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# Preliminary investigation of the impact of mass drug administration on malaria transmission in Zanzibar.

Degree Project in Medicine

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## Abbreviations

95% CI – 95% Confidence Interval

ACD – Active Case Detection

ACT – Artemisinin Combination Therapy

CRTC – Cluster Randomized Controlled Trial

DHAp – Dihydroartemisinin-Piperaquine

DMSO – District Malaria Surveillance Officer

DOT – Directly Observed Treatment

G6PD – Glucose-6-Phosphate Dehydrogenase

HTS – High Transmission Season

IRS – Indoor Residual Spraying

LLIN – Long Lasting Insecticidal Nets

MCN – Malaria Case Notification

MDA – Mass Drug Administration

MEEDS – Malaria Early Epidemic Detection System

MSAT – Mass Screening and Treatment

PCD – Passive Case Detection

PCR – Polymerase Chain Reaction

RACD – Reactive Case Detection

RDT – Rapid Diagnostic Test

RR – Risk Ratio

SLD – Single Low Dose

ZAMEP – Zanzibar Malaria Elimination Programme

ZAMRUKI – Zanzibar Malaria Research Unit Karolinska Institutet

WHO – World Health Organization

## Abstract

**Background.** Mass drug administration (MDA) is the simultaneous treatment of a defined population without diagnostic testing, irrespective of the presence of symptoms. MDA has been suggested as a malaria elimination strategy since today's diagnostic tools are insufficiently sensitive to detect the low-density, asymptomatic infections thought to fuel remaining malaria transmission in low-endemic settings. A cluster-randomized controlled trial (CRCT) assessing MDA on Zanzibar was initiated in April 2016. This report investigates the impact MDA had on malaria transmission during 16 months follow-up.

**Methods.** Eight areas received two rounds of MDA including dihydroartemisinin-piperaquine + single low dose primaquine (0.25 mg/kg) and were compared to eight similar control areas. Data on recorded clinical malaria cases detected at health facilities during the follow-up period was collected through Zanzibar's malaria surveillance-system. Cumulative incidence and crude risk ratios were used to describe observed trends in malaria transmission.

**Results.** During 16 months follow-up MDA fails to achieve a statistically significant lower risk of malaria in the intervention arm compared to the control arm; RR=0.85 (95% CI 0.69-1.03; P=0.10). No risk reduction was observed during the high transmission season immediately after MDA; RR=1.16 (95% CI 0.76-1.75; P=0.49). During the following year's (2017) high transmission season the RR was 0.68 (95% CI 0.52-0.88; P=0.004).

**Discussion.** Previous studies have concluded that MDA has a rapid effect on malaria transmission and that the difficulty is to maintain this effect. No such direct effect could be observed in this report. The reason for the observed reduced malaria risk during the high transmission season of 2017, one year after MDA was conducted, is unclear. Further analyses are needed to confirm the effectiveness MDA had on transmission.



**Keywords.** Malaria, mass drug administration, MDA, elimination, Primaquine

# 1 Introduction

## 1.1 Malaria in a global perspective

Malaria, a parasitic disease transmitted through the bites of female Anopheles mosquitoes, is a curable and preventable disease. Despite that 212 million people worldwide where infected in 2015 according to WHO and 429 000 died due to malaria-related complications the same year (1). Children under the age of 5 account for 70 % of these deaths. The parasite *Plasmodium falciparum* cause 99% of global malaria deaths and is the dominant species on the African continent. Claiming 76% of the world's cases and 75% of all malaria deaths, sub-Saharan Africa is heavily stricken by its malaria burden. However, four other large WHO-regions – the Americas, the Eastern Mediterranean, South-East Asia and the Western Pacific – also pay a heavy toll. Simply put, half of the world's population is at risk and young children and pregnant women are most vulnerable (1). In 2015 the WHO, together with over 400 experts, put together the *Global Technical Strategy for Malaria 2016-2030* (2). It is a strategical plan with guidelines to reduce and eliminate malaria. The goals for 2030 include reducing malaria case incidence by at least 90%, reducing malaria mortality rates by at least 90%, eliminating malaria in at least 35 countries and preventing re-establishment of malaria in all countries that are malaria free. These goals are being made possible through studies of prevention and treatment of Malaria in the endemic regions and one of these outposts, right in the heart of the global malaria fight, is Zanzibar where this study took place.

## 1.2 Clinical features of the disease

The disease is divided into uncomplicated and severe malaria with symptoms spanning from none to organ failure (3). Fever is the most common symptom of malaria and malaria is also the most common cause of fever in high endemic regions (4). Non-specific symptoms like fatigue, headache, myalgia, abdominal discomfort, nausea and vomiting is frequent with paroxysms of fever (can also be continuous). A phase of chills and shaking followed by a warm phase and sweats make up the febrile episodes. Patients with uncomplicated malaria

usually do not develop any severe symptoms other than fever and light anemia. Splenomegaly can be seen after a couple of days of infection and hepatomegaly and jaundice is not uncommon. If treatment with an effective ACT (artemisinin combination therapy) is initiated soon after onset of symptoms the mortality rate of uncomplicated falciparum malaria is low (less 0.1%) but if left untreated severe illness can emerge (5). *P.falciparum* is the most lethal of the malaria parasites and causes the majority of severe malaria cases. Traits of severe malaria are signs of vital organ dysfunction/failure and acidotic breathing due to metabolic acidosis and hyper-parasitaemia (5). Cerebral malaria is a form of severe malaria in which cerebral microcirculation is disrupted due to blood-clotting, causing convulsions, lowered consciousness and coma (4). Severe anemia, as a result of continuous hemolysis and destruction of red blood cells by the parasites and the spleen, is also seen in severe malaria patients. Other feared traits are renal failure, metabolic acidosis, pulmonary edema, acute respiratory distress syndrome (ARDS) and distributive chock. Due to the severity of these symptoms, severe malaria is linked to high mortality (3-5).

## 1.3 Biology of the malaria parasite

Unicellular parasites of the *Plasmodium* genus are the causative agents of malaria. Five *Plasmodium* species are known to cause illness and infection in humans: *P. falciparum, P.vivax, P. ovale, P. malariae* and *P. knowlesi* (6). Adult female *Anopheles* mosquitos carries the parasite in its salivary glands. *Anopheles gambiae,* the most abundant *Anopheles* species in Africa, is antropophilic i.e., prefers human blood (6). During feeding the infectious form of the parasite, the *sporozoites,* are released into the human's circulation and travel to and invades the hepatocytes of the liver. Each sporozoite invades one hepatocyte where they replicate to form a hepatic schizont containing many merozoites. When the hepatic schizont burst it releases up to 40 000 merozoites. *P. vivax* and *P. ovale* can lie dormant as hypnozoites in hepatocytes for two weeks up to more than a year (4). The merozoites from the ruptured schizont enter the circulation and begin infecting red blood cells. Here they feed of

hemoglobin and hide from the immune system. In the red blood cells another asexual replication takes place, beginning with the merozoite entering a ring-stage and developing into a trophozoite. The erythrocyte matures into a schizont containing 6-30 merozoites. Eventually the schizont ruptures and the released merozoites can now attack new erythrocytes, creating a chain reaction. These blood stages of the malaria parasite, and the toxins and waste products leaked from the ruptured erythrocytes, are what causes illness and symptoms. The erythrocytic cycle takes 48 h in *P. falciparum*, *P. vivax* and *P.ovale*, 72 h in *P. malariae* and rapid 24 h in *P. knowlesi* (4). These cycle-times cause the periodicity of the paroxysms of chills and fever observed in malaria patients. The infection is asymptomatic until blood parasite concentrations of around 50 parasites/microliter, or 100 million parasites in an adult's circulation, is reached. This results in an incubation period of generally 12-14 days from the infectious mosquito bite (4).

The intrahepatic and the intraerythrocytic replication cycle are both asexual. Instead of becoming a merozoite, some trophozoites differentiate into sexual male and female gametocytes that can be transmitted back to another feeding *Anopheles* mosquito. The male and female gametocytes fuse into a motile ookinete that invade the mosquito's midgut wall. The ookinete grows, forming an oocyst that eventually ruptures releasing many sporozoites that makes their way to the mosquito's salivary glands; thus, the cycle is complete(4, 7).

### 1.4 Diagnosis and treatment of malaria

WHO accentuates that suspected malaria cases should have a parasitologically verified diagnosis, either by rapid diagnosis test (RDT) or microscopy, before being provided antimalarial treatment. This to prevent overuse and development of drug resistance. Gold standard for parasitologically verified diagnosis is light microscopy of a thick blood smear by an experienced microscopist. This is not always an available method why RDTs have an important place in malaria diagnostics. From a finger prick of blood, the RDT detects

parasite-specific antigens or enzymes and can, to some degree, differentiate between species. RDT is easy to use, cheap and gives the result in 15-20 min and have been found to be a suitable tool at primary health care levels (8). RDTs are an essential tool to diagnose symptomatic cases in both low and high endemic areas alike. However, in low transmission settings, where asymptomatic, low-density infections are relatively more abundant, the limitations in sensitivity reduce the usefulness of RDTs to achieve malaria elimination (9). PCR is the optimal tool in terms of sensitivity to detect malaria infection, but the method has no clinical applicability in today's endemic regions. PCR is expensive and demands advanced laboratory equipment and highly trained operators. PCR might play a more important role in the future when more, previously high endemic areas reach a sustainable level of low transmission and ultimate elimination is the goal (9, 10).

Artemisinin combination therapies (ACTs) are the foundation of modern, highly effective antimalarial treatment. According to the WHO's treatment guidelines, a combination of at least two different antimalarials, with different pharmacodynamics and half-lives, should be used to rapidly and effectively remove parasites and prevent resistance development. ACT consist of a fast acting artemisinin derivate, meant to rapidly decrease blood parasite count, in combination with a slowly eliminated partner drug that eliminates remaining parasites and provide post-treatment prophylaxis (8). Treatment is given for three days and recommended ACTs for uncomplicated *P. falciparum* malaria are: artemether + lumefantrine, artesunate + amodiaquine, artesunate + mefloquine, artesunate + sulfadoxine-pyrimethamine and dihydroartemisinin + piperaquine.

ACTs are highly effective and one of the potential greatest threats to the vision of a malaria free world is the development and spread of Artemisinin resistance (11, 12). Resistance development to Artemisinin has already emerged in the Greater Mekong subregion of Southeast Asia, making this threat a reality.

ACTs are not fully effective on malaria gametocytes, the parasite-stage accountable for the transmissibility back to mosquitos. The WHO guidelines therefore states:

"*In low-transmission areas, give a single dose of 0.25 mg/kg bw primaquine with ACT to patients with P. falciparum malaria (except pregnant women, infants aged < 6 months and women breastfeeding infants aged < 6 months) to reduce transmission. Testing for glucose-6-phosphate dehydrogenase (G6PD) deficiency is not required. Strong recommendation, low-quality evidence.*" (8)

## 1.5 Malaria control, elimination and eradication

Each malaria endemic country must eliminate malaria to reach global malaria eradication. The path to malaria elimination starts with the incorporation of a national malaria control programme. The definition of malaria control is to decrease the burden of malaria (incidence, prevalence, morbidity or mortality) to locally acceptable levels. When the slide positivity rate (SPR) or RDT positivity rate is <5% among febrile patients or the incidence is <5/1000 persons at risk the country has reached a pre-elimination phase. When even further progress in malaria control is achieved and the incidence of local malaria is as low as 1/1000 persons at risk, the country can enter the elimination phase (13). Malaria control, elimination and eradication depend on vector control, rapid diagnosis and treatment and malaria surveillance and case management.

Malaria control programs focus on achieving high population coverage of vector control interventions and ensuring easy access to diagnosis and effective treatment to reduce malaria incidence and mortality. Vector control aims to limit the mosquitos contact with humans and therefore prevent malaria infection and transmission. There are two primary vector control tools: insecticide-treated mosquito nets (ITNs) and indoor residual spraying (IRS) with insecticides. IRS was the core of the Global Malaria Elimination Programme's success in the 1950s and 60s but today's achievements in malaria control are mostly due to the world-wide

deployment of ITN and the long-lasting insecticidal nets (LLINs). WHO recommends that 100% of the population at risk of malaria sleeps under a LLIN but in 2015, 47% did not (1). IRS are effective due to the behavior of the vectors. Most biting occurs indoor during the evening and night hours (hence why LLIN are so important) and after the feeding of blood, the mosquito resides on the walls and ceilings of the house, digesting their meal (6). IRS therefore consists of spraying walls and ceilings with insecticides, killing the vector.

Rapid diagnosis and treatment is important since uncomplicated infection in a fast pace can evolve into severe illness. Sever falciparum malaria is deadly in almost all cases if treatment is not provided. The aim is to initiate ACT treatment within 24-48 hours after onset of symptoms (8). Fast identification and treatment of cases also limit the onward transmission form an individual case.

Malaria surveillance is essential in a country moving towards elimination. The ability to detect each new case and document, track and gather additional information about every new case becomes increasingly important as transmission declines (14). Transmission is highly heterogeneous in malaria endemic regions, especially in low-transmission settings, and cases often stem from small foci of higher transmission. Transmission can also be heterogeneous within these foci, and areas with transmission intensity above average are called hotspots. Hotspots contain the epidemiological and geographical features required for nourishing transmission (15). The ability to detect and map these foci or hotspots, which may sustain transmission, is an important task of malaria surveillance systems, making it possible to mobilize targeted efforts and interventions. A scale-up of the surveillance systems into a core intervention is one of the "three pillars" WHO's *Global Technical Strategy for Malaria 2016-2030* relies on to achieve its goals of 90% reduction in global malaria incidence and mortality (2).

Passive case detection (PCD) is the cornerstone of malaria surveillance. This is the detection of a new cases when they seek medical assistance at health facilities. Active case detection (ACD) is the active search and screening for malaria infection in populations and is important since not all malaria cases, for example asymptomatic carriers, are detected through PCD. As a response to a detected case (either by PCD or ACD) a reactive case detection (RACD) strategy can be carried out. RACD means that individuals, surrounding a diagnosed case, are screened and treated if found positive (14).

As transmission declines, and countries move towards elimination, the relative proportion of low-density/sub-patient infections increases (16). Subpatient malaria infections are defined as parasitaemias detectable by PCR but not by RDT or microscopy. Low-density/sub-patient infections (<100 parasites/microliter of blood) are to a wide extent asymptomatic and are therefore missed by PCD. They also avoid detection by ACD since ACD depends on RDTs for finding infections. With today's tools, asymptomatic and subpatient infections can accumulate into a human parasite reservoir that may maintain malaria transmission through gametocyte development. When the aim is elimination, finding these sub-patient parasite carriers becomes increasingly important since they have been estimated to fuel 20-50% of human-to mosquito transmission in pre-elimination areas (17). To effectively target the sub-patient parasite reservoir is one of the greatest challenges to achieve malaria elimination. The symptomatic and asymptomatic biomass of malaria parasites are higher in hotspots than other areas which allows for targeting of the human parasite reservoir. Focusing efforts and interventions to hotspots may therefore be an effective way to reduce malaria transmission (15). WHO's Global Technical Strategy also points out that targeting the reservoir is an important step in accelerating the pace towards elimination (2).

The ultimate goal of malaria eradication calls for universal access to these core control and elimination tools/strategies in all endemic regions. The endemic regions consist of some of

the poorest and most remote corners of the world making this prospect highly dependent on substantial investments and global commitment. Due to the Global Fund to Fight AIDS, Tuberculosis and Malaria, the President's Malaria Initiative and many others the global funding has increased significantly from US$ 960 million in 2005 to US$ 2.9 billion in 2015 (18, 19).

## 1.6 Targeting the parasite reservoir

Malaria elimination from previously high endemic areas, containing all the right geographical and epidemiological factors to uphold transmission, is no easy task. Despite universal coverage of core interventions and a strong health and surveillance systems, low levels of transmission linger. PCR has pointed out the large pool of asymptomatic/sub-patient parasite carriage that contribute to the remaining transmission in low-transmission settings (20). Finding and treating these carriers should be a priority of any elimination strategy.

There are two major tactics to reach the asymptomatic pool of parasite carriers. Mass screening and treatment (MSAT), where you screen a population and treat those found positive, is one of them. MSAT's weakness in malaria elimination is that it depends on highly sensitive, in-field available, diagnostic tools. RDT's sensitivity (approximately 100 parasites/microliter of blood) has shown to be insufficient to find the asymptomatic/sub-patent parasite carriers, rendering MSAT not a viable option to achieve malaria elimination. That is until in-field suitable diagnostic tools with high enough sensitivity, comparable to PCR, become available (21, 22).

Mass drug administration (MDA) is one way around the issue of finding the sub-patient infections. MDA is mass treatment of all individuals of a population at approximately the same time, regardless of the presence of symptoms and without prior diagnostic testing (23). MDA with antimalarials enables the targeting of the sub-patent human parasite reservoir and

may interrupt transmission in low transmission settings and play an important role in elimination.

## 1.7 Mass drug administration (MDA) for malaria

The high efficacy of ACTs, the transmission blocking properties of primaquine and the presence of sub-patent infections potentially fueling transmission has renewed the interest in MDA for malaria elimination. MDA was used as early as 1910 against Malaria (24). It was used more extensively during the 1950s during the eradication programme, to wide extents in China and Russia and in research across several different endemic settings with mixed success (25). Because of the generally low quality of available data, a lot is still unknown about the specifics and actual effectiveness of MDA. The optimal drug combination, timing (when MDA is administered in relation to the malaria season), number of rounds and for how long MDA should be repeated are key features that need answers (26). Reviews on the subject (27, 28) has concluded that MDA has to be further examined and more high-quality cluster randomized controlled trials are needed. Available data show that MDA has been most successful in low transmission settings aiming for elimination but most studies fail to display a persistent effect lasting longer than six months. Isolated small islands and remote highlands have been most successful. Important aspects of successful MDA are: Community participation and coverage >80%, making the delivery system of MDA a very important factor. Furthermore, high coverage of vector control interventions has been part of most successful MDA regimens. The use of Primaquine (transmission blocking properties) is crucial to effectively target all stages of the malaria parasite's life cycle. Timing of MDA is also of great significance and varies between settings; in areas with seasonal transmission MDA has the greatest chance to interrupt transmission at the lowest point of transmission (25). Since malaria incidence correlate with rainfall, this is usually during the dry season just before the onset of the wet season. Since high coverage is key, timing of MDA also has to be

planned around patterns of population movement (for example to earn alternative income during parts of the year) (25). The time of the year when the population is most stationary gives the best opportunity to achieve high coverage.

Brady *et al*. (2017) (29) address some of the key features of successful MDA in low-transmission settings using mathematical consensus modelling. They conclude that MDA has a suppressing effect on transmission more long-lasting than the prophylactic effect of the given treatment. The relative effect on transmission is more pronounced and long-lasting the lower the starting level of transmission is; MDA should be done at the lowest point of transmission during the year. The effect of MDA is only short-termed and sustained long-term effect depends on strengthened vector control and/or other interventions; otherwise MDA has to be repeated frequently. Simulated MDA effectiveness greatly depends on total yearly population coverage and if that is achieved by one or multiple rounds of MDA is less important. MDA conducted during 2 years was more effective than during 1 year.

In an era of emerging artemisinin resistance, MDA is a controversial method. A restrictive view on the use of ACTs, to only parasitically verified malaria cases, is adopted in WHO's treatment guidelines to prevent artemisinin resistance. Despite that, part of the reborn interest in MDA arise due to the emergence of artemisinin resistance in the in the Greater Mekong subregion of south east Asia. The fear of artemisinin losing its efficacy is wide-spread among the malaria fighting communities and the spread of resistant strains to Africa, and the rest of the endemic regions, would be catastrophic. Therefore, the gathering of WHO's Evidence Review Group in 2015 (23) resulted in MDA being recommended as a "fighting fire with fire" tactic to contain/eradicate resistant strains in the Greater Mekong subregion. They also recommend MDA in low-transmission settings aiming for elimination, since these conditions allow MDA to interrupt remaining low-level transmission to the point where it can't recover.

MDA is also recommended to radically lower malaria mortality and morbidity in epidemic scenarios and other emergency situations, when the health system is overpowered (23).

### 1.7.1 Choice of drugs to be used in MDA

Each round of MDA consists of a full treatment and therefore involves multiple doses over a number of consecutive days. Completion of all doses is essential for MDA (23) and in a MDA study from Gambia (30) the most common reason for not completing the cure was fear of side-effects. The drug combination must therefore be safe and accepted by the receiving communities to achieve high enough coverage and compliance. An easy drug regimen is also preferred to achieve high compliance of all doses.

The optimal drug combination of MDA has yet to be defined but should comprise of an ACT in combination with a potent gametocytocidal drug to effectively remove gametocytes and therefore suppress transmission (23). Primaquine is the only gametocytocidal drug commercially available. The downside of using Primaquine is the risk of adverse side effects, including toxic hemolysis in glucose-6-phosphatese (G6PD) deficient patient. G6PD deficiency is the result of a genetic mutation that, due to natural selection, is more abundant in malaria stricken parts of the world as it gives about 50% protection against severe malaria (31). As stated before, the WHO recommends the addition of SLD Primaquine (0.25 mg/kg) for the treatment of uncomplicated P.falciparum malaria in low-transmission settings. The WHO Evidence Review Group also conclude that SLD primaquine (0.25 mg/kg) is recommended for MDA regimens (23). The risk and degree of hemolysis in G6PD deficient patient are dose and exposure duration dependent and the use of SLD primaquine is therefor, without prior testing, considered safe in G6PD abundant populations (8, 32).

Proposed regimens for MDA is dihydroartemisinin-piperaquine + SLD Primaquine (0.25 mg/kg). The ACT, Dihydroartemisinin-piperaquine (DHAp), is taken once a day for 3 days.

MDA at large scales drastically increase the drug pressure (the circulating amount of the drug) which could be argued gives a natural selection of resistant strains and predispose spread since it wipes out rivaling, sensitive strains (24). To minimize this risk, the drug combination of MDA should not be the same as the first line treatment for individual cases and the addition of a transmission blocking drug, like primaquine, is key to limit the spread of resistant parasites (23). However, if MDA fails to eliminate malaria from the targeted areas, it may drive resistance development (8).

### 1.7.2 Delivery strategy of MDA

Aside from the safety profile of the drug, the delivery system of the drug is equally important to achieve >80% coverage and compliance of MDA. Before MDA is engaged, the communities have to be involved and educated about why and how MDA will be conducted. Information about the drugs and risk of side-effects must be included. This to build a general foundation of trust and understanding. Involvement of elders and local leaders (social, political, religious etc.), who can motivate their community, can further enhance acceptance and participation (23, 25).

The area targeted for MDA should be divided into smaller areas, each for which a team of local health workers/volunteers are responsible. The use of local volunteers will increase community involvement and the delivery teams should administer the drugs using a house-to-house approach. Directly observed therapy (DOT) of all doses is central to achieve compliance goals and the small but many delivery teams will make this possible on large scales. Staff administering the drugs should be educated to recognize adverse events of the drugs and health care workers at health facilities should also be trained to handle these events (like hemolysis) (23, 25). The population must know that they should seek medical attention and can get help at health facilities if adverse events occur.

## 1.8 MDA on Zanzibar

Zanzibar (population 1.3 million), a semi-autonomous archipelago outside the coast of Tanzania, a previously high endemic setting of malaria in sub-Saharan Africa, has now reached pre-elimination status. Between 2003 and 2015 they achieved a 96% reduction in in RDT/microscopy verified P. falciparum prevalence from 10.3% (95% CI 9.3 – 11.4) to 0.43% (95% CI 0.23-0.73). The all-cause mortality in young children (<5 years) declined by 64% (Bjorkman et al, unpublished). This transformation was made possible in only 15 years due to the nation-wide deployment of core malaria interventions; ACT in 2003, RDT in 2005 and LLIN and IRS in 2006. Malaria incidence dropped massively after the implementation of these interventions and hopes of elimination arose. However, in 2007 the decline in transmission leveled out and a remaining low-level transmission has been stable since. Transmission is now also more seasonal and heterogenous with remaining foci of transmission. PCR has revealed an almost 4 times higher parasite prevalence than RDT, indicating the presence of a large sub-patent/asymptomatic parasite reservoir.

In 2013, the Zanzibar Ministry of Health officially changed their target from malaria control to elimination. Zanzibar Malaria Elimination Programme (ZAMEP) has two malaria surveillance systems; Malaria Early Epidemic Detection System (MEEDS) and the Malaria Case Notification (MCN) system. MEEDS is a PCD system to which health facilities report malaria cases once weekly. MCN is both a PCD and ACD system where every new case detected is registered in real time and is later followed up by a District Malaria Surveillance Officer (DMSO) at the house-hold level. Individuals in the index case's household is tested by RDT and, if found positive, treated. The DMSO also collect additional information about those found positive and the information is documented in MCN; information such as recent travel and use of LLIN. If household members are tested positive by RDT, a wider screening of direct neighbors are conducted.

Zanzibar was among the first in sub-Saharan Africa to introduce ACT (artesunate and amodiaquine) as first-line treatment and other interventions on a wide scale (33) and the goal is to provide proof of the feasibility of malaria elimination from a previously high endemic area. A MSAT study in 2015 demonstrated that the sensitivity of RDT was too low to find the low density, asymptomatic infections thought to play a vital role in maintaining transmission (21). As a result, a study to evaluate MDA's potential in achieving elimination on Zanzibar was initiated in 2016. Zanzibar is a good location to assess MDA. It's an island and pre-elimination setting, the remaining transmission is highly seasonal and hotspots of transmission has been identified through the malaria surveillance systems already in place. MDA was used in 2013 as an outbreak response in four hotspot *Shehias* (smallest administrative unit) (34). The outbreak was noticed through MCN when all four Shehias reported over the alert threshold of 5 cases/week, only 2-6 weeks after they received MSAT. One round of MDA was distributed via house-to-house visits and was well accepted; 97% coverage and 90% compliance were reported. MDA's effect on malaria transmission was not assessed.

The malaria transmission of Zanzibar is low and highly seasonal and most cases are recorded during May-September. This period is referred to as the high transmission season occurs shortly after the start of the wet season, ranging from April to June. To assess the effectiveness of MDA in reducing the annual peak in transmission, a cluster-randomized controlled trial (CRCT) with one intervention arm (two rounds of MDA) and one control arm (no MDA) was initiated in April 2016. This report presents the trends in malaria transmission during the 16 months follow-up after the administration of MDA.

# 2 Study aim(s) and objective(s)

## 2.1 Aim

To investigate the impact of two rounds of dihydroartemisinin-piperaquine (DHAp) + single low dose (SLD) primaquine Mass Drug Administration (MDA) on seasonal malaria transmission in areas (Shehias) considered hotspots in Unguja Island, Zanzibar.

## 2.2 Objectives

### 2.2.1 Primary objective

To compare the cumulative malaria incidence in the control (no MDA) and intervention (MDA) arms during the study period May 2016-September 2017.

### 2.2.2 Secondary objective

To compare malaria cumulative incidence in the control and intervention arms during the high transmission seasons in May-September 2015, 2016, 2017, i.e. prior to and after the MDA.

# 3 Method

## 3.1 Study design

This is a follow-up on a CRCT with two arms: an intervention arm with two rounds of MDA, and a control arm with no MDA. Each arm contained eight clusters (Shehias). The study was initiated on 30$^{th}$ April 2016. Two rounds of MDA with DHAp (D-ARTEPP, Guilin Pharmaceutical (Shanghai) Co., Ltd., China) and SLD (0.25mg/kg) primaquine (Primaquine, Remedica Ltd.,Cyprus ) was administered approximately four weeks apart (day 0 and 28), at the anticipated lowest point of malaria transmission before the onset of the annual high transmission season occurring after the start of the rainy season (April-June). A total of 22 teams, consisting of two health care workers and one community guide, delivered MDA during house-to-house visits and the coverage (proportion of the population that received treatment) for each round was determined. SLD primaquine and the first dose of DHAp was taken under direct observation (DOT) in individuals that were present and agreed to treatment. The two remaining doses of DHAp was left with clear instructions. Post MDA surveys were conducted seven days after each round of MDA in a subset of the MDA

households in order to estimate the self-reported compliance to the three-day DHAp treatment regimen. Safety of this combination of MDA was also evaluated by the same post MDA surveys using a structured questionnaire covering adverse events. Passive monitoring of severe adverse events, such as haemolysis, took place at the health facilities connected to the study areas. Before MDA was implemented, the staff of the health facilities received training and education about recognizing and handling adverse events of the included drugs, risk of acute haemolysis being the most important. To boost understanding, acceptance and participation, the communities were informed about the purpose and the procedure of the study before the study was initiated and local village leaders were also involved.

### 3.1.1 Study sites

The majority of Zanzibar's symptomatic malaria cases are recorded on the island Unguja following the main rains which occur between April-June. A total of 16 clusters in Central, South, and West districts, Unguja Island, Zanzibar were randomly selected then randomized; 8 for MDA and 8 for control. Each cluster consisted of a hotspot Shehia, defined as a *Shehia* with an annual malaria incidence of >8/1000 (i.e. >0.8%) calculated as the number of confirmed malaria infections notified at health facilities and during active case detection during January-December 2015/projected *Shehia* population for 2015. The allocation of *Shehias* within the trial arms was conducted using computerized block randomization based on *Shehia* population size. The districts were originally chosen since they have the biggest concentration of hotspot Shehias/district.

### 3.1.2 Sample size of original study

To detect a 50% reduction in annual incidence (from baseline 12/1000 in included Shehias to 6/1000 in the intervention arm) with 80 % power and a mean Shehia population of 1450, eight clusters in each arm were required. This resulted in roughly 12,000 individuals in each arm giving a total sample size of around 24,000.

### 3.1.3 MDA implementation

According to the *MDA implementation report*, the coverage of the first round of MDA was: 91% received DHAp, 87% received SLD Primaquine and 67% took the first dose as DOT. The coverage of the second round of MDA was: 88% received DHAp, 81% received SLD Primaquine and 48% took the first dose as DOT. A post MDA survey in a subset of the households of the MDA Shehias assessed the compliance to the full treatment to 84% and 96% in the first and second round respectively.

## 3.2 Study procedure and data collection for this report (16 months follow-up)

In November 2016, six months after the second round of MDA, the short-term impact of MDA was evaluated by comparing crude cumulative incidence of malaria in the MDA and control Shehias during May-November (the CRCT's primary objective). The crude malaria cumulative incidence was calculated as: the number of verified malaria cases from the Shehias of the study arm divided by the estimated population size of the study arm. The malaria cases were detected by PCD at health facilities and monitored through MCN. MCN contains information about in which Shehia a malaria positive individual lives. MCN therefore allows tracking of malaria cases from the intervention and control arm irrespectively of where on Zanzibar the case is confirmed. The populations of the two study arms were estimated using a population enumeration census from 2012 with an annual growth rate of 2.8%. This study assessed the long-term impact of MDA by comparing cumulative incidence in the control and MDA Shehias six and 12 months after MDA and during the entire study period May 2016-September 2017 (16 months). Comparison of cumulative incidence in MDA and Control Shehias during high transmission seasons May-Sep 2015, 2016 and 2017 was also conducted.

Data of malaria cases among the residents of the study Shehias, reported at health facilities across Zanzibar, was downloaded from the MCN database for the period May 2016-Sep 2017. Data from period May-Sep 2015 was also obtained for the secondary objective. Data was

checked for inconsistencies and errors; doublets of the same malaria case (malaria case ID), diagnosis date, Shehia info and travel history info. Through this MCN data analyses regarding MDA´s long-term effectiveness in reducing malaria transmission in the study Shehias were done. Data of all malaria cases reported at health facilities on the whole island Unguja was also downloaded from the MEEDS database for the time-period week 1 2014 to week 40 2017. This data was obtained to cross-check the MCN data and calculate RDT testing and positivity rates at health facilities. Testing rate for each year was calculated as the number of patients tested with RDT divided by the total number of patients seeking help at health facilities. Positivity rate for each year was calculated as the number of patients testing positive for malaria by RDT divided by the total number that were tested. Furthermore, available rainfall data from 2014-2017 was also acquired from Tanzania Meteorological Agency.

### 3.3 Statistical analysis

The MCN data was cleaned and processed using Excel 2016. Further statistical analyzes were conducted in IBM SPSS Statistics 25. Descriptive statistics was used to describe the study populations and the study outcomes. The cumulative incidences of malaria in MDA and control Shehias during a specific time period were compared by calculating the crude risk ratio (RR) for malaria between MDA and control Shehias for that specific time period. Kaplan-Meier hazard plots of cumulative malaria incidence in MDA vs control areas were produced to visualize the primary objective's result. The RRs for the different time periods was produced by crosstabulation in SPSS and the Kaplan-Meier hazard plots were produced by Kaplan-Meier survival analysis in SPSS. The statistical significance threshold was set at 0.05.

A significant portion of Zanzibar's malaria cases are thought to be imported. To assess MDA's effect on local transmission (i.e., malaria acquired inside the study Shehias), RR analyses were repeated after exclusion of all potentially imported malaria cases (cases with

history of travel overnight outside of Zanzibar and/or outside Shehia within 30 days of malaria diagnosis).

### 3.4 Ethical considerations

This is a follow-up on a study that was conducted with the ethical approval from Zanzibar Medical Research Ethical Committee (ZAMREC). All participants were also informed of the purpose and benefits of the study, as well as potential risks and side-effects of MDA. Written consent of participation in the study were obtained from heads of households. It was emphasized that participation was non-compulsory. All study activities were conducted in conformity with the local culture and customs.

MDA is a controversial method. The ethics of exposing whole populations of the potential side effects of drugs is debatable. Primaquine is known for serious side effects like toxic hemolysis in G6PD-deficient patients. Despite this, WHO recommends the addition of SLD primaquine to all *P. falciparum* cases, in low-transmission settings, without screening for deficiency. The WHO Evidence Review Group has also suggested the use of SLD primaquine in MDA regimens (23). The risk and degree of hemolysis in G6PD deficient patient are dose and exposure duration dependent and the use of SLD primaquine (0.25 mg/kg) is therefor considered safe without prior testing in G6PD abundant populations (8, 32).

WHO recommends the verification of a parasitic infection, before malaria treatment is administered, to reduce drug overuse as a factor contributing to drug resistance development. MDA oppose that recommendation and might be a source of progressing resistance development. To minimize this risk, the drug combination of MDA should not be the same as the first line treatment for individual cases and the addition of a transmission blocking drug, like primaquine, is key to limit the spread of resistant strains (23). However, if MDA fails to eliminate malaria from the targeted areas it may drive resistance development (8).

# 4 Results

## 4.1 Cumulative malaria incidence after MDA

MDA was initiated on the 30th April 2016, therefore the follow-up period starts 1 May 2016 and MCN data was collected up to 31 August 2017; for a total of 16 months follow-up. A total of 389 RDT confirmed malaria cases from the study Shehias were detected by PCD at health facilities, and were reported in MCN during this time period. 173 cases were from the intervention arm (MDA) and 216 were from the control arm. Characteristics of the recorded cases can be seen in table 1. Figure 1 displays the weekly number of cases reported in the two study arms respectively during the study period. Figure 1 also displays when the two rounds of MDA were administered and the different follow-up periods of this report.

**Tabell 1. Characteristics of clinical malaria cases (detected at health facilities and reported through MCN) in the two study arms during the study period May 2016-Sep 2017.**

| VARIABLE | MDA SHEHIAS | CONTROL SHEHIAS |
|---|---|---|
| **NUMBER OF MALARIA CASES** | 173 | 216 |
| **MEDIAN AGE (YEARS)** | 15 | 19.5 |
| **AGE GROUPS (YEARS)** | | |
| <5 (%) | 18.5 | 8.8 |
| 5-9 (%) | 7.5 | 10.7 |
| 10-14 (%) | 12.1 | 10.2 |
| 15-19 (%) | 12.1 | 13.4 |
| 20-29 (%) | 16.8 | 19.4 |
| 30-39 (%) | 5.8 | 7.9 |
| 40-49 (%) | 4.6 | 6.0 |
| 50-59 (%) | 1.2 | 5.6 |
| 60- (%) | 2.3 | 4.2 |
| MISSING INFO (%) | 19.1 | 13.9 |
| **SEX** | | |
| FEMALE (%) | 26.0 | 31.5 |
| MALE (%) | 44.5 | 50.9 |
| MISSING INFO (%) | 29.5 | 17.6 |
| **SLEPT UNDER LLIN NIGHT BEFORE TESTING POSITIVE** | | |
| YES (%) | 37.6 | 44.4 |
| NO (%) | 23.1 | 24.5 |
| MISSING INFO (%) | 39.3 | 31.0 |
| **HISTORY OF TRAVEL OVERNIGHT WITHIN 30 DAYS** | | |
| YES (%) | 28.3 | 22.7 |
| NO (%) | 71.7 | 77.3 |

Abbreviations: MDA, mass drug administration; MCN, malaria case notification; LLIN, long lasting insecticidal net.

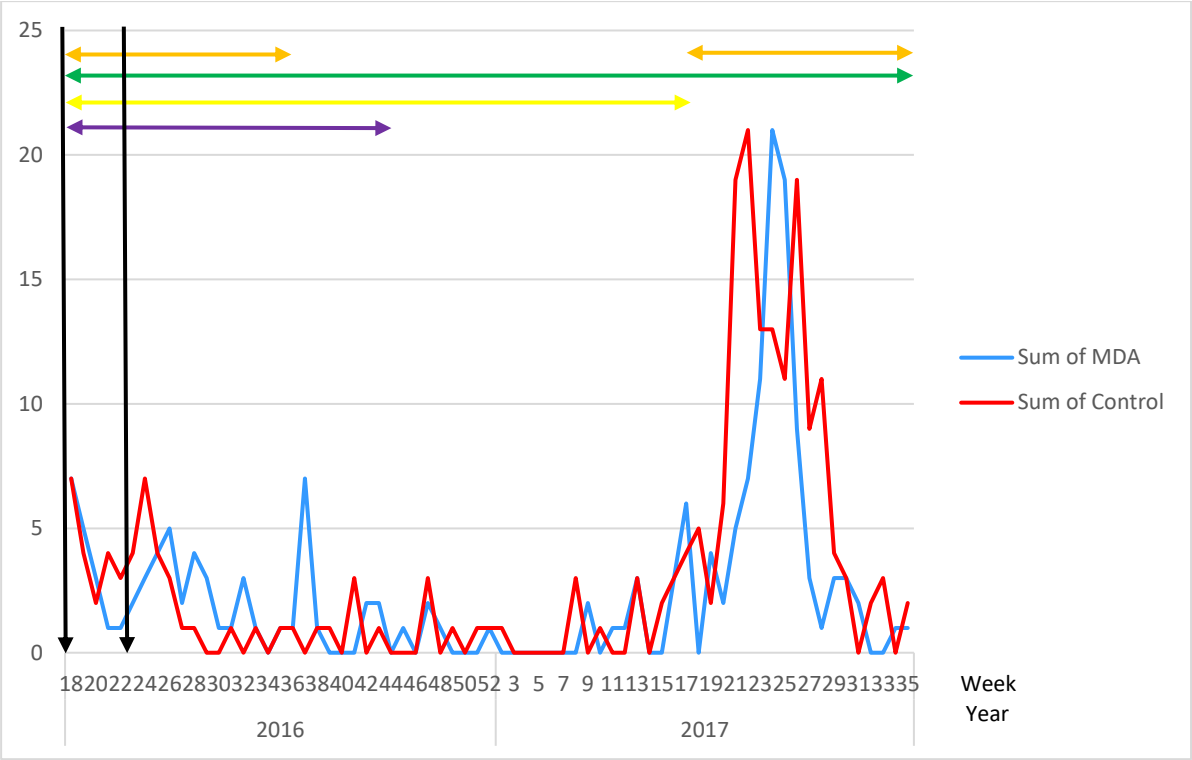


**Figure 1.** **Weekly clinical malaria cases (detected at health facilities and reported through MCN) among the residents of the MDA and control Shehias during the time period May 2016-Sep 2017 (16 months follow-up). Black arrows mark the administration of the two rounds of MDA (week 18 and 22), orange arrows mark the high transmission season of 2016 and 2017 (week 18-36). Green arrow marks the 16 month follow-up, yellow arrow marks the 12 month follow-up and purple arrow marks the 6 month follow-up.**

The 2016 population sizes of the study arms were estimated from the 2012 population enumeration survey to be 11 576 in MDA Shehias and 12 239 in control Shehias. The 173 recorded malaria cases in the intervention arm is therefore equal to a cumulative incidence of 1.49% during 16 months follow-up which translates into 11 cases/1000 person-years. The 216 cases from the control arm during the same time period is equal to a cumulative incidence of 1.76% or 13 cases/1000 person-years. Cumulative incidence for 6 and 12 months post MDA was also analyzed and the number of cases, population sizes and cumulative incidences during different time-periods can be found in table 2. A visualization of the cumulative incidence of malaria in the study arms during the 16 months following administration of MDA can be seen in the Kaplan-Meier hazard plot displayed in figure 2 A).

The cumulative incidence of malaria in MDA Shehias was divided by the cumulative incidence in control Shehias to generate a crude risk ratio (RR) of malaria between MDA and control Shehias for each specific time period. The RR of malaria between MDA and control Shehias was 1.27 (0.87-1.85; P=0.21) after 6 months, 1.17 (95% CI 0.86-1.61; P=0.32) after 12 months, and 0.85 (95% CI 0.69-1.03; P=0.10) after 16 months post MDA (i.e., equal to a non-significant 15% reduction in risk of malaria in the MDA arm compared to the control arm. RRs during different time-periods can be seen in table 2.

A significant portion of Zanzibar's malaria cases are thought to be imported. The RR between MDA and control Shehias for locally acquired malaria (inside the study Shehias) was 1.32 (95% CI 0.80-2.17; P=0.27) after 6 months, 1.08 (0.71-1.65; P=0.71) after 12 months and 0.79 (95% CI 0.62-0.99; P=0.04) after 16 months post MDA. Cumulative incidences of locally acquired malaria during different time-periods and the matching RRs can be found in table 2. Figure 2 B) displays a Kaplan-Meier hazard plot of cumulative incidence of locally acquired malaria during 16 months post MDA. Figure 2 B) (local transmission) shows the same trends as figure 2 A) (all cases) with a greater dispersion between the two graphs at the end of the study period, representing the 21% lower risk in MDA Shehias compared to control during the full 16 months follow-up period (RR=0.79; P=0.04).

**Table 2. Overview of cumulative incidence of clinical malaria (detected at health facilities and reported through MCN) in the two study arms and crude risk ratios (RR) of clinical malaria between the MDA and control arm during 16 months follow-up (May 2016-Sep 2017) and during high transmission season May-Sep 2015, 2016 and 2017. Significant p values are marked in bold.**

| TIME PERIOD | STUDY ARM | MALARIA CASES | POPULATION SIZE | CUMULATIVE INCIDENCE (%) | RISK RATIO (RR) | 95% CONFIDENCE INTERVAL | SIGNIFICANCE |
|---|---|---|---|---|---|---|---|
| 6 MONTHS | MDA | 60 | 11 576 | 0.52 | 1.27 | 0.87-1.85 | P = 0.21 |
| | Control | 50 | 12 239 | 0.41 | Reference | | |
| 12 MONTHS | MDA | 81 | 11 576 | 0.70 | 1.17 | 0.86-1.61 | P = 0.32 |
| | Control | 73 | 12 239 | 0.60 | Reference | | |
| 16 MONTHS | MDA | 173 | 11 576 | 1.49 | 0.85 | 0.69-1.03 | P = 0.10 |
| | Control | 216 | 12 239 | 1.76 | Reference | | |
| 6 MONTHS | MDA | 35 | 11 576 | 0.30 | 1.32 | 0.80-2.17 | P = 0.27 |
| LOCAL CASES | Control | 28 | 12 239 | 0.23 | Reference | | |
| 12 MONTHS | MDA | 44 | 11 576 | 0.38 | 1.08 | 0.71-1.65 | P = 0.71 |
| LOCAL CASES | Control | 43 | 12 239 | 0.35 | Reference | | |
| 16 MONTHS | MDA | 124 | 11 576 | 1.07 | 0.79 | 0.62-0.99 | **P = 0.04** |
| LOCAL CASES | Control | 167 | 12 239 | 1.36 | Reference | | |
| HTS 2015 | MDA | 127 | 11 260 | 1.13 | 1.23 | 0.96-1.59 | P = 0.11 |
| | Control | 109 | 11 906 | 0.92 | Reference | | |
| HTS 2016 | MDA | 47 | 11 576 | 0.41 | 1.16 | 0.76-1.75 | P = 0.49 |
| | Control | 43 | 12 239 | 0.35 | Reference | | |
| HTS 2017 | MDA | 92 | 11 900 | 0.77 | 0.68 | 0.52-0.88 | **P = 0.004** |
| | Control | 143 | 12 582 | 1.14 | Reference | | |
| HTS 2015 | MDA | 85 | 11 260 | 0.75 | 1.11 | 0.82-1.50 | P = 0.50 |
| LOCAL CASES | Control | 81 | 11 906 | 0.68 | Reference | | |
| HTS 2016 | MDA | 33 | 11 576 | 0.29 | 1.40 | 0.83-2.35 | P = 0.21 |
| LOCAL CASES | Control | 25 | 12 239 | 0.20 | Reference | | |
| HTS 2017 | MDA | 80 | 11 900 | 0.67 | 0.68 | 0.52-0.90 | **P = 0.007** |
| LOCAL CASES | Control | 124 | 12 582 | 0.99 | Reference | | |

Abbreviations: MCN, malaria case notification; HTS, high transmission season; RR, risk ratio.

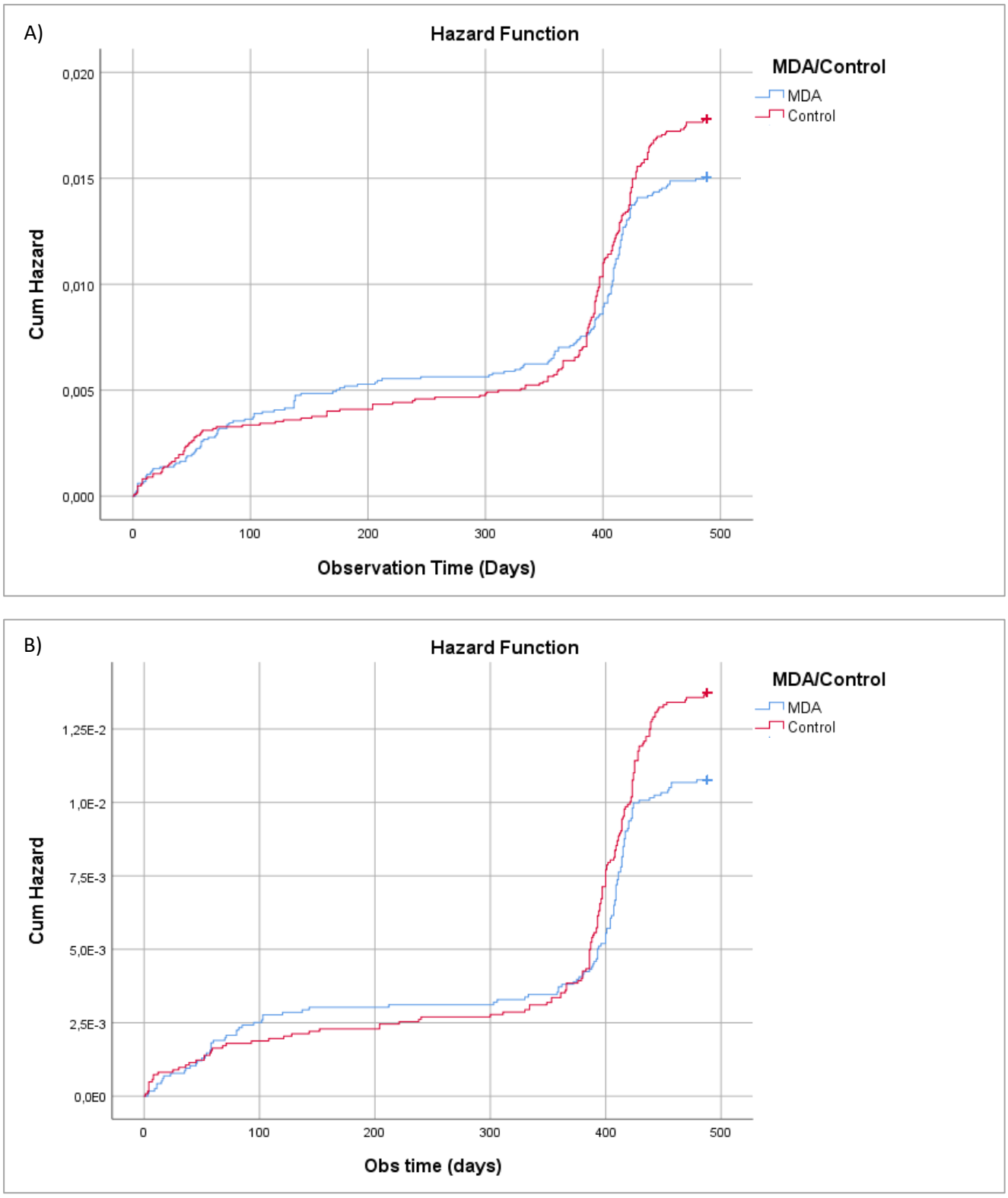


**Figure 2.** Kalplan-Meier hazard plots of cumulative incidence of clinical malaria in the two study arms during the time period May 2016-Sep 2017 (488 days). Panel A) displays all clinical malaria cases detected at health facilities. In panel B) all potentially imported malaria cases have been excluded to display only cases infected by local transmission inside the study Shehias.

## 4.2 Cumulative malaria incidence during high transmission season 2015, 2016 and 2017

The majority of Zanzibar's malaria cases occur during the high transmission season ranging between May-September. The secondary objective of this report was therefore to investigate whether MDA had an impact on seasonal malaria transmission. In 2015, the year before MDA, there were 236 cases of malaria from the study Shehias (detected by PCD and reported to MCN) during the high transmission season. During the same period in 2016 there were only 90 cases and during 2017 there were 235 cases. The distribution of cases between MDA and Control Shehias can be seen in table 2. Figure 3 displays the weekly number of cases in MDA and Control Shehias respectively during the high transmission season 2015, 2016 and 2017. There were slightly more cases and higher cumulative incidence in MDA Shehias during the high transmission season 2015 and 2016, although the difference was not significant. In 2015, the RR of malaria between MDA and Control Shehias during the high transmission season was 1.23 (95% CI 0.96-1.59; P=0.11) and in 2016 the RR was 1.16 (95% CI 0.76-1.75; P=0.49). During the high transmission season 2017 there were fewer cases and lower cumulative incidence in MDA Shehias compared to control and the RR was 0.68 (95% CI 0.52-0.88; P=0.004) equal to 32% reduced risk of malaria in MDA Shehias compared to control. The cumulative incidences and the RRs for the different time periods can be found in table 2.

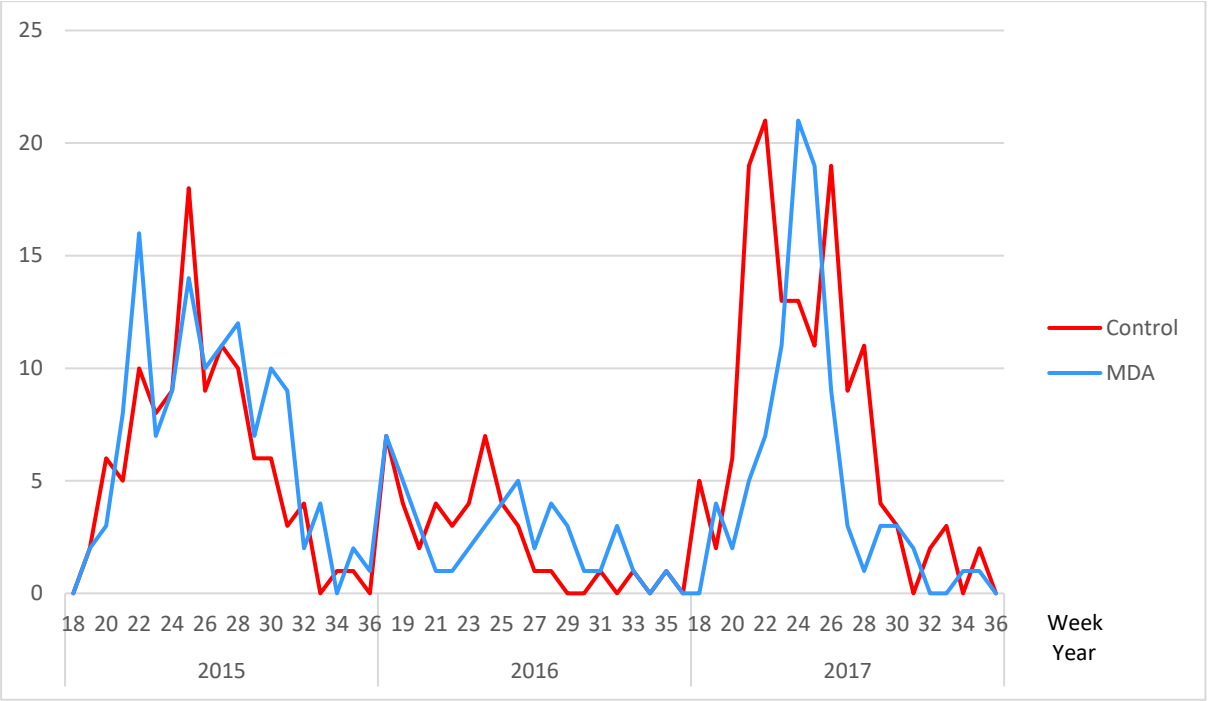


**Figure 3. Weekly clinical malaria cases (detected at health facilities and reported through MCN) among the residents of the MDA and control Shehias during high transmission season (May-Sep, week 18-36) 2015, 2016 and 2017.**

Similar trends apply for local transmission during the studied high transmission seasons. There was no significant difference between MDA and control Shehias in 2015 (RR=1.11; P=0.50) and 2016 (RR=1.4; P=0.21). During the high transmission season 2017 there was 32% significantly lower risk of locally acquired malaria in MDA Shehias compared to control (RR=0.68; P=0.007). Cumulative incidences and RRs can be found in table 2.

## 4.3 Cross-checking and validation of MCN data

The results of this report are derived from MCN data. As observed previously in the results section, there seems to be generally lower incidence of malaria in both MDA and control Shehias during high transmission season 2016 (table 2 and figure 4) compared to 2015 and 2017. Lower incidence was not expected in both study arms post MDA. To cross-check the MCN data, it was compared with MEEDS data from the health facilities in the study Shehias. The MCN data allows tracking of all residents of the study Shehias that are tested positive for malaria, regardless of where on Zanzibar they are tested. The MEEDS data provides information about all cases that are tested positive for malaria at the health facilities of the

study Shehias; MEEDS data therefore captures some cases that are not residents of the study Shehias. Since most of the residents of the study Shehias are assumed to seek medical attention at the health facilities in the study Shehias, MCN and MEEDS data should be similar. This is the case and can be observed in figure 4. This suggests that the MCN data is accurate.

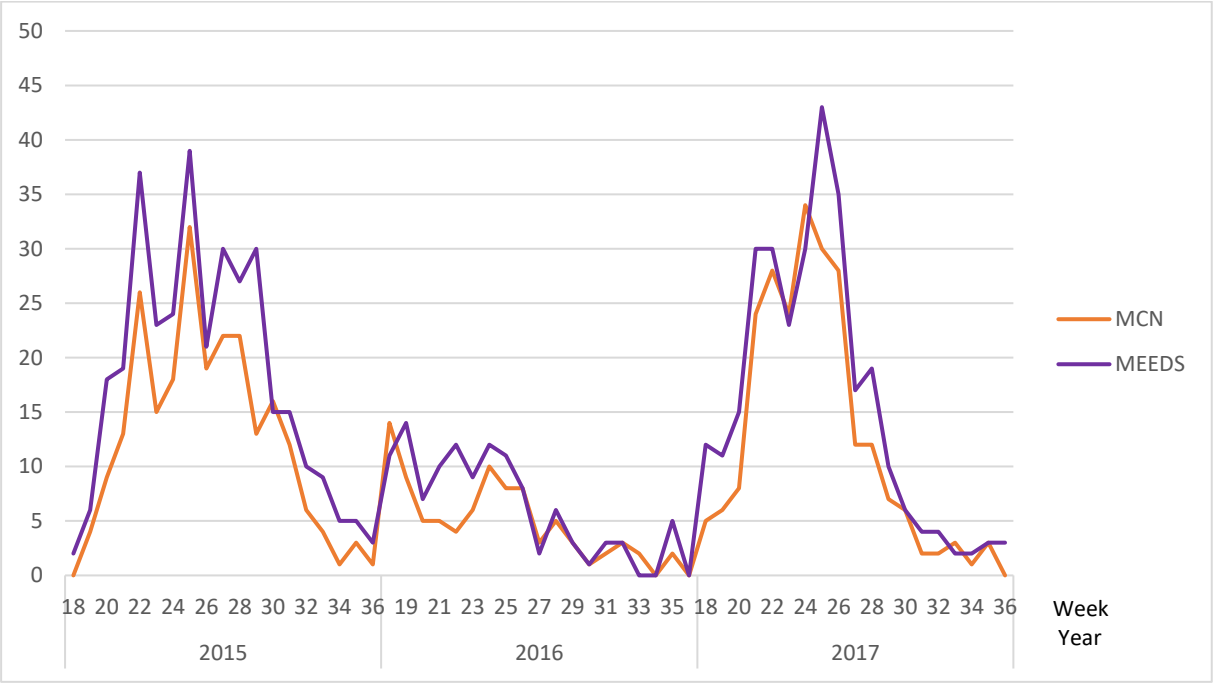


**Figure 4. Comparison of MCN and MEEDS data from the study Shehias (MDA+control) during high transmission season (May-Sep, week 18-36) 2015, 2016 and 2017. MCN data displays the weekly number of clinical malaria cases among the residents of the study Shehias (detected at health facilities across Zanzibar). MEEDS data displays the weekly number of cases tested positive at the health facilities in the study Shehias.**

The generally lower malaria incidence observed in the MCN and MEEDS data from the study Shehias during High transmission season 2016 could be a result of reduced RDT testing rate at health facilities. To check if this was the case MEEDS data was used since it contains information about how many patients that seeks medical help, how many that are tested with RDT and how many that are found malaria positive. With this data the RDT testing rate and RDT positivity rate of different years was analyzed (see table 3). There was no drop in RDT

testing rate in the study Shehias or on Unguja in 2016 but a reduction in RDT-positivity rate can be observed not only in the study Shehias but on whole Unguja as well.

**Table 3. RDT testing and positivity rate at health facilities (HF) on Unguja and in the study Shehias.**

| AREA | TESTING RATE % (95% CI) | POSITIVITY RATE % (95% CI) |
|---|---|---|
| **UNGUJA HF** | | |
| 2014 | 26.8 (26.7-27.0) | 1.7 (1.6-1-7) |
| 2015 | 25.6 (25.5-25.7) | 2.0 (1.9-2.1) |
| 2016 | 30.4 (30.3-30.5) | 1.2 (1.1-1.2) |
| 2017 (40 WEEKS) | 31.7 (31.6-31.8) | 1.4 (1.4-1.5) |
| | | |
| **MDA/CONTROL HF** | | |
| 2014 | 22.4 (22.1-22.7) | 2.9 (2.7-3.2) |
| 2015 | 24.7 (24.4-25.1) | 3.1 (2.8-3.4) |
| 2016 | 25.2 (24.8-25.6) | 1.6 (1.4-1.8) |
| 2017 (40 WEEKS) | 29.9 (29.4-30.3) | 2.9 (2.7-3.3) |

Abbreviations: MDA, mass drug administration; HF, health facilities.

MEEDS data from whole Unguja (visualized in figure 5) showed that there was generally lower malaria incidence on the whole island during the high transmission season 2016 compared to other years. Figure 5 displays the weekly number of malaria cases reported to MEEDS by all health facilities on Unguja from week 1 2014 to week 40 2017. Figure 5 also displays the average weekly amount of rainfall (mm) of the six districts of Unguja. A reduction in rainfall during the main rains of the rainy season could be one reason behind the generally lower malaria incidence during the high transmission season of 2016 but no such reduction in rainfall could be visually observed; see figure 6.

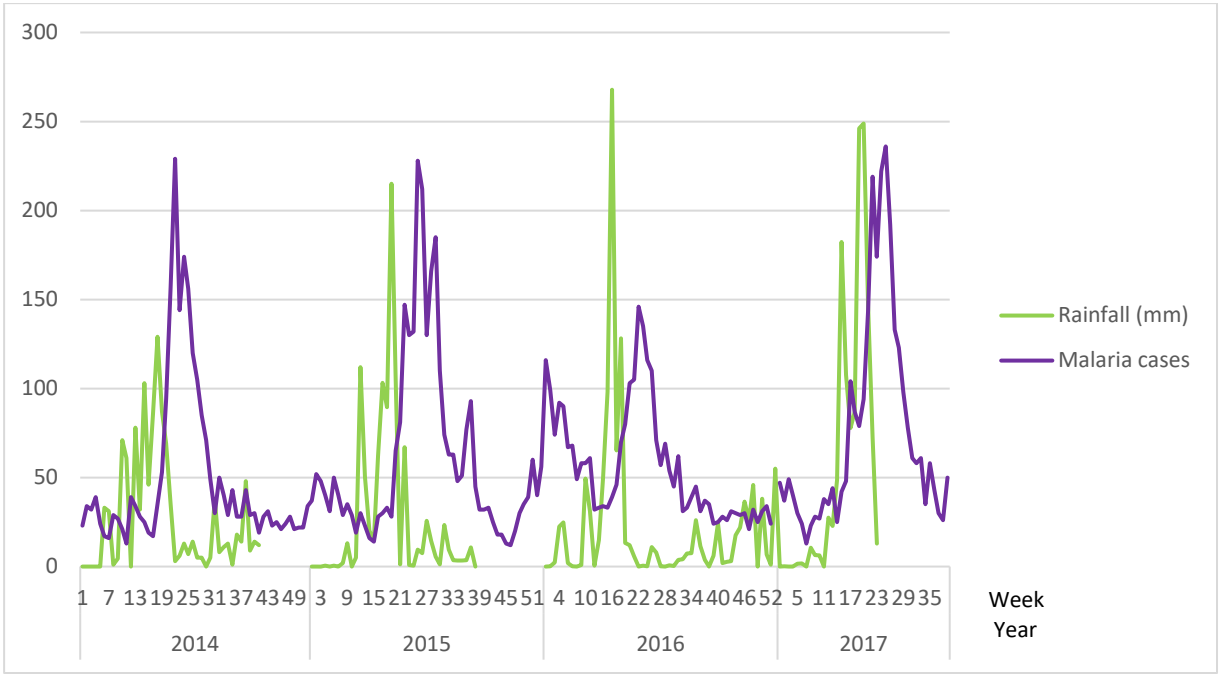


**Figure 5.** **Weekly reported clinical malaria cases (detected at health facilities) on the island Unguja (MEEDS data) in relation to rainfall data (average weekly rainfall (mm) of the six districts of Unguja).**

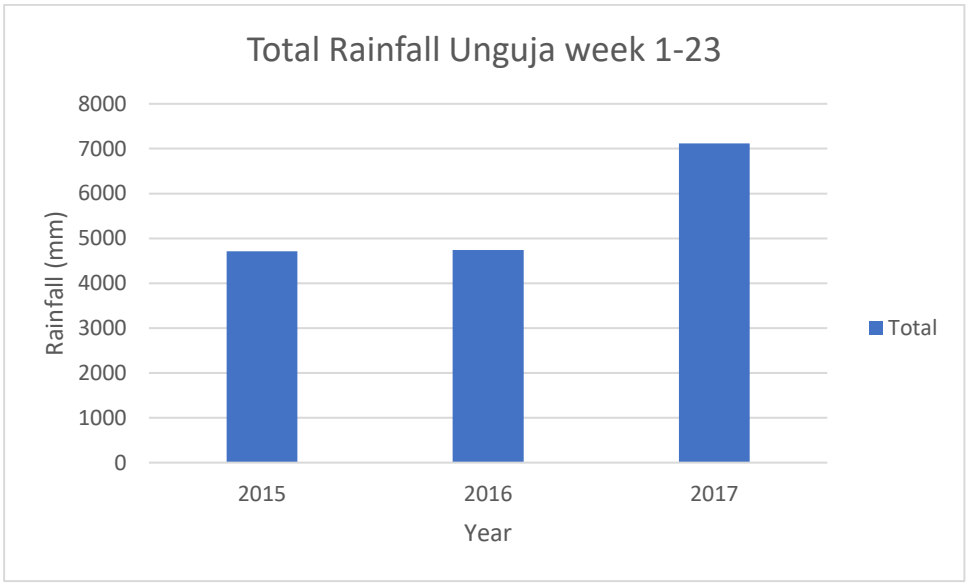


**Figure 6.** **Total amount of rainfall (mm) of the six districts of Unguja during the 23 first weeks of 2015, 2016 and 2017; thus including the main rainy season of each year.**

## 5 Discussion

This report is and initial follow-up of a CRCT which aims to assess use of MDA as a malaria elimination strategy in Zanzibar. In April 2016, eight areas (Shehias) of relatively higher transmission (hotspots) were provided two rounds of DHAp + SLD Primaquine (0.25mg/kg)

MDA and cumulative malaria incidence was compared with eight similar control areas. The two rounds of MDA were conducted just before the onset of the annual malaria High transmission season occurring after the start of the rainy season. During the 16 months follow-up period of this report (May 2016-Sep 2017) MDA fails to achieve a significantly lower cumulative incidence and risk of malaria in the MDA Shehias compared to control (RR=0.85; P=.10). No significant effect was either observed during the first 6 or 12 months of follow-up. There was no evidence in these preliminary investigations that MDA had had an impact on malaria transmission.

The cumulative incidences of the study arms during high transmission season 2015 (the year before MDA) were analyzed to provide a baseline or pre-intervention comparison between MDA and control Shehias. Malaria incidence was slightly higher in the intervention arm at the baseline of the study, although the difference was not significant (RR = 1.23; 95% CI 0.96-1.59; P=0.11). The high transmission season of 2016 starts directly after the administration of the first round of MDA (administered in the end of April 2016). The cumulative incidence was lower in both study arms during this period but still slightly higher in the intervention arm; RR=1.16 (95% CI 0.76-1.75; P=0.49). Not until the high transmission season of 2017, one year after MDA, a 32% lower risk of malaria can be observed in the MDA Shehias compared to control; RR=0.68 (95% CI 0.52-0.88; P=.004). The combined results imply that there is a transition from non-significantly higher to significantly lower malaria transmission in the MDA Shehias after MDA was conducted, if only high transmission season 2015, 2016 and 2017 are considered. This trend is also reflected in the cumulative incidence during the full 16 month follow-up, although the difference is still not significant in the end; RR=0.85 (95% CI 0.69-1.03; P=0.10). Furthermore, there was no evidence of an immediate short-term impact of MDA, neither when comparing the cumulative incidence during the first six nor 12 months; RR= 1.27 (95% CI 0.87-1.85; P=0.21) and RR=

1.17 (95% CI 0.86-1.61; P=0.32) respectively. This is visualized very well in the Kaplan-Meier hazard plots of cumulative incidence of malaria seen in figure 2 A). In this figure, one can see that the cumulative incidence builds up fast in the control arm, but even faster in the intervention arm during the first time of the observation period and then levels out; this first slope represents the high transmission season 2016 (first 123 days), when the incidence of malaria is higher than during the rest of the year. Not until the onset of the high transmission season 2017 (last 123 days) the graphs (cumulative incidence) starts to once again accelerate upwards. Here it is easy to see that the cumulative incidence is higher in the control Shehias.

To assess if MDA had an effect on local transmission all analyses were repeated after exclusion of all potentially imported cases. In general, the same trends apply for local transmission with the exception that a significant lower risk of clinical malaria can be seen during the full 16 months follow-up. During this period the risk of locally acquired malaria was 21% lower in MDA Shehias compared with control; RR=0.79 (95% CI 0.62-0.99; P=0.04). The Kaplan Meier hazard plot of local transmission in figure 2 B) clearly visualizes that no significant difference in local transmission was observed until the end of the study period during high transmission season 2017; RR=0.68 (95% CI 0.52-0.90 P=0.007). The weakness of excluding all potentially imported cases is that not all of them are actually imported. Many of the excluded cases could have acquired the infection through local transmission since the exclusion criteria – history of travel the last 30 days – does not specify if they traveled to a high-risk area of malaria. The imported cases are excluded with high sensitivity but very low specificity.

Literature on the subject of MDA against malaria are united in their opinion that MDA gives and immediate effect on prevalence and incidence of malaria and that maintaining this effect is the issue (23, 27-29). The results of this report don't support this conclusion, it rather suggests that the effect of MDA could be observed first in the long run. That is if the observed

significant lower risk of malaria towards the end of the study period (high transmission season 2017) is in fact because of MDA. The analyses and results of this report are insufficient to determine if this is the case and further analyses are needed to determine the impact MDA had on malaria transmission. These analyses must take into consideration the study design of a CRCT. This report is only an initial investigation on the malaria transmission after MDA was conducted.

If the reduction in risk towards the end of the study period actually is due to MDA, what would cause such a delay in observable effect? To display an effect, using the methodological aspects of this report, MDA fist had to equalize the potentially (non-significant) higher risk of malaria in the MDA Shehias that could be observed during high transmission season 2015 (before MDA was administered) and during high transmission season 2016 (after MDA was administered). This report uses crude RRs to assess the effect of MDA and in theoretical numbers this equalization in risk would mean bringing an RR>1 down to RR=1. When the risk of malaria is equal in the study arms (RR=1), the intervention (MDA in this case) can start to display an effect. This is why more sophisticated analysis are needed, taking into consideration the study design of a CRCT and adjusting for baseline differences between the study arms.

Another theory of why there is a delay in the effect of MDA, indicating a long-term effect, is that MDA may has been conducted too late in 2016. If the number of mosquitoes had started to rise earlier than expected (before MDA was administered) and they were already infected from biting humans carrying gametocytes, the mosquitos could still infect the same amount of people as in the control areas. MDA then depleted much of the sub-patient/asymptomatic gametocyte carriage in the MDA Shehias, meaning that not as big portion of the mosquitos of high transmission season 2017 became infected; therefore the effect of MDA could be observed first during the high transmission season one year after MDA. In theory this could

be the case but the prophylactic effect of the Piperaquine component of DHAp should have protected the population of the MDA Shehias during high transmission season 2016 from infection by already infected mosquitos.

About 60% of the population of Zanzibar is rural and farming is the most common source of income (6). MDA was conducted at the beginning of the wet season when the population in theory should be more stationary due to increased farming capacity (25). To conduct MDA during this time of year therefore predisposes high community coverage since a greater part of the population is home. The coverage of the first round of MDA was: 91% received DHAp, 87% received SLD Primaquine and 67% took the first dose as DOT. The coverage of the second round of MDA was: 88% received DHAp, 81% received SLD Primaquine and 48% took the first dose as DOT. A post MDA survey in a subset of the households of the MDA Shehias assessed the compliance to the full treatment to 84% and 96% in the first and second round respectively. According to these figures, the execution of the MDA study was highly successful and the high acceptance that these figures indicate can be a result of a well-informed receiving end and a good delivery strategy.

With high coverage and compliance achieved, why can't a more pronounced effect be seen in the MDA Shehias compared to control. All study Shehias had similar coverage of vector control interventions at the baseline of the survey and no other malaria control activities (such as mass distribution of LLIN, mass IRS or other wide-scale interventions) were conducted in the study Shehias during the period of this report. The malaria incidence fell dramatically in both the intervention and control arms during high transmission season 2016 (immediately after MDA was conducted) compared to the high transmission season 2015 and 2017. Since the Shehias are small and in close proximity of each other, a dilution effect of MDA could have occurred, meaning that also the control Shehias benefited from the effect of MDA. This might be the case but the results of figure 5 shows that the transmission (incidence) was

generally lower on the whole island Unguja during the high transmission season of 2016 compared to other years. If a dilution effect occurred, the effect of MDA generally lowered malaria transmission on whole Unguja which is not very probable. A more likely explanation is that there were a synergy of entomological and ecological factors contributing to the generally lower transmission observed on Unguja during high transmission season 2016. This reduction in incidence, occurring at the time when MDA was conducted, makes the results of this report hard to evaluate. The original study includes PCR measured malaria prevalence from both when MDA was conducted and three months after MDA. These results may provide valuable information about the effect the two rounds of MDA had on the asymptomatic/sub-patient parasite carriage in the MDA Shehias.

There has been a renewed interest in MDA during the last couple of years due to emerging ACT resistance and the difficulties in targeting the asymptomatic/sub-patient parasite reservoir thought to maintain 20-50% of remaining transmission in low transmission settings. MDA is one of the few methods available today to target these individuals. To determine the impact of MDA, high quality CRCT are needed; few studies of this kind have been conducted (27). In 1999 the first CRCT assessing the effect of MDA against malaria was conducted in an area of high transmission in the Gambia (35). In this study, a single round of MDA with artesunate and a single dose of sulfadoxine-pyrimethamine fails to reduce the malaria incidence among children and the prevalence among adults compared to control during 20 weeks of follow-up. This drug combination does not affect mature gametocytes and this in combination with the study taking place in a high transmission setting are possible reasons for a not detectable effect.

This report highlights the difficulties of conducting a CRCT in a low transmission setting where transmission is unpredictable. Unpredictable variations in transmission and prevalence has been observed in other regions where MDA were tested. A MDA CRCT from Tanzania

could not evaluate the effect of MDA since the transmission had almost completely disappeared from the area prior the administration of MDA (36).

In 2015, another CRCT using two rounds of DHAp MDA was conducted in Zambia (37). The short-term impact of MDA was evaluated 5 months after the intervention and a non-significant 70% lower cumulative infection incidence could be observed in the areas receiving MDA compared with control (crude incidence rate ratio = 0.30 (95% CI 0.06–1.49; P = .14)). A reduction in PCR measured parasite prevalence of 87% (adjusted odds ratio = 0.13 (95% CI 0.02–0.92; P = .04) compared with control was achieved. This study also suffered from lower incidence than expected in the control arm. As a result, the statistical power to detect significant differences were reduced.

The three studies discussed above all reached a coverage >80%. Despite that the effect of MDA is not entirely certain. MDA is recommended in the Greater Mekong subregion to contain artemisinin resistant strains and prohibit the spread to other regions. A pilot study from this area was recently published (38). It used three rounds of MDA with DHAp + SLD primaquine and the MDA intervention was implemented together with distribution of LLIN and rapid diagnosis and treatment via a “malaria post”. The control areas also received LLIN distribution and the rapid diagnosis and treatment intervention which led to big reductions in malaria prevalence (PCR) in the control areas. The study design therefore makes it hard to conclude the impact of MDA alone; they report 88% prevalence reduction in MDA villages and 85% prevalence reduction in control villages. In addition, being a pilot, the study provides low power to detect the effect of MDA.

# 6 Conclusions and implications

There is no evidence in these preliminary investigations that two rounds of MDA had an effect on malaria transmission in the eight hotspot Shehias on Zanzibar. However, this report provides insufficient information to fully determine the impact MDA had. Further analyses

are needed to fully understand the transmission observed after MDA was conducted. These analyses should adjust for variations between the study arms at baseline and take into consideration the study design of a CRCT. The results of this report consist of descriptive statistics and represents only an initial investigation/evaluation of the malaria transmission using crude risk ratios. During the high transmission season 2016 there were an unexpected reduction in malaria incidence on the whole island where this study took place. Since most of the yearly malaria cases occur during this period, this reduction in incidence makes it hard to evaluate the effect of MDA since there were few cases in both the intervention and control arm respectively. Previous studies have concluded that the effect of MDA on malaria transmission is rapid and the difficulty is to maintain this effect. No such direct effect could be observed in this report. The significantly lower risk of malaria observed in the intervention arm during the high transmission season 2017, one year after MDA was administered, cannot be attributed to the effect of MDA based on these preliminary findings.

However, MDA may still play a vital role in Zanzibar's and other malaria elimination areas' efforts to combat malaria, and the results of this report at this stage can't be used to neither recommend nor discard the use of MDA as a malaria elimination strategy.

# 7 Populärvetenskaplig sammanfattning

## Massmedicinering mot malaria på Zanzibar

Malaria är trots att det är en botbar och förebyggbar sjukdom en stor börda i många delar av världen; särskilt i regionerna av Afrika som ligger söder om Sahara. Att utrota malaria anses möjligt och massmedicinering av malariamedicin är en av metoderna som kan spela en stor roll för att lyckas. Detta projekt utvärderar massmedicinering för att uppnå utrotning av malaria på Zanzibar, en ögrupp utanför Tanzania. Massmedicinering innebär att alla i ett område får botande doser läkemedel oavsett om dem är sjuka eller inte. Denna metod övervägs eftersom många bär malariaparasiter utan att få symptom på sjukdom och kan därför

ovetande sprida sjukdomen vidare. Massmedicinering innebär att man inte behöver identifiera dessa symptomfria bärare, vilket är svårt med de verktyg som idag används för att ställa diagnosen malaria.

I april 2016 delades det ut två omgångar massmedicinering mot malaria i åtta områden på Zanzibar. Antalet nya fall av malaria i dessa områden jämfördes med antalet fall i åtta liknande områden som inte fick någon massmedicinering. Mycket är okänt om massmedicinering mot malaria varför studier som denna är viktiga.

Denna rapport är en uppföljning på studien som genomfördes förra året på Zanzibar. Resultaten i denna rapport visar inga antydningar på att massmedicinering har haft en effekt på antalet nya malariafall som uppstod under de 16 månader som följde massmedicineringen. Tidigare studier har visat att massmedicinering ger en snabb effekt på förekomsten av malaria och att problemet är att underhålla dessa resultat i det långa loppet. Resultaten i denna rapport visar på en minskad risk av malaria först ett år efter massmedicineringen genomfördes, denna effekt tros därför inte vara på grund av massmedicinering utan på grund av andra orsaker. För att fullt utvärdera effekten som massmedicineringen på Zanzibar hade måste ytterligare och mer avancerade analyser genomföras än vad som ligger till grund för resultatet i denna rapport. Massmedicinering kan fortfarande vara ett kraftfullt verktyg på vägen mot att utrota malaria.

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