.73%)	1			
Night	5/5 (100%)	58/915 (6.34%)	0.77 (0.53-1.12)	0.175	0.98 (0.72-1.34)	0.887
Whole blood	6/6 (100%)	87/1234 (7.05%)	1			
Serum replacement	6/6 (100%)	261/2160 (12.08%)	1.73 (1.33-2.25)	0.001	1.02 (0.81-1.28)	0.887

Note: n/N Proportion of mosquitoes scored positive by microscopy for oocysts on day 8 post-infection. (n) number of infected mosquitoes. (N) number of dissected mosquitoes.

The odds ratio (OR) was estimated using logistic regression, adjusting for feeding days, circadian rhythm, and serum replacement as fixed effects, with biological replicates (participants) as random effects. The incidence risk ratio (IRR) was estimated using a negative binomial distribution with a log link function, applying the same fixed and random effect adjustments.

4.2 Discussion

Understanding factors influencing mosquito infectiousness through DMFA using gametocytes from naturally infected participants is crucial for malaria transmission research and TBI evaluation. This study examined the infectiousness of *P. falciparum* wild isolates to *An. gambiae s.s.* mosquitoes pre and post AL treatment, the impact of the time of DMFA conduct (day vs. night) and serum replacement versus autologous serum. We demonstrated that pre-AL treatment, all 6 participants enrolled for DMFAs (100%) infected mosquitoes, whereas only 1 in 3 (33%) infected mosquitoes 9 days post-treatment. A significant reduction in infection prevalence and oocyst intensity on infected mosquitoes was observed on day 9 compared to day 0. To the best of our knowledge, this is the first study to assess the impact of AL 9 days post-treatment, revealing a substantial decline in mosquito infectiousness compared to baseline.

4.2.1 Effects of AL on Individual Infectiousness to Mosquitoes

This study reveals that the proportion of infected mosquitoes on day 9 was significantly lower than that on day 0, indicating a noteworthy decline in infection prevalence 9 days post-treatment. To the best of our knowledge, this is the first study to assess the impact of AL 9 days post-treatment, revealing a substantial decline in mosquito infectiousness compared to baseline.

The difference may be due to AL's inability to clear mature gametocytes present at recruitment (Bousema *et al*., 2006; Makanga, 2014) allowing some participants to remain infectious post-treatment (Sutherland *et al*., 2005). These findings align with previous studies showing that AL reduces gametocyte density, mosquito infection rates, and oocyst intensity (Bradley *et al*., 2019; Mahamar *et al*., 2024; Makanga, 2014). For example, Sutherland *et al.* reported that not only did AL decrease the rate of gametocyte carriage, but also gametocyte carriage time, 42 days post treatment and parallel to infectivity of gametocytes to mosquitoes compared to

participants administered with Chloroquine combined with sulfadoxine-pyrimethamine (CQSP) (Sutherland *et al*., 2005). Also, Goncalves *et al.* showed that only one participant transmitted infection to mosquitoes 7 days post-treatment compared to participants AL and primaquine drug (Gonçalves *et al*., 2016). In a more recent trial in Mali, no infections by day 7 were reported compared to participants administered with Sulfadoxine–pyrimethamine and amodiaquine (Mahamar *et al*., 2024).

Several factors contribute to post-treatment variability, including individual differences in gametocyte clearance (Makanga, 2014; WWARN Gametocyte Study Group, 2016). Additionally, rapid male gametocyte clearance post-treatment may render infections non-transmissible (Jm *et al*., 2018). Additionally, immature gametocytes (stages I-IV) sequestered in the bone marrow or spleen require more time to mature (Smalley *et al*., 1981; Thomson & Robertson, 1935). Artemether lumefantrine targets both asexual and immature gametocyte stages (Bousema *et al*., 2010; Chen *et al*., 1994; Sinclair *et al*., 2009; Smithuis *et al*., 2010) and lumefantrine further inhibits gametocyte development by making them sterile, which leads to a low mosquito infection rate (van Pelt-Koops *et al*., 2012). However, eligible gametocytemic carriers carrying stage V of *P. falciparum* gametocyte that can be recruited for evaluation of TBIs are scarce in the field. This is because asymptomatic carriers do not show any ill symptoms, which makes them not seek health care, thus mass screening is required to at least 100 individuals and be able to obtain 20% of the eligible participants. Thus, it is essential to optimise assays by using pre-treated participants as a source of gametocytes for the evaluation of TBI. Furthermore, this finding has broader implications for other ACTs, as their effects can vary in different settings. In East Africa, including Tanzania, AL is administered to all participants diagnosed with a *Plasmodium* infection. Although AL has shown effectiveness in reducing gametocyte density and oocyst intensity, some studies have demonstrated that submicroscopic gametocyte densities persist and transmit infections in West Africa (Gonçalves *et al*., 2016). Thus, addressing the dynamics of gametocyte infectiousness following AL treatment is crucial not only for understanding individual-level transmission potential but also to critically evaluate TBI for designing and informing malaria control strategies, in settings approaching elimination, where reducing the infectious reservoir is critical for interrupting transmission.

4.2.2 Effects of Circadian Rhythms and Mosquitoes' Infectiousness

We found no significant difference in infection rates between mosquitoes fed during the day and those fed at night. This aligns with previous findings on cultured *P. falciparum* gametocyte infectivity in *Anopheles gambiae* susceptible strain (Habtewold, 2022), but contrasts with recent findings on sporozoites, where high infection was observed in mice infected during the night rather than during daytime through DSF rather than DMFA (Bento *et al*., 2025). Additionally, a trial in Mali showed that direct skin feeding (DSF) led to higher infection rates at dusk compared to dawn (Coulibaly *et al*., 2017). This variation may be due to differences in the experimental setup. For example, in the Mali study, dusk feeding was conducted on day 0, while dawn feeding took place two days later (Coulibaly *et al*., 2017).

The discrepancies in mosquito infectiousness may result from both human and mosquito factors. In mosquitoes, circadian rhythms regulate key behaviours such as mating, host-seeking, sugar foraging, and oviposition (Das & Dimopoulos, 2008; Jones *et al*., 1967; Sheppard *et al*., 2017). These rhythms may also influence mosquito immunity to *P. falciparum* gametocytes, which is affected by gut microbiota composition (Gendrin *et al*., 2015; Habtewold, 2022). *An. gambiae* midgut microbiota levels fluctuate throughout the day, with the lowest levels recorded at midnight compared to midday (Gendrin *et al*., 2015). At night, active feeding may trigger mechanisms that reduce normal gut flora, increasing susceptibility to malaria parasites (Habtewold, 2022). However, our study used wild malaria isolates with low gametocyte density, likely contributing to the absence of differences in oocyst intensity. In humans, some studies indicate that *Plasmodium falciparum* gametocytes may exhibit time-of-day-dependent patterns in exflagellation readiness and transmission potential, possibly aligning with mosquito feeding behaviour to maximise transmission success (Reece *et al*., 2017; Scheneider, 2018). These rhythms may be regulated by the parasite's intrinsic molecular clock or host cues such as melatonin, body temperature, or nutrient availability.

4.2.3 Effect of Serum Replacement on Mosquito Infection Rate

As anticipated, a higher proportion of infected mosquitoes was observed in the serum replacement group compared to the whole blood group. This aligns with a previous systematic review, which found that serum replacement enhances mosquito infectivity across 212 membrane-feeding experiments (Bousema *et al*., 2012). This effect is likely due to the removal

of transmission-blocking attributes such as antibodies, drug metabolites, and other factors that are known to reduce mosquitoes' infectivity. Contrary to previous studies, we found no significant difference in oocyst intensity between the two groups (Bousema *et al*., 2012). Bousema *et al*. (2012) observed higher oocyst intensity in serum-replaced blood compared to whole blood. The similarity in oocyst intensity from our study findings may result from low gametocyte density among recruited participants. This may reduce the impact of serum replacement on the gametocytes' infectiousness. Although it has been reported that the mosquito infection rate does not have a direct linear correlation with gametocyte density in human blood (Bousema & Drakeley, 2011; Da *et al*., 2015; Tadesse *et al*., 2018). The effect of serum replacement highlighted by this finding informs researchers that as malaria endemicity decreases, immunity is likely to wane. Therefore, whether or not performing serum replacement is unlikely to affect oocyst intensity.

In addition to antibodies, various host-derived factors found in whole blood can hinder malaria transmission to mosquitoes. These factors include complement proteins, pro-inflammatory cytokines such as tumour necrosis factor-α and interleukin-6 (Naotunne *et al*., 1993) and acute-phase reactants, all of which may decrease gametocyte viability or disrupt gamete fertilisation and ookinete development in the mosquito midgut. Residual antimalarial drugs and their metabolites, particularly long-acting compounds like lumefantrine and piperaquine, slow down the rate of parasite development after ingestion (Peatey *et al*., 2009; Sinden *et al*., 2012). Furthermore, oxidative stress markers and alterations in red blood cell membranes may negatively affect gametocyte function and infectivity (Percário *et al*., 2012). Replacing serum with malaria-naive or pooled control serum likely reduces the influence of these inhibitory components, creating a more conducive and standardised environment for evaluating transmission potential.

4.2.4 Limitations of the Study

First, thick smears for microscopy were not collected on day 9 to confirm the presence of gametocytes, making it difficult to assess gametocyte dynamics and clearance rates. Second, the small sample size of gametocytemic donors, particularly in the AL post-treatment group, where a decline in infectivity was observed, limits the findings. Notably, at least one individual remained infectious 9 days pot treatment. While the study provides valuable insights to enhance bioassays for evaluating transmission-blocking drugs and vaccines among gametocyte

participants, larger sample sizes are necessary to confirm these findings. Third, there was a lack of molecular quantification regarding the persistence of sub microscopic gametocytes.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study confirms that artemether–lumefantrine (AL) markedly reduces human infectiousness to mosquitoes, emphasising the importance of selecting gametocytemic participants who have not recently received AL treatment. Feeding mosquitoes at night does not enhance infection rates, hence, evaluations can be done day or night time, whereas serum replacement leads to a higher proportion of infected mosquitoes.

5.2 Recommendations

Future studies should aim to assess the drug susceptibility of parasite isolates used in DMFAs alongside targeted sequencing approaches to detect resistance-associated mutations. This will help better understand the interactions between the drugs and different parasite genotypes. Additionally, studies on feeding time points should evaluate both pyrethroid-resistant mosquitoes to provide more comprehensive insights.

REFERENCES

Alkema, M., Smit, M. J., Marin-Mogollon, C., Totté, K., Teelen, K., van Gemert, G. J., van de Vegte-Bolmer, M., Mordmüller, B. G., Reimer, J. M., Lövgren-Bengtsson, K. L., Sauerwein, R. W., Bousema, T., Plieskatt, J., Theisen, M., Jore, M. M., & McCall, M. B. B. (2024). A Pfs48/45-based vaccine to block Plasmodium falciparum transmission: Phase 1, open-label, clinical trial. *BMC Medicine, 22(*1), 1-12. https://doi.org/10.1186/s12916-024-03379-y

Bennink, S., Kiesow, M. J., & Pradel, G. (2016). The development of malaria parasites in the mosquito midgut. *Cellular Microbiology, 18*(7), 905–918.

Bento, I., Parrington, B. A., Pascual, R., Goldberg, A. S., Wang, E., Liu, H., Borrmann, H., Zelle, M., Coburn, N., Takahashi, J. S., Elias, J. E., Mota, M. M., & Rijo-Ferreira, F. (2025). Parasite and vector circadian clocks mediate efficient malaria transmission. *Nature Microbiology, 10*(4), 882–896. https://doi.org/10.1038/s41564-025-01949-1

Bousema, S. P., Gouagna, L. C., Drakeley, C. J., Tostmann, A., Houben, R., Githure, J. I., Ord, R., Sutherland, C. J., Omar, S. A., & Sauerwein, R. W. (2006). Moderate effect of artemisinin-based combination therapy on transmission of Plasmodium falciparum. *The Journal of Infectious Diseases, 193*(8), 1151–1159. https://doi.org/10.1086/503051

Bousema, T., Dinglasan, R. R., Morlais, I., Gouagna, L. C., van Warmerdam, T., Awono-Ambene, P. H., Bonnet, S., Diallo, M., Coulibaly, M., Tchuinkam, T., Mulder, B., Targett, G., Drakeley, C., Sutherland, C., Robert, V., Doumbo, O., Touré, Y., Graves, P. M., Roeffen, W., … Churcher, T. S. (2012). Mosquito feeding assays to determine the infectiousness of naturally infected Plasmodium falciparum gametocyte carriers. *PloS One, 7*(8), e42821. https://doi.org/10.1371/journal.pone.0042821

Bousema, T., & Drakeley, C. (2011). Epidemiology and infectivity of Plasmodium falciparum and Plasmodium vivax gametocytes in relation to malaria control and elimination. *Clinical Microbiology Reviews, 24*(2), 377–410. https://doi.org/10.1128/CMR.00051-10

Bousema, T., Okell, L., Shekalaghe, S., Griffin, J. T., Omar, S., Sawa, P., Sutherland, C., Sauerwein, R., Ghani, A. C., & Drakeley, C. (2010). Revisiting the circulation time of Plasmodium falciparum gametocytes: Molecular detection methods to estimate the duration of gametocyte carriage and the effect of gametocytocidal drugs. *Malaria Journal, 9,* 1-11. https://doi.org/10.1186/1475-2875-9-136

Bradley, J., Soumare, H., Mahamar, A., Diawara, H., Roh, M., Delves, M., Drakeley, C., Churcher, T., Dicko, A., Gosling, R., & Bousema, T. (2019). Transmission-blocking effects of primaquine and methylene blue suggest P. falciparum gametocyte sterilisation rather than effects on sex ratio. *Clinical Infectious Diseases, 69*(8), 1436-1439. https://doi.org/10.1093/cid/ciz134

Butcher, G. A. (1997). Antimalarial drugs and the mosquito transmission of Plasmodium. *International Journal for Parasitology, 27*(9), 975–987.

Carter, L. M., Pollitt, L. C., Wilson, L. G., & Reece, S. E. (2016). Ecological influences on the behaviour and fertility of malaria parasites. *Malaria Journal, 15*(1), 1-13.

CDC. (2023). *Life Cycle of Anopheles Species Mosquitoes | Mosquitoes | CDC.* https://www.cdc.gov/mosquitoes/about/life-cycles/anopheles.html

Chawla, J., Oberstaller, J., & Adams, J. H. (2021). Targeting gametocytes of the malaria parasite Plasmodium falciparum in a functional genomics era: Next steps. *Pathogens*, *10*(3), 1-22.

Chen, P. Q., Li, G. Q., Guo, X. B., He, K. R., Fu, Y. X., Fu, L. C., & Song, Y. Z. (1994). The infectivity of gametocytes of Plasmodium falciparum from patients treated with artemisinin. *Chinese Medical Journal, 107*(9), 709–711.

Christian P. (2016). P*lasmodium falciparum gametocyte transit through the cutaneous microvasculature: A new target for malaria transmission blocking vaccines?* PubMed. https://pubmed.ncbi.nlm.nih.gov/27184760/

Churcher, T. S., Bousema, T., Walker, M., Drakeley, C., Schneider, P., Ouédraogo, A. L., & Basáñez, M. G. (2013). Predicting mosquito infection from Plasmodium falciparum gametocyte density and estimating the reservoir of infection. *eLife, 2,* 1-12. https://doi.org/10.7554/eLife.00626

Coalson, J. E., Walldorf, J. A., Cohee, L. M., Ismail, M. D., Mathanga, D., Cordy, R. J., Marti, M., Taylor, T. E., Seydel, K. B., Laufer, M. K., & Wilson, M. L. (2016). High prevalence of Plasmodium falciparum gametocyte infections in school-age children using molecular detection: Patterns and predictors of risk from a cross-sectional study in southern Malawi. *Malaria Journal, 15*(1), 1-17. https://doi.org/10.1186/s12936-016-1587-9

Coulibaly, M. B., Gabriel, E. E., Sinaba, Y., Sylla, D., Sacko, A., Sylla, L., Coulibaly, B., Hume, J. C. C., Baber, I., Assadou, M. H., Sagara, I., Wu, Y., Healy, S. A., Doumbo, O., Traore, S. F., & Duffy, P. E. (2017). Optimizing Direct Membrane and Direct Skin Feeding Assays for Plasmodium falciparum Transmission-Blocking Vaccine Trials in Bancoumana, Mali. *The American Journal of Tropical Medicine and Hygiene, 97*(3), 719–725.

Da, D. F., Churcher, T. S., Yerbanga, R. S., Yaméogo, B., Sangaré, I., Ouedraogo, J. B., Sinden, R. E., Blagborough, A. M., & Cohuet, A. (2015). Experimental study of the relationship between Plasmodium gametocyte density and infection success in mosquitoes; implications for the evaluation of malaria transmission-reducing interventions. E*xperimental Parasitology, 149,* 74–83. https://doi.org/10.1016/j.exppara.2014.12.010

Das, S., & Dimopoulos, G. (2008). Molecular analysis of photic inhibition of blood-feeding in Anopheles gambiae. *BMC Physiology, 8,* 1-19. https://doi.org/10.1186/1472-6793-8-23

Dong, Y., Das, S., Cirimotich, C., Souza-Neto, J. A., McLean, K. J., & Dimopoulos, G. (2011). Engineered anopheles immunity to Plasmodium infection. *PLoS Pathogens, 7*(12), 1-12. https://doi.org/10.1371/journal.ppat.1002458

Gendrin, M., Rodgers, F. H., Yerbanga, R. S., Ouédraogo, J. B., Basáñez, M. G., Cohuet, A., & Christophides, G. K. (2015). Antibiotics in ingested human blood affect the mosquito microbiota and capacity to transmit malaria. *Nature Communications, 6*(1), 1-7.

Gonçalves, B. P., Kapulu, M. C., Sawa, P., Guelbéogo, W. M., Tiono, A. B., Grignard, L., Stone, W., Hellewell, J., Lanke, K., Bastiaens, G. J. H., Bradley, J., Nébié, I., Ngoi, J. M., Oriango, R., Mkabili, D., Nyaurah, M., Midega, J., Wirth, D. F., Marsh, K., … Bousema, T. (2017). Examining the human infectious reservoir for Plasmodium falciparum malaria in areas of differing transmission intensity. *Nature Communications, 8*(1), 1-11.

Gonçalves, B. P., Tiono, A. B., Ouédraogo, A., Guelbéogo, W. M., Bradley, J., Nebie, I., Siaka, D., Lanke, K., Eziefula, A. C., Diarra, A., Pett, H., Bougouma, E. C., Sirima, S. B., Drakeley, C., & Bousema, T. (2016). Single low dose primaquine to reduce gametocyte carriage and Plasmodium falciparum transmission after artemether-lumefantrine in children with asymptomatic infection: A randomised, double-blind, placebo-controlled trial. *BMC Medicine, 14*(1), 1-11. https://doi.org/10.1186/s12916-016-0581-y

Goodman, A. L., & Draper, S. J. (2010). Blood-stage malaria vaccines—Recent progress and future challenges. *Annals of Tropical Medicine and Parasitology, 104*(3), 189–211. https://doi.org/10.1179/136485910X12647085215534

Habtewold, T., Tapanelli, S., Masters, E. K., Windbichler, N., & Christophides, G. K. (2022). The circadian clock modulates Anopheles gambiae infection with Plasmodium falciparum. *PLoS One*, *17*(12), 1-15.

Habtewold, T., Tapanelli, S., Masters, E. K. G., Hoermann, A., Windbichler, N., & Christophides, G. K. (2019). Streamlined SMFA and mosquito dark-feeding regime significantly improve malaria transmission-blocking assay robustness and sensitivity. *Malaria Journal, 18*(1), 1-11.

Hawking, F. (1967). The 24-hour periodicity of microfilariae: Biological mechanisms responsible for its production and control. Proceedings of the Royal Society of London. Series B. *Biological Sciences, 169*(1014), 59-76.

Hillyer, J. F., Barreau, C., & Vernick, K. D. (2007a). Efficiency of salivary gland invasion by malaria sporozoites is controlled by rapid sporozoite destruction in the mosquito haemocoel. *International Journal for Parasitology, 37(*6), 673–681.

Hillyer, J. F., Barreau, C., & Vernick, K. D. (2007b). Efficiency of salivary gland invasion by malaria sporozoites is controlled by rapid sporozoite destruction in the mosquito haemocoel. *International Journal for Parasitology, 37*(6), 673–681.

Hofer, L. M., Kweyamba, P. A., Sayi, R. M., Chabo, M. S., Maitra, S. L., Moore, S. J., & Tambwe, M. M. (2023). Malaria rapid diagnostic tests reliably detect asymptomatic Plasmodium falciparum infections in school-aged children that are infectious to mosquitoes. *Parasites and Vectors, 16*(1), 1-10. https://doi.org/10.1186/s13071-023-05761-w

Hofer, L. M., Kweyamba, P. A., Sayi, R. M., Chabo, M. S., Mwanga, R., Maitra, S. L., Somboka, M. M., Schnoz, A., Golumbeanu, M., Schneeberger, P. H. H., Ross, A., Habtewold, T., Nsanzabana, C., Moore, S. J., & Tambwe, M. M. (2024). Additional blood meals increase sporozoite infection in Anopheles mosquitoes but not Plasmodium falciparum genetic diversity. *Scientific Reports, 14*(1), 1-13. https://doi.org/10.1038/s41598-024-67990-y

Roth, J. M., Sawa, P., Omweri, G., Osoti, V., Makio, N., Bradley, J., Wambua, J., Mosha, J. F., Drakeley, C., & Mens, P. F. & Mens, P. F. (2018). Plasmodium falciparum gametocyte dynamics after pyronaridine–artesunate or artemether–lumefantrine treatment. *Malaria Journal, 17*(1), 1-11.

Jones, M. D. R., Hill, M., & Hope, A. M. (1967). The Circadian Flight Activity of the Mosquito, Anopheles gambiae: Phase Setting by the Light Regime. *Journal of Experimental Biology, 47*(3), 503–511. https://doi.org/10.1242/jeb.47.3.503

Kaindoa, E. W., Matowo, N. S., Ngowo, H. S., Mkandawile, G., Mmbando, A., Finda, M., & Okumu, F. O. (2017). Interventions that effectively target Anopheles funestus mosquitoes could significantly improve control of persistent malaria transmission in south-eastern Tanzania. *PloS One, 12*(5), 1-19. https://doi.org/10.1371/journal.pone.0177807

Kone, A., van de Vegte-Bolmer, M., Siebelink-Stoter, R., van Gemert, G. J., Dara, A., Niangaly, H., Luty, A., Doumbo, O. K., Sauerwein, R., & Djimde, A. A. (2010). Sulfadoxine-pyrimethamine impairs Plasmodium falciparum gametocyte infectivity and Anopheles mosquito survival. *International Journal for Parasitology, 40*(10), 1221–1228.

Kristan, M., Lines, J., Nuwa, A., Ntege, C., Meek, S. R., & Abeku, T. A. (2016). Exposure to deltamethrin affects development of Plasmodium falciparum inside wild pyrethroid resistant Anopheles gambiae s.s. Mosquitoes in Uganda. *Parasites and Vectors, 9*(1), 1-9. https://doi.org/10.1186/s13071-016-1384-x

Kweyamba, P. A., Hofer, L. M., Kibondo, U. A., Mwanga, R. Y., Sayi, R. M., Matwewe, F., Austin, J. W., Stutz, S., Moore, S. J., Müller, P., & Tambwe, M. M. (2023). Sub-lethal exposure to chlorfenapyr reduces the probability of developing Plasmodium falciparum parasites in surviving Anopheles mosquitoes. *Parasites and Vectors, 16*(1), 1-9. https://doi.org/10.1186/s13071-023-05963-2

Kyrou, K., Hammond, A. M., Galizi, R., Kranjc, N., Burt, A., Beaghton, A. K., Nolan, T., & Crisanti, A. (2018). A CRISPR–Cas9 gene drive targeting doublesex causes complete population suppression in caged Anopheles gambiae mosquitoes. *Nature Biotechnology, 36*(11), 1062–1066. https://doi.org/10.1038/nbt.4245

Leite, L. N., Bascuñán, P., Dotson, E. M., & Benedict, M. Q. (2024). Considerations for Rearing and Maintaining Anopheles in the Laboratory. *Cold Spring Harbor Protocols, 2024*(3), pdb.top107802. https://doi.org/10.1101/pdb.top107802

Lensen, A., Bril, A., van de Vegte, M., van Gemert, G. J., Eling, W., & Sauerwein, R. (1999). Plasmodium falciparum: Infectivity of cultured, synchronized gametocytes to mosquitoes. *Experimental Parasitology, 91*(1), 101–103. https://doi.org/10.1006/expr.1998.4354

Mahamar, A., Smit, M. J., Sanogo, K., Sinaba, Y., Niambele, S. M., Sacko, A., Dicko, O. M., Diallo, M., Maguiraga, S. O., Sankaré, Y., Keita, S., Samake, S., Dembele, A., Lanke, K., Ter Heine, R., Bradley, J., Dicko, Y., Traore, S. F., Drakeley, C., … Stone, W. (2024). Artemether-lumefantrine with or without single-dose primaquine and sulfadoxine-pyrimethamine plus amodiaquine with or without single-dose tafenoquine to reduce Plasmodium falciparum transmission: A phase 2, single-blind, randomised clinical trial in Ouelessebougou, Mali. *The Lancet. Microbe, 5*(7), 633–644. https://doi.org/10.1016/S2666-5247(24)00023-5

Makanga, M. (2014). A review of the effects of artemether-lumefantrine on gametocyte carriage and disease transmission. *Malaria Journal, 13,* 1-15. https://doi.org/10.1186/1475-2875-13-291

Makanga, M., & Krudsood, S. (2009). The clinical efficacy of artemether/lumefantrine (Coartem®). *Malaria Journal, 8*(Suppl 1), S1-S5. https://doi.org/10.1186/1475-2875-8-S1-S5

Matowo, N. S., Martin, J., Kulkarni, M. A., Mosha, J. F., Lukole, E., Isaya, G., Shirima, B., Kaaya, R., Moyes, C., Hancock, P. A., Rowland, M., Manjurano, A., Mosha, F. W., Protopopoff, N., & Messenger, L. A. (2021). An increasing role of pyrethroid-resistant Anopheles funestus in malaria transmission in the Lake Zone, Tanzania. *Scientific Reports, 11,* 1-13

Meibalan, E., Barry, A., Gibbins, M. P., Awandu, S., Meerstein-Kessel, L., Achcar, F., Bopp, S., Moxon, C., Diarra, A., Debe, S., Ouédraogo, N., Barry-Some, I., Badoum, E. S., Fagnima, T., Lanke, K., Gonçalves, B. P., Bradley, J., Wirth, D., Drakeley, C., … Bousema, T. (2019). Plasmodium falciparum Gametocyte Density and Infectivity in Peripheral Blood and Skin Tissue of Naturally Infected Parasite Carriers in Burkina Faso. *The Journal of Infectious Diseases, 223*(10), 1822–1830. https://doi.org/10.1093/infdis/jiz680

Mlugu, E. M., Minzi, O., Kamuhabwa, A. A. R., & Aklillu, E. (2020). Prevalence and Correlates of Asymptomatic Malaria and Anemia on First Antenatal Care Visit among Pregnant Women in Southeast, Tanzania. *International Journal of Environmental Research and Public Health, 17*(9), 1-16. https://doi.org/10.3390/ijerph17093123

MoHSW. (2006). *National guidelines for diagnosis and treatment of malaria. Dar es Salaam: Ministry of Health and Social Welfare.* chrome-extension://efaidnbmnnnibp cajpcglclefindmkaj/https://www.nmcp.go.tz/storage/app/uploads/public/643/90d/29e/64390d29ef4b0392189644.pdf

Mukabana, L. N., Mshani, I. H., Gachohi, J., Minja, E. G., Jackson, F. M., Kahamba, N. F., Pinda, P. G., Muyaga, L., Msaky, D. S., Ngowo, H. S., Mambo, S. N., Olwendo, A., Bisanzio, D., & Okumu, F. O. (2025). Heterogeneous malaria transmission patterns in southeastern Tanzania driven by socio-economic and environmental factors. Malaria Journal, 24(1), 1-12. https://doi.org/10.1186/s12936-025-05418-2

Murdock, C. C., Moller-Jacobs, L. L., & Thomas, M. B. (2013). Complex environmental drivers of immunity and resistance in malaria mosquitoes. *Proceedings of the Royal Society B: Biological Sciences, 280*(1770), 1-10. https://doi.org/10.1098/rspb.2013.2030

National Bureau of Statistics (NBS) [Tanzania], Office of the Chief Government Statistician (OCGS) [Zanzibar], Ministry of Health (MoH) [Tanzania Mainland], Ministry of Health (MoH) [Zanzibar], & ICF. (2023). *Tanzania demographic and health survey and malaria indicator survey 2022: Final report.* ICF. https://www.dhsprogram.com/publications/publication-FR382-DHS-Final-Reports.cfm

Naotunne, T. S., Karunaweera, N. D., Mendis, K. N., & Carter, R. (1993). Cytokine-mediated inactivation of malarial gametocytes is dependent on the presence of white blood cells and involves reactive nitrogen intermediates. *Immunology, 78*(4), 555–562.

Ngasala, B., Chiduo, M. G., Bushukatale, S., Mmbando, B. P., Makene, T., Kamugisha, E., Ahmed, M., Mandara, C. I., Francis, F., Mahende, M. K., Kavishe, R. A., Muro, F., Ishengoma, D. S., Mandike, R., Molteni, F., Chacky, F., Kitojo, C., Greer, G., Bishanga, D., … Mohamed, A. (2024). Efficacy and safety of artemether-lumefantrine for the treatment of uncomplicated falciparum malaria in mainland Tanzania, 2018. *Malaria Journal, 23*(1), 1-12. https://doi.org/10.1186/s12936-024-04926-x

Ngotho, P., Soares, A. B., Hentzschel, F., Achcar, F., Bertuccini, L., & Marti, M. (2019). Revisiting gametocyte biology in malaria parasites. F*EMS Microbiology Reviews, 43*(4), 401–414. https://doi.org/10.1093/femsre/fuz010

Odero, J. O., Nambunga, I. H., Wangrawa, D. W., Badolo, A., Weetman, D., Koekemoer, L. L., Ferguson, H. M., Okumu, F. O., & Baldini, F. (2023). Advances in the genetic characterization of the malaria vector, Anopheles funestus, and implications for improved surveillance and control. *Malaria Journal, 22*(1), 1-14.

Omondi, P., Burugu, M., Matoke-Muhia, D., Too, E., Nambati, E. A., Chege, W., Musyoka, K. B., Thiongo, K., Otinga, M., Muregi, F., & Kimani, F. (2019). Gametocyte clearance in children, from western Kenya, with uncomplicated Plasmodium falciparum malaria after artemether-lumefantrine or dihydroartemisinin-piperaquine treatment. Malaria Journal, 18(1), 1-9. https://doi.org/10.1186/s12936-019-3032-3

Ouattara, S., Hien, D., Nao, E., Pare, P., Guissou, E., Cohuet, A., Morlais, I., Yerbanga, R. S., Dabiré, R., Ouédraogo, J. B., Mouline, K., & Lefevre, T. (2024). A simple, field-applicable method to increase the infectivity of wild isolates of Plasmodium falciparum to mosquito vectors. *Malaria Journal, 23,* 1-9. https://doi.org/10.1186/s12936-024-04969-0

Peatey, C. L., Skinner-Adams, T. S., Dixon, M. W. A., McCarthy, J. S., Gardiner, D. L., & Trenholme, K. R. (2009). Effect of antimalarial drugs on Plasmodium falciparum gametocytes. *The Journal of Infectious Diseases, 200*(10), 1518–1521.

Percário, S., Moreira, D. R., Gomes, B. A. Q., Ferreira, M. E. S., Gonçalves, A. C. M., Laurindo, P. S. O. C., Vilhena, T. C., Dolabela, M. F., & Green, M. D. (2012). Oxidative Stress in Malaria. *International Journal of Molecular Sciences, 13*(12), 16346–16372. https://doi.org/10.3390/ijms131216346

Reece, S. E., Prior, K. F., & Mideo, N. (2017). The Life and Times of Parasites: Rhythms in Strategies for Within-host Survival and Between-host Transmission. J*ournal of Biological Rhythms, 32*(6), 516–533. https://doi.org/10.1177/0748730417718904

Richie, T. L., & Saul, A. (2002). Progress and challenges for malaria vaccines. *Nature, 415*(6872), 694–701. https://doi.org/10.1038/415694a

Rijo-Ferreira, F., & Takahashi, J. S. (2019). Genomics of circadian rhythms in health and disease. *Genome Medicine, 11*(1), 1-16. https://doi.org/10.1186/s13073-019-0704-0

Rijo-Ferreira, F., Takahashi, J. S., & Figueiredo, L. M. (2017). Circadian rhythms in parasites. *PLOS Pathogens, 13*(10), 1-9. https://doi.org/10.1371/journal.ppat.1006590

Rund, S., O'Donnell, A., Gentile, J., & Reece, S. (2016). Daily Rhythms in Mosquitoes and Their Consequences for Malaria Transmission. *Insects, 7*(2), 1-20.

Reece, S. E., Prior, K. F., & Mideo, N. (2017). The life and times of parasites: Rhythms in strategies for within-host survival and between-host transmission. *Journal of biological rhythms*, *32*(6), 516-533.

Schneider, P., Rund, S. S., Smith, N. L., Prior, K. F., O'Donnell, A. J., & Reece, S. E. (2018). Adaptive periodicity in the infectivity of malaria gametocytes to mosquitoes. *Proceedings of the Royal Society B*, *285*(1888), 1-9. https://doi.org/10.1098/rspb.2018.1876

Sheppard, A. D., Rund, S. S., George, G. F., Clark, E., Acri, D. J., & Duffield, G. E. (2017). Light manipulation of mosquito behaviour: Acute and sustained photic suppression of biting activity in the Anopheles gambiae malaria mosquito. *Parasites and Vectors, 10*(1), 1-14. https://doi.org/10.1186/s13071-017-2196-3

Sinclair, D., Zani, B., Donegan, S., Olliaro, P., & Garner, P. (2009). Artemisinin-based combination therapy for treating uncomplicated malaria. T*he Cochrane Database of Systematic Reviews, 2009*(3), 1-220.

Sinden, R. E., Carter, R., Drakeley, C., & Leroy, D. (2012). The biology of sexual development of Plasmodium: The design and implementation of transmission-blocking strategies. M*alaria Journal, 11,* 1-11. https://doi.org/10.1186/1475-2875-11-70

Sinka, M. E., Bangs, M. J., Manguin, S., Rubio-Palis, Y., Chareonviriyaphap, T., Coetzee, M., Mbogo, C. M., Hemingway, J., Patil, A. P., Temperley, W. H., Gething, P. W., Kabaria, C. W., Burkot, T. R., Harbach, R. E., & Hay, S. I. (2012). A global map of dominant malaria vectors. *Parasites and Vectors, 5,* 1-11. https://doi.org/10.1186/1756-3305-5-69

Siria, D. J., Batista, E. P. A., Opiyo, M. A., Melo, E. F., Sumaye, R. D., Ngowo, H. S., Eiras, A. E., & Okumu, F. O. (2018). Evaluation of a simple polytetrafluoroethylene (PTFE)-based membrane for blood-feeding of malaria and dengue fever vectors in the laboratory. *Parasites and Vectors, 11*(1), 1-10. https://doi.org/10.1186/s13071-018-2823-7

Smalley, M. E., Abdalla, S., & Brown, J. (1981). The distribution of Plasmodium falciparum in the peripheral blood and bone marrow of Gambian children. T*ransactions of the Royal Society of Tropical Medicine and Hygiene, 75*(1), 103–105. https://doi.org/10.1016/0035-9203(81)90019-5

Smalley, M. E., & Sinden, R. E. (1977). Plasmodium falciparum gametocytes: Their longevity and infectivity. *Parasitology, 74*(1), 1–8. https://doi.org/10.1017/s0031182000047478

Smith, R. C., Vega-Rodríguez, J., & Jacobs-Lorena, M. (2014). The Plasmodium bottleneck: Malaria parasite losses in the mosquito vector. *Memórias Do Instituto Oswaldo Cruz, 109*(5), 644–661. https://doi.org/10.1590/0074-0276130597

Smithuis, F., Kyaw, M. K., Phe, O., Win, T., Aung, P. P., Oo, A. P. P., Naing, A. L., Nyo, M. Y., Myint, N. Z. H., Imwong, M., Ashley, E., Lee, S. J., & White, N. J. (2010). Effectiveness of five artemisinin combination regimens with or without primaquine in uncomplicated falciparum malaria: An open-label randomised trial. *The Lancet. Infectious Diseases, 10*(10), 673–681. https://doi.org/10.1016/S1473-3099(10)70187-0

Spitzen, J., & Takken, W. (2005). Malaria mosquito rearing-maintaining quality and quantity of laboratory-reared insects. *Proceedings of the Netherlands Entomological Society Meeting, 16*, 95-100.

Stone, W. J. R., Eldering, M., van Gemert, G.-J., Lanke, K. H. W., Grignard, L., van de Vegte-Bolmer, M. G., Siebelink-Stoter, R., Graumans, W., Roeffen, W. F. G., Drakeley, C. J., Sauerwein, R. W., & Bousema, T. (2013). The relevance and applicability of oocyst prevalence as a read-out for mosquito feeding assays. *Scientific Reports, 3,* 1-8. https://doi.org/10.1038/srep03418

Stone, W., Mahamar, A., Smit, M. J., Sanogo, K., Sinaba, Y., Niambele, S. M., Sacko, A., Keita, S., Dicko, O. M., Diallo, M., Maguiraga, S. O., Samake, S., Attaher, O., Lanke, K., Ter Heine, R., Bradley, J., McCall, M. B. B., Issiaka, D., Traore, S. F., … Dicko, A. (2022). Single low-dose tafenoquine combined with dihydroartemisinin-piperaquine to reduce Plasmodium falciparum transmission in Ouelessebougou, Mali: A phase 2, single-blind, randomised clinical trial. *The Lancet. Microbe, 3*(5), e336–e347.

Sutherland. (2005). *Reduction of malaria transmission to Anopheles mosquitoes with a six-dose regimen of co-artemether-PubMed*. https://pubmed.ncbi.nlm.nih.gov/15839740/

Sutherland, C. J., Ord, R., Dunyo, S., Jawara, M., Drakeley, C. J., Alexander, N., Coleman, R., Pinder, M., Walraven, G., & Targett, G. A. T. (2005). Reduction of Malaria Transmission to Anopheles Mosquitoes with a Six-Dose Regimen of Co-Artemether. *PLOS Medicine, 2*(4), 1-9. https://doi.org/10.1371/journal.pmed.0020092

Tadesse, F. G., Slater, H. C., Chali, W., Teelen, K., Lanke, K., Belachew, M., Menberu, T., Shumie, G., Shitaye, G., Okell, L. C., Graumans, W., Van Gemert, G.-J., Kedir, S., Tesfaye, A., Belachew, F., Abebe, W., Mamo, H., Sauerwein, R., Balcha, T., … Bousema, T. (2018). The Relative Contribution of Symptomatic and Asymptomatic Plasmodium vivax and Plasmodium falciparum Infections to the Infectious Reservoir in a Low-Endemic Setting in Ethiopia. *Clinical Infectious Diseases, 66*(12), 1883–1891.

Thomson, J. G., & Robertson, A. (1935). The structure and development of plasmodium falciparum gametocytes in the internal organs and peripheral circulation. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 29*(1), 31–40.

van der Kolk, M., de Vlas, S. J., & Sauerwein, R. W. (2006). Reduction and enhancement of Plasmodium falciparum transmission by endemic human sera. *International Journal for Parasitology, 36*(10–11), 1091–1095. https://doi.org/10.1016/j.ijpara.2006.05.004

van der Kolk, M., De Vlas, S. J., Saul, A., van de Vegte-Bolmer, M., Eling, W. M., & Sauerwein, R. W. (2005a). Evaluation of the standard membrane feeding assay (SMFA) for the determination of malaria transmission-reducing activity using empirical data. *Parasitology, 130*(Pt 1), 13–22. https://doi.org/10.1017/s0031182004006067

van der Kolk, M., De Vlas, S. J., Saul, A., van de Vegte-Bolmer, M., Eling, W. M., & Sauerwein, R. W. (2005b). Evaluation of the standard membrane feeding assay (SMFA) for the determination of malaria transmission-reducing activity using empirical data. *Parasitology, 130*(Pt 1), 13–22. https://doi.org/10.1017/s0031182004006067

van Pelt-Koops, J. C., Pett, H. E., Graumans, W., van der Vegte-Bolmer, M., van Gemert, G. J., Rottmann, M., Yeung, B. K. S., Diagana, T. T., & Sauerwein, R. W. (2012). The Spiroindolone Drug Candidate NITD609 Potently Inhibits Gametocytogenesis and Blocks Plasmodium falciparum Transmission to Anopheles Mosquito Vector. *Antimicrobial Agents and Chemotherapy, 56*(7), 3544–3548. https://doi.org/10.1128/AAC.06377-11

WHO. (2010). *WHO Guidelines for malaria.* https://www.who.int/publications/i/item/guidelines-for-malaria

WHO. (2023). *World malaria report 2023*. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2023

Witmer, K., Dahalan, F. A., Delves, M. J., Yahiya, S., Watson, O. J., Straschil, U., Chiwcharoen, D., Sornboon, B., Pukrittayakamee, S., Pearson, R. D., Howick, V. M., Lawniczak, M. K. N., White, N. J., Dondorp, A. M., Okell, L. C., Chotivanich, K., Ruecker, A., & Baum, J. (2020). Transmission of Artemisinin-Resistant Malaria Parasites to Mosquitoes under Antimalarial Drug Pressure. *Antimicrobial Agents and Chemotherapy, 65*(1), e00898-20. https://doi.org/10.1128/AAC.00898-20

WWARN Gametocyte Study Group. (2016). Gametocyte carriage in uncomplicated Plasmodium falciparum malaria following treatment with artemisinin combination therapy: A systematic review and meta-analysis of individual patient data. *BMC Medicine, 14,* 1-18. https://doi.org/10.1186/s12916-016-0621-7

Yimam, Y., Nateghpour, M., Mohebali, M., & Abbaszadeh Afshar, M. J. (2021). A systematic review and meta-analysis of asymptomatic malaria infection in pregnant women in Sub-Saharan Africa: A challenge for malaria elimination efforts. *PloS One, 16*(4), 1-15. https://doi.org/10.1371/journal.pone.0248245

Yu, S., Wang, J., Luo, X., Zheng, H., Wang, L., Yang, X., & Wang, Y. (2022). Transmission-Blocking Strategies Against Malaria Parasites During Their Mosquito Stages. *Frontiers in Cellular and Infection Microbiology*, *12*, 1-14.

APPENDICES

Appendix 1: Informed consent form

PART I: Information sheet

Study Title: **Impact of artemether-lumefantrine treatment, circadian rhythm and serum replacement on the infectiousness of wild *P. falciparum* gametocytes to *An. gambiae* sensu stricto mosquitoes.**

Subject's description: Male and female aged 6-45 years

Principal Investigator: Dorin Mmasi

Co- Investigators: Dr Mgeni Mohamed Tambwe

Prof. Sarah Moore

Study site: Ifakara Health Institute, Bagamoyo Branch

INTRODUCTION

My name is..... (name of the PI), and I work for Ifakara Health Institute. Malaria is a disease affecting both males and females of different age groups. The disease is caused by malaria parasites that live in the blood and are transmitted by some kinds of female mosquitoes when they bite people. We are doing a study on how best we can prevent transmission from malaria-infected humans to mosquitoes. We invite you to participate in this study. Before you decide to participate, feel free to talk to your parents or caregivers about the study. Please feel free to ask any of the study personnel any questions or clarifications that will allow you to understand clearly the nature of the study

PURPOSE OF THE STUDY

The purpose of this study is to effect of Antimalarial treatment, serum replacement, circadian rhythm on the infectiousness of wild *P. falciparum* gametocytes to *An. gambiae sensu stricto* mosquitoes

STUDY SITE

The study will be conducted at IHI Bagamoyo in Kingani, in the Bagamoyo district.

HOW IS MY CHILD SELECTED?

The participants will be selected from Kibindu, Chamgoi, Miono, and Wamimkoko villages located in the Bagamoyo district. Healthy school children with no signs or symptoms of malaria will be screened for malaria parasites. Those who will meet the inclusion criteria will be invited to participate. The study will include 3 or more gametocyte carriers.

WHICH ARE THE PROCEDURES THAT WILL BE PERFORMED ON MY CHILD?

I. If you and your child agree to participate, we will ask both of you to sign an informed consent form, and then the following procedures will be performed on your child.
II. We test for the malaria parasite by taking a drop of blood from the finger. If your child is found to have malaria parasites, they will be taken to the IHI Bagamoyo laboratory within 24 hours under the supervision of the parent/guardian and the teacher.
III. At the IHI Bagamoyo laboratory, we will ask your child to allow a nurse to draw 5 mL of blood (equivalent to one teaspoonful).
IV. Then, your child will receive treatment for malaria for free, and you will be allowed to go home.
V. The blood will then be fed to the mosquitoes we have in our laboratory.
VI. A child who has been administered an AL drug will be invited again to donate blood on day 9. The blood will be fed to the mosquitoes we have in our laboratory.

WHAT IS THE DURATION OF THE STUDY AND PARTICIPATION?

The duration of study will be one or two months, depending on the availability of individuals with gametocytes, but your child will participate for a maximum of 24 hours in three different days at the Bagamoyo insectary laboratory.

WHAT ARE THE BENEFITS TO MY CHILD?

There are no direct benefits to participating in this study. The findings will provide a better understanding and contribute to the body of knowledge on the transmission of the disease and in searching for new methods to block transmission from infected humans to female *Anopheles* mosquitoes. It is expected that two children out of 10 will test positive for malaria. Yet the risk of being infected remains high for the others. If your child tests positive for malaria parasites, they will be given malaria treatment for free. This treatment will eliminate the presence of the parasites in your child's blood. Yet, it does not prevent him or her from being infected again, so it is advisable to continue using mosquito nets and insecticides.

ARE THERE ANY RISKS, BURDEN OR DISCOMFORT ASSOCIATED WITH MY CHILD'S PARTICIPATION?

Your child may experience pain or discomfort as a study nurse will draw a blood sample for feeding mosquitoes. This pain or bleeding does not need any medication and will stop as soon as the blood sampling process is completed. A blood sample may be drawn while your child is sitting. All materials to be used in blood sample collection are sterile; therefore, there is no chance of introducing any infection into your child's body.

WILL MY CHILD RECEIVE ANY PAYMENT?

There will be no payment made for your child's participation in the screening. However, if your child is brought to Bagamoyo for blood drawing, you will receive TSH 20,000/= as compensation to cover the meal and transportation back to school/home. A teacher who will escort a child participant will be given Tsh. 10,000/= to cover the meal and transportation back to school. A teacher will receive this amount of money regardless of the number of school children to be escorted at the IHI insectary for mosquitoes feeding on that particular day.

MAY I REFUSE TO PARTICIPATE OR WITHDRAW MY CHILD FROM THE STUDY?

The decision for your child to participate in this study is completely voluntary. You can change your mind at any time, and this will not affect any medical attention that your child might need if they have malaria parasites before exiting the study.

ARE MY CHILD'S DATA AND SAMPLES KEPT UNDER CONFIDENTIALITY?

All the information collected from this study will be confidential and used only for this research project. Your child's name will not be used on labels, on laboratory samples or in any report of this study. The information that may link participants with the study number will be kept in a locked cabinet, only accessed by principal investigators. All blood samples will be used up during the study.

IS THERE ANY ETHICAL/REGULATORY APPROVAL FOR CONDUCTING THIS STUDY?

Permission to conduct this study has been received from the following regulatory authority: Ifakara Health Institute-Institutional Ethics Review Board (IRB) certificate number IHI/IRB/No: 20-2024 and National Institute for Medical Research (NIMR) certificate number BD.242/437/01C/164, after being satisfied that the volunteer safety is followed.

IF YOU HAVE ANY QUESTIONS ABOUT THIS TRIAL

The principal investigator, co-investigator or any other member of the study team may be contacted in case of any question or need of clarification related to this study or related to your participation at any time before signing the consent form and during the study period.

i. **Principal Investigator**: Dorin Joachim Mmasi +255 620 222 761
ii. **Co-investigator**: Dr Mgeni Mohamed +255 713737447
iii. **Secretary to the IHI-IRB:** Dr Mwifadhi Mrisho, mobile phone +255 788 766676.
iv. **Secretary to the NatHREC**: Dr Mwanaidi Kafuye of the National Institute of Medical Research, landline telephone 0222121400

Appendix 2: Parent consent form

PART II: Certificate of Consent

PARENT / GUARDIAN CONSENT FORM

I,_____therefore, consent for my child to participate in the research study entitled **"Impact of artemether lumefantrine, circadian rhythm and serum replacement on the infectiousness of wild *P. falciparum* gametocytes to *An. gambiae* sensu stricto mosquitoes".** I attest to having received the information that describes the study procedures, including blood sampling, and have received an additional extensive explanation about the content of this information sheet from a study person.

I attest to having understood the aim, procedures, benefits, such as giving malaria treatment for free and risks, such as pain during blood sampling and that I was given sufficient time to ask all my questions related to the procedures, benefits and risks of participation and have received satisfactory answers. If I have any more questions, I understand that I may contact the study team at the telephone numbers given on the information sheet.

I attest to understanding that I have the right to withdraw my consent and choose to stop the participation of my child at any time. If I have any more questions, I understand that I may contact the principal investigator Dorin Mmasi, at +255 620 222 761.

I understand I will be given a copy of the information sheet and consent form to keep for my records. I VOLUNTARILY consent for my child to participate.

CHILD'S PARTICIPANT PARTICULARS		
First Name	Middle Name	Surname
Date: |__|__|/|__|__|/|__|__|__|__|(dd/mm/yyyy)	Time: |__|__|:|__|__| (hrs)	Signature:
PARENT/GUARDIAN		
First Name	Middle Name	Surname
Date: |__|__|/|__|__|/|__|__|__|__|(dd/mm/yyyy)	Time: |__|__|:|__|__| (hrs)	Signature:

If parent/legal representative is Illiterate:

Put fingerprint

Here

WITNESS		
I have witnessed the accurate reading of the consent form to the parent/legal representative of the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely		
First Name	Middle Name	Surname
Date: |__|__|/|__|__|/|__|__|__|__|(dd/mm/yyyy)	Time: |__|__|:|__|__| (hrs)	Signature:
PERSON GIVING CONSENT EXPLANATION i) I confirm that the parent/legal representative has given consent freely ii) I confirm that the child has provided verbal assent to participate in this study.		
First Name	Middle Name	Surname
Date: |__|__|/|__|__|/|__|__|__|__|(dd/mm/yyyy)	Time: |__|__|:|__|__| (hrs)	Signature:

Appendix 3: Child assent form

PART III: Certificate of Consent

CHILDS ASSENT

I_____do hereby consent to participate in the research study titled **"Impact of artemether-lumefantrine treatment, circadian rhythm and serum replacement on the infectiousness of wild *P. falciparum* gametocytes to *An. gambiae* sensu stricto mosquitoes".**

I attest to having received the information that describes the study procedures, including blood sampling, and have received an additional extensive explanation about the content of this information sheet from a study person.

I attest to having understood the aim, procedures, benefits, such as giving malaria treatment for free and risks, such as pain during blood sampling and that I was given sufficient time to ask all my questions related to the procedures, benefits and risks of participation and have received satisfactory answers. If I have any more questions, I understand that I may contact the study team at the telephone numbers given on the information sheet. I attest to understanding that I have the right to withdraw my consent and choose to stop the participation of my child at any time.

If I have any more questions, I understand that I may contact the principal investigator, Dorin Mmasi +255 620222761. I understand I will be given a copy of the information sheet and consent form to keep for my records. I VOLUNTARILY consent for my child to participate.

CHILD'S PARTICIPANT PARTICULARS		
First Name	Middle Name	Surname
Date: |__|__|/|__|__|/|__|__|__|__|(dd/mm/yyyy)	Time: |__|__|:|__|__| (hrs)	Signature:
PARENT/GUARDIAN		
First Name	Middle Name	Surname
Date: |__|__|/|__|__|/|__|__|__|__|(dd/mm/yyyy)	Time: |__|__|:|__|__| (hrs)	Signature:

If parent/legal representative is Illiterate:

Put fingerprint

Here

WITNESS I have witnessed the accurate reading of the consent form to the parent/legal representative of the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely		
First Name	Middle Name	Surname
Date: |__|__|/|__|__|/|__|__|__|__|(dd/mm/yyyy)	Time: |__|__|:|__|__| (hrs)	Signature:
PERSON GIVING CONSENT EXPLANATION iii) I confirm that the parent/legal representative has given consent freely iv) I confirm that the child has provided verbal assent to participate in this study.		
First Name	Middle Name	Surname
Date: |__|__|/|__|__|/|__|__|__|__|(dd/mm/yyyy)	Time: |__|__|:|__|__| (hrs)	Signature:

Appendix 4: Adult consent form

PART II: Certificate of Consent

ADULT PARTICIPANT'S CONSENT FORM

I, _____do hereby consent for my child to participate in the research study entitled **"Impact of artemether-lumefantrine treatment, circadian rhythm and serum replacement on the infectiousness of wild *P. falciparum* gametocytes to *An. gambiae* sensu stricto mosquitoes".**

I attest to have received the information that describes the study procedures, including blood sampling, and have received additional extensive explanation about the content of this information sheet from a study person.

I attest to have understood the aim, procedures, benefits such as giving malaria treatment for free and risks such as pain during blood sampling and that I was given sufficient time to ask all my questions related to the procedures, benefits and risk of participation and have received satisfactory answers. If I have any more questions, I understand that I may contact the study team on the telephone numbers given on the information sheet.

I attest to understanding that I have the right to withdraw my consent and choose to stop the participation of my child at any time.

If I have any more questions, I understand that I may contact the Principal Investigator, Dorin Mmasi, +255 620222761.

I understand I will be given a copy of the information sheet and consent form to keep for my records. I VOLUNTARILY consent for my child to participate.

CHILD'S PARTICIPANT PARTICULARS		
First Name	Middle Name	Surname
Date: |__|__|/|__|__|/|__|__|__|__|(dd/mm/yyyy)	Time: |__|__|:|__|__| (hrs)	Signature:

If parent/legal representative is Illiterate:

Put fingerprint

Here

WITNESS I have witnessed the accurate reading of the consent form to the parent/legal representative of the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely		
First Name	Middle Name	Surname
Date: |__|__|/|__|__|/|__|__|__|__|(dd/mm/yyyy)	Time: |__|__|:|__|__| (hrs)	Signature:
PERSON GIVING CONSENT EXPLANATION i) I confirm that the parent/legal representative has given consent freely ii) I confirm that the child has provided verbal assent to participate in this study.		
First Name	Middle Name	Surname
Date: |__|__|/|__|__|/|__|__|__|__|(dd/mm/yyyy)	Time: |__|__|:|__|__| (hrs)	Signature:

Appendix 5: Case Report Form

Case Report Form – Diagnostic Data sheet

Subject ID ☐

Informed consent obtained: written ☐ Oral ☐ Date……...

Subject Information:

Age in years ——————

Gender. ☐ Male ☐
Female

Village……

Taken anti malaria for the past one month: Yes ☐ No ☐

*1.*mRDT Positive: Plasmodium falciparum ☐ Plasmodium Pan ☐ Negative ☐

Microscopy Asexuals:

Read1: WBCs 200/Nr.Parasites: WBCs500/Nr.Gametocytes:

Read2: WBCs 200/Nr.Parasites: WBCs500/Nr.Gametocytes:

Read3: WBCs 200/Nr.Parasites: WBCs500/Nr.Gametocytes:

Asexual by microscopy: Total parasites dived by 3(reads~~) ×40=~~ /µl blood Asexual by microscopy: Total parasites dived by 3(reads) ×~~16= /µ~~l blood

Appendix 6: Direct membrane feeding assay form

DIRECT FEEDING ASSAY FORM	
Protocol Code: AL study	**Study Title Abbreviated**: Impact of AL, circadian rhythm and serum replacement on the infectiousness of wild *P. falciparum* gametocytes to *An. gambiae* sensu stricto mosquitoes
Subject's ID number: |___|___|___|___|___|-|___|___|	
Subject's Initials: |___|___|___|	
Instructions: *Date Format*=(**dd-mm-yy**) *Time Format*=(**hh:mm**)Hours	□=*Check Appropriate*

DIRECT MEMBRANE FEEDING ASSAY PROCEDURE RECORD	
Date of performing direct feeding procedure	|___|___|-|___|___|-|___|___|

Is patient an adult and gave separate consent for Direct membrane feeding procedure?	☐ 1=YES	☐ 2=NO
If Yes, date of signing direct feeding ICF	|___|___|-|___|___|-|___|___|	
Is patient still willing to participate in the direct feeding procedure?	☐ 1=YES	☐ 2=NO

Paper cup's identification number	|___|___|
Number of mosquitoes in a paper cup	|___|___|

DIRECT FEEDING ASSAY PROCEDURE		
Mosquitoes should feed for the duration of 15 to 20 minutes		
Starting time (T_{start})	End time (T_{end})	Total duration (T_{end} - T_{start})
|___|___|:|___|___|	|___|___|:|___|___|	|___|___| minutes
Number of fully fed mosquitoes after direct feeding assay		|___|___|
Optional Comments:		

Supervised by initial |___|___|___|	Time |___|___|:|___|___|

DIRECT FEEDING ASSAY FORM	
Protocol Code: AL study	**Study Title Abbreviated**: Impact of AL, circadian rhythm and serum replacement on the infectiousness of wild *P. falciparum* gametocytes to *An. gambiae* sensu stricto mosquitoes
Subject's Initials: |___|___|___|	Subject's ID number: |___|___|___|___|___|-|___|___|

Instructions: *Date Format*=(**dd-mm-yy**) *Time Format*=(**hh:mm**)Hours □=*Check Appropriate*

DIRECT FEEDING ASSAY MOSQUITOES DISSECTION RECORD	
Date of performing mosquitoes dissection (7 days after direct feeding assay)	|___|___|-|___|___|-|___|___|

Number of mosquitoes alive 7 days after direct feeding assay	|___|___|

NUMBER OF OOCYSTS PER MOSQUITO

Mosquito	**Number of oocysts**	**Mosquito**	**Number of oocysts**
1st	|___|___|	**19th**	|___|___|
2nd	|___|___|	**20th**	|___|___|
3rd	|___|___|	**21st**	|___|___|
4th	|___|___|	**22nd**	|___|___|
5th	|___|___|	**23rd**	|___|___|
6th	|___|___|	**24th**	|___|___|
7th	|___|___|	**25th**	|___|___|

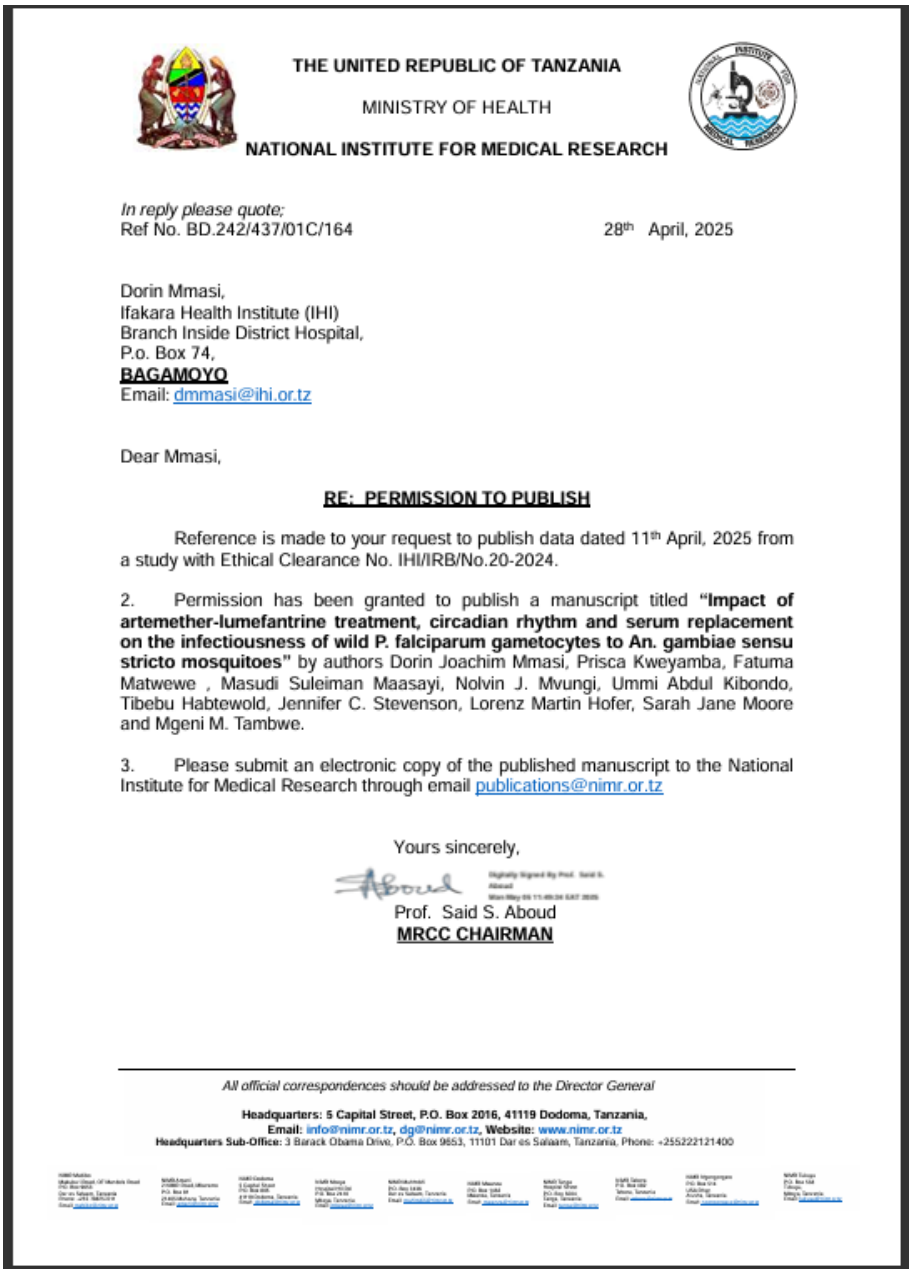
8th	|___|___|	**26th**	|___|___|
9th	|___|___|	**27th**	|___|___|
10th	|___|___|	**28th**	|___|___|
11th	|___|___|	**29th**	|___|___|
12th	|___|___|	**30th**	|___|___|
13th	|___|___|	**31st**	|___|___|
14th	|___|___|	**32nd**	|___|___|
15th	|___|___|	**33rd**	|___|___|
16th	|___|___|	**34th**	|___|___|
17th	|___|___|	**35th**	|___|___|
18th	|___|___|	**36th**	|___|___|

Optional Comments:

Supervised by initial |___|___|___|

Time |___|___|:|___|___|

Appendix 7: Certificate of ethical approval



THE UNITED REPUBLIC OF TANZANIA

MINISTRY OF HEALTH

NATIONAL INSTITUTE FOR MEDICAL RESEARCH

In reply please quote;
Ref No. BD.242/437/01C/164

28th April, 2025

Dorin Mmasi,
Ifakara Health Institute (IHI)
Branch Inside District Hospital,
P.o. Box 74,
BAGAMOYO
Email: dmmasi@ihi.or.tz

Dear Mmasi,

RE: PERMISSION TO PUBLISH

Reference is made to your request to publish data dated 11th April, 2025 from a study with Ethical Clearance No. IHI/IRB/No.20-2024.

2. Permission has been granted to publish a manuscript titled **"Impact of artemether-lumefantrine treatment, circadian rhythm and serum replacement on the infectiousness of wild P. falciparum gametocytes to An. gambiae sensu stricto mosquitoes"** by authors Dorin Joachim Mmasi, Prisca Kweyamba, Fatuma Matwewe , Masudi Suleiman Maasayi, Nolvin J. Mvungi, Ummi Abdul Kibondo, Tibebu Habtewold, Jennifer C. Stevenson, Lorenz Martin Hofer, Sarah Jane Moore and Mgeni M. Tambwe.

3. Please submit an electronic copy of the published manuscript to the National Institute for Medical Research through email publications@nimr.or.tz

Yours sincerely,

Prof. Said S. Aboud
MRCC CHAIRMAN

All official correspondences should be addressed to the Director General

Headquarters: 5 Capital Street, P.O. Box 2016, 41119 Dodoma, Tanzania,
Email: info@nimr.or.tz, dg@nimr.or.tz, **Website:** www.nimr.or.tz
Headquarters Sub-Office: 3 Barack Obama Drive, P.O. Box 9653, 11101 Dar es Salaam, Tanzania, Phone: +255222121400

Plot 463, Kiko Avenue, Mikocheni | P.o. Box 78,373 Dar es Salaam, Tanzania | Phone: +255222774756 Email: irb@ihi.or.tz

ISO 9001:2015 certified

IFAKARA HEALTH INSTITUTE
research | training | services

INSTITUTIONAL REVIEW BOARD

April 19, 2024

National Institute for Medical Research
P O Box 9653
Dar Es Salaam
Email; headquarters@nimr.or.tz

Dorin Joachim Mmasi
Ifakara Health Institute
P.O.Box 74
Bagamoyo

IHI/IRB/No: 20-2024

INSTITUTIONAL CLEARANCE CERTIFICATE FOR CONDUCTING HEALTH RESEARCH

On 27th March 2024, the Ifakara Health Institute Review Board (IHI-IRB) reviewed from study titled: ***"Exploring the effect of Antimalarial treatment, serum replacement, circadian rhythm on mosquito infectiousness using gametocytes from naturally infected individuals"***, submitted by the Principal Investigator, **Dorin Mmasi.** The study has been approved for implementation after IRB consensus. This certificate thus indicates that; the above- mentioned study has been granted an Institutional Ethics Clearance to conduct this study in **Bagamoyo District Council**.

The Principal Investigator of the study must ensure that, the following conditions are fulfilled during or after the implementation of the study:

1. PI should submit a six-month progress report and the final report at the end of the project
2. Any amendment, which will be done after the approval of the protocol, must be communicated as soon as possible to the IRB for another approval
3. All research must stop after the project expiration date, unless there is prior information and justification to the IRB.
4. There should be plans to give feedback to the community on the findings
5. The PI should seek permission to publish findings from NIMR
6. The approval is valid until **27th March 2025**

The following documents were reviewed and approved:

1. IHI-IRB Application Form 2. Responses to the reviewer's comments3. Signed cover letter from Investigator 4. Protocol 5. Instruments for data collection 6. Up to date Curriculum Vitae of PI and Co-Investigator.

The IRB reserves the right to undertake field inspections to check on the protocol compliance

Chairperson

IRB Secretary

RESEARCH OUTPUTS

(i) Publication

Mmasi, D. J., Kweyamba, P. A., Matwewe, F., Maasayi, M. S., Mvungi, N. J., Kibondo, U. A., Habtewold, T., Stevenson, J. C., Hofer, L. M., Moore, S. J., & Tambwe, M. M. (2025). Impact of artemether-lumefantrine treatment, circadian rhythm and serum replacement on the infectiousness of wild *P. falciparum* gametocytes to *An. gambiae sensu stricto* mosquitoes. *Frontiers in Malaria*, *3*, 1609614. https://doi.org/10.3389/fmala.2025.1609614

(ii) Poster Presentation