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STUDY PROTOCOL

REVISED Scale up of a *Plasmodium falciparum* elimination program and surveillance system in Kayin State, Myanmar [version 2; referees: 2 approved]

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Abstract

Background: Myanmar has one of the largest malaria burdens in the Greater Mekong Subregion (GMS). Throughout the GMS, *Plasmodium falciparum* parasites are increasingly resistant to artemisinin combination therapies. Given that there are no current alternative treatment therapies, one proposed solution to the threat of untreatable *P. falciparum* malaria is to eliminate the parasite from the region. Several small-scale elimination projects have been piloted in the GMS, including along the Myanmar-Thailand border. Following the success of the pilot elimination project along the Myanmar-Thailand border, there was a scale up to a broad area of Eastern Kayin State, Myanmar. Here we describe the establishment of the scale up elimination project in Easter Kayin State.
Methods: The scale up relied on geographic reconnaissance and a geographic information system, community engagement, generalized access to community-based early diagnosis and treatment, near real-time epidemiological surveillance, cross sectional malaria prevalence surveys and targeted mass drug administration in villages with high prevalence of *P. falciparum* malaria. Molecular markers of drug resistance were also monitored in individuals with symptomatic and asymptomatic infections.
Discussion: This protocol illustrates the establishment of an elimination project and operational research in a remote, rural area encompassing several armed groups, multiple political organizations and a near-absent health care infrastructure. The establishment of the project relied on a strong rapport with the target community, on-the-ground knowledge (through geographic surveys and community engagement), rapid decision making and an approach that was flexible enough to quickly adapt to a complex landscape. The elimination project is ongoing, now over three years in operation, and assessment of the impact of this operational research will follow. This project has relevance not only for other malaria elimination projects but also for operational research aimed at eliminating other diseases.

Open Peer Review

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	Invited Referees	
	1	**2**
REVISED **version 2** published 22 Dec 2017		
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Abbreviations
ACT: artemisinin combination therapy; CE: community engagement; MDA: mass drug administration; MP: malaria post; MPW: malaria post worker; GIS: geographic information system; qPCR: quantitative PCR; RDT: rapid diagnostic test

Introduction
Malaria is endemic in Myanmar and the Greater Mekong Subregion (GMS) and it is a major cause of morbidity. The main Plasmodial species involved are *Plasmodium falciparum* and *Plasmodium vivax*. Transmission is seasonal and caused by multiple diverse Anopheles vectors[1–3]. In several parts of the GMS *P. falciparum* has become resistant to almost all available antimalarials, including artemisinin, artemisinin derivatives and partner drugs[4]. There are recent reports of a single dihydroartemisinin-piperaquine resistant strain that first emerged on the Thailand-Cambodian border (in Pailin Province) has now spread to southern Vietnam, after previously spreading into northeastern Thailand and southern Laos[5]. The emergence and subsequent spread of drug and multi-drug resistant *P. falciparum* malaria presents a major threat to the region and the rest of the world. Given the paucity of new drugs, one proposed solution is to attempt elimination of *P. falciparum* malaria before complete resistance to antimalarials is widespread. Several projects throughout the region have been established in order to test the feasibility of targeted *P. falciparum* elimination, including projects in Vietnam, Cambodia, Laos and Myanmar (https://clinicaltrials.gov/ct2/show/NCT01872702). Most rely on a combination of public health interventions, usually based on community engagement, early diagnosis and treatment and targeted mass drug administration (MDA).

Between 2012 and 2014, a series of malaria prevalence surveys were conducted on the Thailand-Myanmar border using a highly sensitive quantitative PCR (qPCR) approach[6,7]. Several villages with high prevalence of subclinical infections were chosen for pilot elimination work using a combination of village health workers, community engagement (CE) and MDA, including dihydroartemisinin-piperaquine with a single low dose of primaquine[6]. In four study villages on the Myanmar side of the border, the safety and acceptability of this intervention were carefully evaluated and the impact was measured through repeated (three monthly) mass blood screenings[8–10]. Detailed entomological evaluations were also conducted throughout the 24 months of the pilot study[11]. The results indicated that the strategy is safe and effective in rapidly eliminating the sub-microscopic reservoir of malaria parasites and in reducing transmission to mosquito vectors[12].

These encouraging results motivated a scale up of the elimination project to much of Eastern Kayin State, Myanmar. Here we describe the logistics and establishment of this malaria elimination program in Eastern Kayin State, Myanmar from 2014 through 2017. The impact of the interventions are monitored through the analysis of observational data collected through longitudinal passive case detection at community-based malaria diagnosis and treatment centers (malaria posts (MPs)) and through cross-sectional blood screenings conducted in targeted villages. This protocol helps to fill an important gap in the literature on operational research programs aimed at eliminating malaria[13–15].

Methods
The target area consists of four townships of Kayin State, Myanmar: Myawaddy, Kawkareik, Hlaingbwe and Hpapun (Figure 1). Much of the area has been in civil conflict for over half a century, with no accurate census or map[16] since World War II. In order to establish an operational malaria elimination project, it was therefore crucial to first develop an understanding of the geography and demography and to engage with the communities.

These reconnaissance efforts were then followed up by the establishment of a dense network of MPs, epidemiological and drug resistance surveillance systems, surveys for measuring prevalences of malaria in villages, and targeted MDA in cases where high prevalences of malaria were identified (Figure 2).

Mapping and geographic information system (GIS)
In order to understand the settlement demography and geography of the region, the area was systematically mapped using field teams and satellite-enabled geo-referencing devices. The devices simultaneously used GPS (global position system) and GLONAS (Globalnaya navigatsionnaya sputnikovaya sistema) satellites to increase accuracy of geographical coordinates (latitude and longitude). Mapping teams were composed of community members familiar with the region who were trained in the field by an experienced geographer and assistants. Politically sensitive areas and conflict zones were first approached with CE experts to gain local and regional support for the mapping activities.

From December of 2013 to December 2016, three waves of mapping were conducted. The first survey focused on whether or not malaria services existed in a community, whether or not they were properly staffed and stocked, names of villages, and the number of houses in a village. A second wave of surveys (conducted in 2014) aimed to correct any missing geographic points that were missing from the first wave, to fill in any gaps in the target area map, and to identify the locations of referral clinics. In 2015–2016, a third wave of mapping and surveys included a small set of economic indicators, including basic questions about agricultural development, transportation capabilities, electricity and water sources.
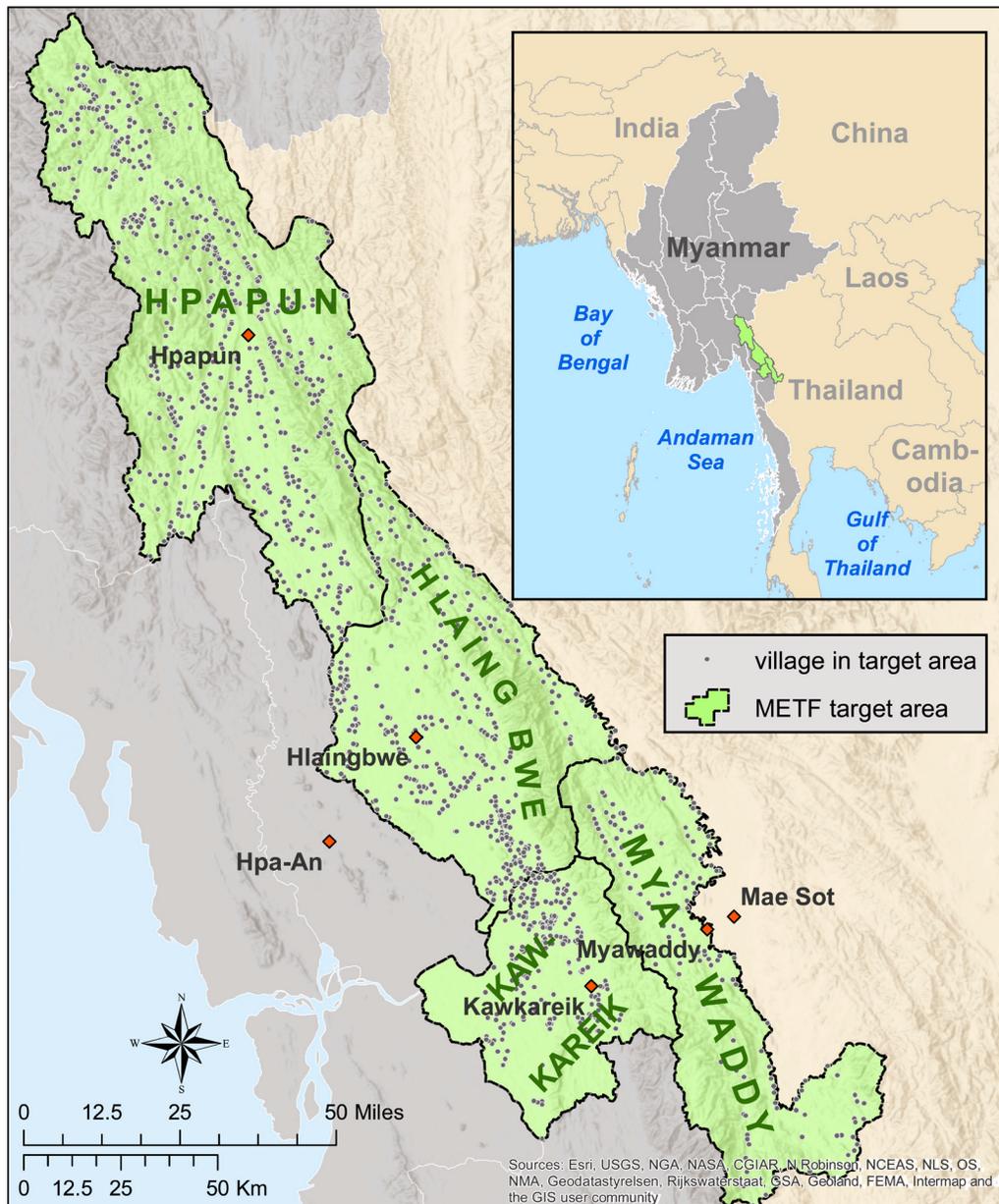
Figure 1. Map of target area and villages within the target area. METF, Malaria Elimination Task Force.

Data from the forms were entered into spread sheets and merged with geographic references downloaded from each GPS unit. These data formed the basic architecture for the project's GIS. R statistical software (version 3.1.0) and Python programming language were used for data tabulations and merging; mapping of the data was primarily done using ArcGIS10.2, and QGIS 2.4 was used for creating and manipulating spatial shape files. The core GIS data were stored in a file geodatabase (file type .gdb). Each mapped village was assigned an arbitrary numeric identification code and all information relating to a village (reports, samples, logistics) was labelled using this identification code.

Community engagement (CE)

The malaria elimination project relied on widespread participation and cooperation within and between communities[9]. The project was therefore heavily dependent on the ability to properly engage with target communities. A CE team was formed at the beginning of the project and team members helped facilitate all aspects of the project.

The CE team created and distributed community engagement materials in order to sensitize and explain the project to villagers. Materials included posters and audio announcements (statements
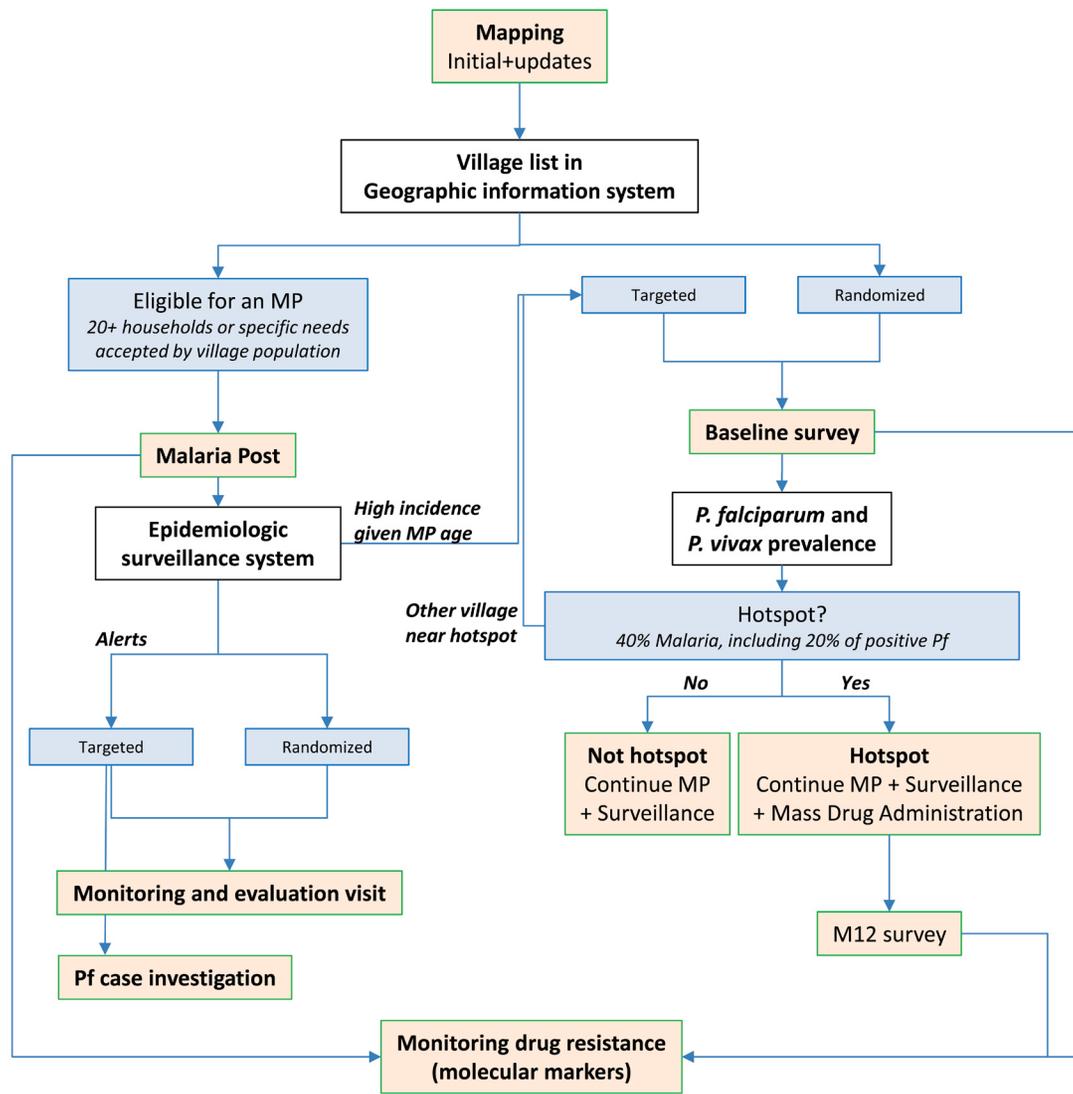
Figure 2. Flow-chart of the different components of Malaria Elimination Task Force elimination strategy and of the allocation of village-level interventions. MP, Malaria Post.

that were created by the CE team but given to village headmen to announce in periodic village announcements). METF posters, in S'kaw Karen and Burmese languages, that encourage people to visit malaria posts when they feel sick were placed in malaria posts (MPs) throughout the target area.

MPs were typically established in batches. Prior to malaria post worker (MPW) training, the CE team asked for a meeting with local health workers, village headmen, and other leaders. During this initial meeting the elimination program, MP system and CE were all explained to local leaders. Prior to malaria prevalence surveys the CE team met with township-level health care leaders and village headmen who were asked for permission to conduct the surveys. Survey planning relied heavily on village headmen, who notified and gathered the participants on the specified days

and times. Results were communicated to the communities and authorities after laboratory analysis of samples was completed.

In villages chosen for MDA, the CE team arrived a few days prior to the beginning of MDA in order to organize and set up new meetings with leaders and villagers to explain the medication, potential side effects, and the regimen that would be followed[9]. Large, village-scale meetings were combined with group discussions and activities specific for different population groups (e.g. women, school children, farmers or soldiers). During the MDA, the CE team members participated in the MDA process and participated in everyday village life, addressing individual and collective concerns in formal or informal discussions. No specific incentives were provided in exchange for participation, but a mobile clinic was available for all community members

during the MDA period, and long lasting insecticide-treated nets (LLINs) were available to those who didn't already possess them. The MP system allowed for feedback to flow back to headquarters after this seven day period.

In situations where villagers presented concerns or resistance to either surveys or MDA there were further discussions with important community, village and township leaders. A few villagers voiced concerns with regard to loss of blood that were alleviated when medical experts explained that the amount of blood being taken was minimal and would not have an effect on villagers. There were also political concerns since the team signed a memorandum of understanding with the central government and since many of these communities have been involved in a longstanding civil conflict with the military. Through meetings between the CE team and villagers, the villagers came to understand that this program was not from a central government organization, but rather an outside organization that works under the auspices of all locally and nationally relevant organizations. While participation varied in some communities, ultimately no communities completely refused to participate in MDA. Some surveys (15) could not be conducted because of a lack of willing participants. In this case, clinical incidence and consultation rates were closely monitored to ensure that population continued to trust and consult the MP in case of fever.

Malaria posts (MPs)
MPs were established in all communities that accepted the responsibility. An original assumption of 800 villages requiring a malaria post was revised to 1200 following reconnaissance efforts. Physical structures were not provided, but each MP had a trained, dedicated and salaried MPW, antimalarials, rapid diagnostic test (RDT) kits, and a few other basic supplies, such as paracetamol, pregnancy tests, scales, a banner to signal MP location, and stationery. MPWs were selected by the village headman and the community, and attended a five-day training that covered malaria case management, referral and reporting systems and CE. Trainings were followed by a course completion test. A manual (in Karen and Burmese) was provided for reference, summarizing procedures, treatment algorithms (Figure 3) and dosing tables (Supplementary File 1).

Fever cases were systematically tested using a *P. falciparum-P. vivax* RDT (SD Bioline P.f/P.v, Standard diagnostics/Alere, Republic of Korea). Uncomplicated *P. falciparum* infections were treated with a fixed dose formulation of artemether - lumefantrine (AL) for three days (5 to 24mg/kg of artemether and 29-144mg/kg of lumefantrine). Pregnant women were treated with quinine clindamycin (quinine 10mg/kg and clindamycin 5mg/kg TID) for seven days in the first trimester and AL in the second and third trimesters of pregnancy. A single low dose of primaquine (0.25 mg/kg) was given to prevent further transmission, except in cases of pregnancy, children younger than six months and lactating mothers. Chloroquine 25mg base/kg over three days was used for the treatment of *P. vivax*. Administered doses were determined by the patient's body weight. An additional blood sample was collected on filter-paper (3×1cm diameter dried blood spots) from *P. falciparum* positive cases in order to monitor antimalarial drug resistance profile.

MP data were sent on a weekly basis, with each MP reporting: all cases of fever by age groups (0-4y, 5-14y and ≥ 15y); all RDT results by age groups (0 – 4, 5 – 14, 15 plus); all malaria treatments by species, gender and age group; the number of severe malaria cases referred, the number of pregnant women with malaria, and the number of deaths attributable to malaria; remaining stocks of artemisinin combination therapies (ACTs) and RDTs (Supplementary File 2).

At the beginning of the project there was little-to-no cell phone coverage in the entire target area, but by 2017 most of the target area (excluding parts of Hpapun and Hlaingbwe Townships) was covered. Data collection in areas with no cell phone coverage relied on runners who collected the forms and transported them (using any convenient transportation means) to the nearest place where they could be entered into an online data system (Voozanoo). In areas where access to a GSM (Global System for Mobile Communications) network was available, MP weekly data reports were entered using a smartphone application specifically developed using Android open source materials (DroidDB and BarcodeScanner, Syware Inc, free runtime developer license 4.1). Used on relatively inexpensive smartphones (80 US$), this application allowed to scan the MP identification code from a barcode sticker thus limiting typing errors, to capture the data in an entry interface matching the reporting form, and to convert it in a standardized format SMS (short messaging service, e.g. text message). In the receiving phone, SMS were extracted as an Excel file (SMS To File Free_1.2_3) and aggregated to the database. After aggregation, MP data completion and correctness was regularly assessed by searching for duplicate reports, missing weekly reports, through double entry of a subset of records and through integrated weekly GIS routines that link records to spatial references.

MP activities were continuously monitored via the weekly data reporting system and by a dedicated monitoring and evaluation team, which was recruited to make investigative visits, using standardized questionnaires (Supplementary File 3), to randomly selected MPs and MPs that ceased to transmit data, or reported no activity or stock outs. Every month, systematic quality control of RDTs from 15 randomly selected MPs was performed at central headquarters in Mae Sod, Thailand (Figure 1). A data evaluation algorithm was established in order to monitor data quality. Missing reports, abnormal data reporting, spikes in clinical malaria cases, or potential problems in stock inventories were checked weekly. After potential data entry errors were excluded, the malaria post supervisors in charge of any problematic MPs were contacted to assess the situation and the required response.

Malaria prevalence surveys
Procedures. Cross sectional surveys were conducted at the village level using an ultrasensitive high-volume qPCR assay in order to estimate the prevalence of *P. falciparum* and *P. vivax* malaria[8]. To ensure that the selected villages were representative of the area, a grid (with 20km wide by 30km long cells) was superimposed on a map of the target area and each cell of the grid was assigned a number of surveys, sufficient to reach roughly 25% survey coverage (from an original village count
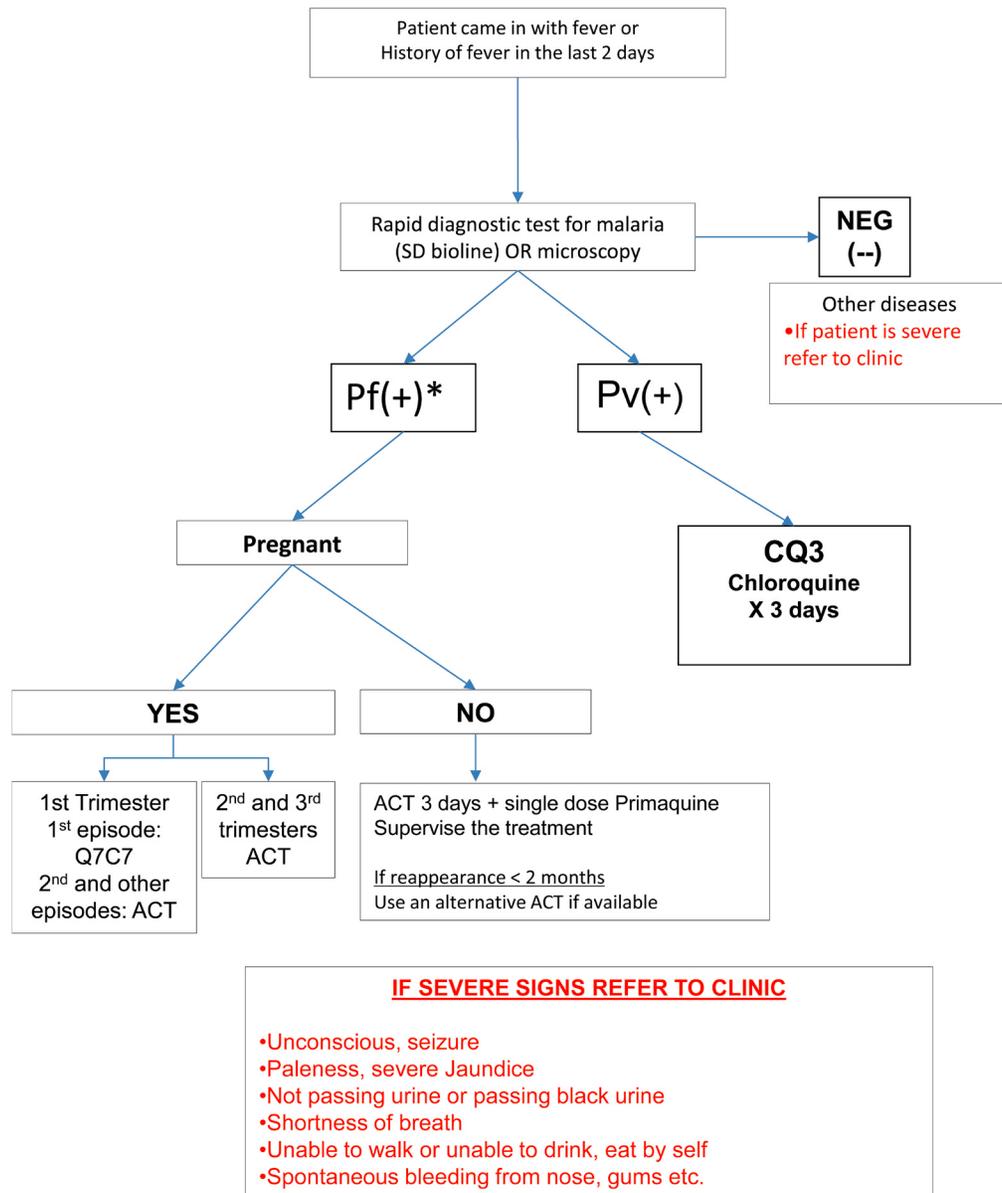
Figure 3. Fever case management algorithm at Malaria Posts. *Pf(+) represents all *Plasmodium falciparum* positive infections, including mixed *P. falciparum* and *P. vivax*. ACT, artemisinin combination therapy.

of 1000). Villages within each grid cell were then randomly selected using sampling functions in STATA v14.1 ("sample") and R v3.4.0 ("sample()"). Surveys were originally planned in 250 randomly selected villages. An additional 60 suspected hotspot villages were included later, based on geographic proximity to a detected hotspot and/or higher-than-expected incidence given the duration of MP activity in the village, leading to oversampling in high prevalence areas. Follow-up cross sectional surveys were conducted > 12 months post-MDA to assess the impact following the same procedures.

Following CE, survey teams approached village headmen to aid in selecting villagers for possible participation in a survey. In most cases no village census was available. Survey teams took samples from adults who agreed to participate, attempting to balance samples across sex and broad age groups, until reaching the sample size needed based on the full village population (Supplementary File 4 and Supplementary File 5). This sample size represented a significant proportion of the village population. Assuming that 50% of inhabitants were older than 18, which was verified in complete census obtained during MDA interventions, the sample

size often comprised between 30 and 50% (baseline) and 50–80% (M12) of adult village population (Supplementary File 5). In follow up surveys (> M12) and in very small villages (e.g. 20 houses) it was necessary to sample multiple people from the same household. Participants were asked to provide 2mL blood by venous puncture after providing written informed consent. Samples were collected in EDTA (Ethylenediamine-tetraacetic acid) tubes, stored in an icebox and brought back to the field laboratory within 48h of collection. A RDT was performed in the field for all participants to the survey, and proposed to the rest of the village population. All RDT-positive malaria cases were provided with adequate treatment by the MP. An additional blood sample was collected on filter-paper (3×1cm diameter dried blood spots) from *P. falciparum* RDT-positive cases in order to monitor antimalarial drug resistance profile.

Malaria detection in the laboratory. Upon arrival in the laboratory an aliquot of whole blood was used for malaria detection by RDT (SD Bioline P.f/P.v) and microscopy (thin and thick smears). The remaining blood was centrifuged and packed red blood cells were used for extraction and detection of malaria parasites by ultrasensitive qPCR[8]. Briefly, 500 µl packed red blood cells were manually extracted using QIAamp DNA blood midi kits (Qiagen) and rehydrated in 25 µl of the provided AE buffer. *Plasmodium* specific monoplex PCR assays were performed using an ABI 7500 fast machine (Applied Biosystems) with five-fold serial dilutions of *P. falciparum* 3D7 standard at dilutions of 50,000 to 16 parasites/ml. The serum, buffy coat and residual packed red blood cells were kept at -80°C.

The detection by qPCR was conducted in two steps: 1) detection of Plasmodium infection using a highly sensitive genus-specific marker; 2) determination of *P. vivax* or *P. falciparum* species in Plasmodium positive samples using less sensitive species-specific markers[17]. The species of some Plasmodium positive samples could therefore remain uncharacterized. In these instances microscopy and RDT results were used to attribute the species. If both RDT and microscopy were negative, malaria was attributed to either *P. falciparum* or *P. vivax* according to the relative proportion of each species already detected in samples with complete qPCR results.

Sample and data management. Individual samples were identified using a barcode label that was used to trace field data and results obtained for the different malaria detection tests performed in the laboratory (RDT, microscopy, qPCR). All paper-recorded survey data (participant demographic information, field RDT result, laboratory RDT and microscopy examination results) were entered in a Microsoft Access (Access 2010 version 14.0) database. Result outputs from qPCR analysis were merged directly in the Access database. Double entry of survey results was performed for 39/272 baseline surveys (14%) and ten percent of the remaining results were checked to confirm minimal error rates.

Sample size. The within-village sample size was calculated, taking into account feasibility constraints (small village sizes, sample conservation and cold chain) and the expected precision of estimates. For baseline surveys (month zero, M0), the targeted sample size was calculated to measure a 40% malaria prevalence with a +/-10% precision of the 90% CI (Supplementary File 4). For M12 (month 12) surveys, the targeted sample size was calculated to measure a 90%-decrease from baseline *P. falciparum* prevalence (Supplementary File 5). If the expected M12 prevalence was ≤2%, the sample size was calculated to achieve a 95% CI-width of +/-100% of expected value. If the expected M12 prevalence was >2%, the sample size was calculated to achieve a 95% CI-width of +/-50% of expected value (e.g. for a village with baseline prevalence 30%, M12 survey aimed at a sample size sufficient to measure a 3% prevalence with a 95% CI interval of +/-1.5%).

Hotspot definition. A village was operationally classified as a malaria "hotspot" when the 90% CI upper limit of the sum of *P. falciparum* and *P. vivax* prevalence estimate was ≥40% and the corresponding value of the proportion of *P. falciparum* in the positive samples was ≥20%. Such villages were targeted for mass drug administration.

Targeted mass drug administration (MDA)

Drug regimen and exclusion criteria. The ACT regimen used in MDA consisted of dihydroartemisinin (7mg/kg) plus piperaquine (55 mg/kg) (DP) with a single low dose of primaquine (0.25mg/kg). Women in their first trimester of pregnancy, children under one year of age, individuals with previous drug allergies and villagers who refused to participate were excluded from MDA. Women within reproductive age (roughly 14 – 44 years old) and of unknown pregnancy status (self-reported being unsure of pregnancy status) were screened with a urinary human chorionic gonadotropin (HCG) test kit. Women in their second and third trimester of a pregnancy, as well as breastfeeding mothers, were eligible for DP treatment but were excluded from the single dose of primaquine.

Procedures. After obtaining written informed consent, each eligible participant's medical history was briefly reviewed and a clinical examination was conducted. Those who met the inclusion criteria were administered a three-day course of DP with a single low dose of primaquine on the first day and this was repeated over three consecutive months (Supplementary File 3). All doses were directly observed. Participants who were unable to attend to the next take(s) were provided the remaining doses to complete unsupervised treatment. The MDA team stayed in each MDA village for seven days per visit to document side effects, to address concerns and to treat other minor illnesses. All adverse events (AE) that were reported by MDA participants within one week of taking an MDA course were carefully recorded and treated when necessary at a mobile clinic set up during the MDA period or referred to the nearest appropriate health care facility. All drug administration data (date, weight, dosage) were recorded in a logbook as well as reasons for non-participation collected using a standardized questionnaire (Supplementary File 6). All MDA data were checked onsite by the field teams and reviewed after the end of the 3 months. Logbooks and AE sheets were entered in an Access database (Access 2010 version 14.0).

MDA participation was calculated as the total number of individuals completing one, two or three 3-day treatment courses over the 3 months of MDA intervention, divided by the total number of individuals recorded as present in the village at least on one occasion during the three months of activity, excluding visitors staying <2 weeks. MDA efficacy was assessed by prevalence surveys conducted ≥ 12 months after the start of MDA and by monitoring the incidence of clinical episodes at the MP.

Antimalarial resistance monitoring

Antimalarial resistance monitoring was conducted at two partner laboratories: one in the Faculty of Tropical Medicine at Mahidol University and the other at the Sanger Institute, both using dried blood spots from *P. falciparum* positive cases at MPs.

Assessment of mutations in PfKelch13 (associated with artemisinin resistance). Polymorphisms in the *PfKelch* gene were assessed by nested PCR amplification covering the full length of the gene (total 2181 bp)[18], followed by DNA sequencing using an ABI sequencing platform (Macrogen Inc, South Korea). Cross contamination was monitored by adding negative control samples in every run. Sequencing results were aligned against *PfKelch13* of reference strain 3D7 (putative 9PF13_0238 NCBI Reference Sequence (3D7): XM_001350122.1), using Bioedit software (Abbott, CA, USA). Polymorphic patterns were assessed by two individuals blinded to the origin of the sample.

Markers of ACT partner drug resistance. *PfPlasmepsin2* and *Pfmdr1* copy numbers were quantified using Taqman real time PCR on a Corbett Rotor-Geneä Q (Corbett Research, Australia), following previous reports[18,19]. Amplification was performed in triplicate on a total volume of 10 μL as multiplex PCR using Quantitec Multiplex PCR no ROX (QIAgen, Germany). Every amplification run contained 9 replicates of calibrators and triplicates without template as negative controls. β-tubulin served as an internal standard for the amount of sample DNA added to the reactions. Copy numbers were calculated using the formula: copy number= 2^{-DDCt} ; with $DD\ C_t$ denoting the difference between $D\ C_t$ of the unknown sample and $D\ C_t$ of the reference sample.

Tests for piperaquine resistance markers were also carried out on *P. falciparum* DNA sequence data from 216 clinical samples collected in the same region between 2013 and 2015, included in the MalariaGEN *P. falciparum* Community Project. DNA was extracted directly from blood samples taken from patients at admission time, after leukocyte depletion by CF11 filtration to minimize human DNA. Selected samples, having >50 ng DNA and <80% human DNA contamination, were sequenced on the Illumina HiSeq platform following the manufacturer's standard protocols[19]. Paired-end sequencing reads of length 200–300 bp were obtained, generating approximately 1 Gbp of read data per sample. Polymorphism discovery, quality control and sample genotyping followed a process described in detail elsewhere[20]. Three tests for piperaquine resistance markers were performed, and samples were considered sensitive if all three tests yielded negative results: 1) Position 2,504,560 on chromosome 10 was genotyped to assess the presence of the exo-E415G mutation[21];

2) Sequencing reads were searched for the breakpoint sequence (ATGATTACGATAATCACACTGTTGGTTTCGCCCTT) that characterizes plasmepsin 2-3 amplifications associated with piperaquine resistance in Cambodia[21]; and 3) Copy number was assigned to plasmepsin 2-3 from a genome-wide analysis of sequencing read coverage, using a procedure based on a Gaussian hidden Markov model (HMM), described in detail elsewhere[22,23].

The same sequencing data were used to estimate copy number for the *pfmdr1* gene, using a method previously described in detail[24]. Briefly, the sequencing read coverage was normalized for each sample, and the *pfmdr1* copy number was estimated by calculating the ratio between the coverage at a number of positions within that gene, and the median coverage of a set of 56 reference positions at various loci across the genome. To improve estimates, the reference positions were chosen in genes with similar characteristics to *pfmdr1*: similar GC content, level of evolutionary conservation, exon length, median coverage, and low variation in relative coverage across the MalariaGEN dataset. Each sample's reference coverage was estimated as the median of coverage at the 56 positions, while seven positions in *pfmdr1* were analogously used to determine *pfmdr1* coverage, and hence the copy number estimation.

Statistical analyses

Incidence rates of clinical *P. falciparum* or *P. vivax* malaria episodes (cases per 1,000 population per unit of time) and 95% Poisson confidence intervals were calculated using weekly MP data reports. Weekly incidence was calculated for each village and also aggregated over space and time (e.g. by month or year and by village tract or by township).

Total malaria, *P. falciparum*, and *P. vivax* prevalence and 95% binomial Wilson confidence intervals (corrected for finite population size) were calculated using the results of surveys analyzed by high volume ultrasensitive qPCR.

Statistical clustering of both incidence and prevalence was assessed using a range of geostatistical and cartographic approaches. Analytic approaches for analyzing spatial autocorrelation of population standardized incidence and prevalence data included the global Moran's I statistic, local indicators of spatial autocorrelation, Kulldorff's scan statistic[25], variography and spatial correlograms. It was necessary to carefully interpret results and to look for congruity between approaches because of the skewed nature of the data (with many villages having low prevalence and few having high prevalence). Choropleth maps were created for incidence at the village tract level and point maps were created and used for operational needs (e.g. to design stocking or survey strategies and to assess potential gaps in health care coverage). Statistical analyses were done using STATA v14.1 (STATA Corp), R v3.4.0 (The R Foundation) and the Spatial Analysis in Macroecology software package (v4.0).

The evaluation of the impact of the program was conducted by monitoring the temporal trends in incidence at township, village tract and village level and by measuring the proportion of villages

and village tracts achieving and sustaining low *P. falciparum* case incidence. The specific impact of MDA on malaria prevalence in hotspot villages was measured by comparing the baseline prevalences to follow-up survey prevalences 12 months after MDA.

Discussion

This project illustrates the scale up of an elimination program in a region encompassing remote and rugged terrain, a complex political landscape, ongoing areas of active conflict, and a near-absent pre-existing health care infrastructure. The key interventions of the project included the establishment of a dense network of community level early malaria diagnosis and treatment clinics (MPs) and targeted MDA.

There are several limitations to this protocol. This program is organized at the village level, which is an operationally relevant unit for programming and implementing interventions, but may overlook malaria dynamics at higher scales (e.g. hotspots encompassing more than a single village). This issue was addressed in part by surveying neighbouring villages of hotspots in order to define their actual size. The definition of hotspots was based on assumptions from previous surveys and studies in the region. It is probably useful to revisit and refine it in the light of the results obtained at large scale by this program. Geostatistical approaches such as the use of Kulldorff's scan statistic[25] may be useful for defining hotspots of infections and drug resistant strains.

Other limitations were related to operating a large program in remote and politically complicated areas. Monitoring the impact of the project relied mostly on observational data from the MPs, with the exception of specific MDA activity, for which additional surveys were conducted. More detailed studies were not possible because of the magnitude of the intervention area, as well as occasional barriers to accessing field sites because of active conflict, natural disasters or political sensitivities.

Another major operational constraint was that interventions (MP openings; baseline surveys; MDA) could not be simultaneously conducted in all locations across the target area. As a result, for example, baseline surveys could not be conducted at the same season or after the same duration of MP opening in all villages. Likewise, MDA was performed before one of the two main transmission seasons (rainy season or cold season), but not necessarily the same season. There was no specific follow-up of control villages, since the operational goal was elimination and given potential ethical implications of not treating hotspot communities. The step-wedge nature of the deployment will, however, allow some degree of comparison of incidence between villages with MDA occurring sooner or later after MP opening.

The prevalence surveys, which relied on an ultrasensitive qPCR approach, posed a significant challenge or constraint for this program. Blood samples needed to be quickly processed at a laboratory meaning that particularly remote areas with difficult access were difficult or impossible to survey. The ongoing

development of a new generation of high sensitivity RDTs could dramatically simplify the measurement of *P. falciparum* prevalence[26]. Likewise, incidence-based or risk-based targeting of prevalence surveys could also narrow down surveys to suspected hotspots. In a programmatic setting, these would allow for more efficient, faster, and decentralized decision-making with regard to interventions other than MPs.

The project relied on an adaptive strategy, focussing on the rapid establishment of a geographically referenced health care infrastructure (the MP network), drawing from on-the-ground knowledge of the area (CE and geography), utilising existing systems when possible (community-based partners) and quickly filling gaps where necessary. The project has now entered its fourth year and results from the first three years will be published soon. It was important to find a balance between approaches that were scalable but also took into account the local contexts and complexities of the target area. The strategies employed here and lessons learned through this project can be applied to eliminate malaria in other settings and for other infectious disease elimination programs.

Ethical statement

All individuals participating in blood surveys and MDA provided written informed consent. Consent forms were translated into S'kaw Karen, the most commonly spoken native language in the target area. The forms were back-translated by native speakers at SMRU and corrected when necessary prior to use in the field. All CE team members are fluent in the language and were able to explain the forms and the project in S'kaw Karen to villagers.

This project was approved through the Ethics Review Committee on Medical Research Involving Human Subjects from the Republic of the Union of Myanmar, Ministry of Health and Sports, Department of Medical Research (Lower Myanmar): 73/Ethics 2014.

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Supplementary material

Supplementary File 1: Malaria post manual (in English).

Click here to access the data.

Supplementary File 2: Weekly Malaria Post data report form.

Click here to access the data.

Supplementary File 3: Malaria post monitoring and evaluation questionnaire.

Click here to access the data.

Supplementary File 4: Table for baseline qPCR survey sample size calculation.

Click here to access the data.

Supplementary File 5: Table for M12 qPCR survey sample size calculation.

Click here to access the data.

Supplementary File 6. Mass Drug Administration data collection logbook (reduced from A3 format) is shown in *part A*. Having all 3 months of follow-up on the same sheet was important for the teams to be able to follow participation accurately. This included drug uptake and gathering standardized data on non-participation, detailed in *part B*.

Click here to access the data.

References

1. Oo TT: **Biology and vector competence of the anopheline mosquitoes of myanmar with special consideration of *anopheles dirus*.** Ruperto-Carola University of Heidelberg, Germany; 2003.
Publisher Full Text

2. Sinka ME, Bangs MJ, Manguin S, *et al.*: **The dominant *Anopheles* vectors of human malaria in the Asia-Pacific region: occurrence data, distribution maps and bionomic précis.** *Parasit Vectors.* BioMed Central Ltd; 2011; **4**: 89, [cited 2013 Dec 12].
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

3. Manguin S, editor: **Anopheles mosquitoes - new insights into malaria vectors.** Rijeka, Croatia: InTech; 2013.
Publisher Full Text

4. Phyo AP, Nkhoma S, Stepniewska K, *et al.*: **Emergence of artemisinin-resistant malaria on the western border of Thailand: a longitudinal study.** *Lancet.* 2012; **379**(9830): 1960–6.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

5. Imwong M, Suwannasin K, Kunasol C, *et al.*: **The spread of artemisinin-resistant *Plasmodium falciparum* in the Greater Mekong subregion: a molecular epidemiology observational study.** *Lancet Infect Dis.* 2017; **17**(5): 491–497.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

6. Imwong M, Nguyen TN, Tripura R, *et al.*: **The epidemiology of subclinical malaria infections in South-East Asia: findings from cross-sectional surveys in Thailand-Myanmar border areas, Cambodia, and Vietnam.** *Malar J.* BioMed Central; 2015; **14**: 381.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

7. Lwin KM, Imwong M, Suangkanarat P, *et al.*: **Elimination of *Plasmodium falciparum* in an area of multi-drug resistance.** *Malar J.* BioMed Central; 2015; **14**: 319.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

8. Imwong M, Hanchana S, Malleret B, *et al.*: **High-throughput ultrasensitive molecular techniques for quantifying low-density malaria parasitemias.** *J Clin Microbiol.* 2014; **52**(9): 3303–9.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

9. Kajeechiwa L, Thwin MM, Nosten S, *et al.*: **Community engagement for the rapid elimination of malaria: the case of Kayin State, Myanmar [version 1; referees: 2 approved].** *Wellcome Open Res.* 2017; **2**: 59.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

10. Kajeechiwa L, Thwin MM, Shee PW, *et al.*: **The acceptability of mass administrations of anti-malarial drugs as part of targeted malaria elimination in villages along the Thai-Myanmar border.** *Malar J.* BioMed Central; 2016; **15**(1): 494.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

11. Ya-Umphan P, Cerqueira D, Parker DM, *et al.*: **Use of an Anopheles Salivary Biomarker to Assess Malaria Transmission Risk Along the Thailand-Myanmar Border.** *J Infect Dis.* 2017; **215**(3): 396–404.
PubMed Abstract | **Publisher Full Text**

12. Landier J, Kajeechiwa L, Thwin MM, *et al.*: **Safety and effectiveness of mass drug administration to accelerate elimination of artemisinin-resistant falciparum malaria: A pilot trial in four villages of Eastern Myanmar [version 1; referees: 1 approved].** *Wellcome Open Res.* 2017; **2**: 81.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

13. Moonen B, Cohen JM, Snow RW, *et al.*: **Operational strategies to achieve and maintain malaria elimination.** *Lancet.* 2010; **376**(9752): 1592–603.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

14. Zhou SS, Rietveld AE, Velarde-Rodriguez M, *et al.*: **Operational research on malaria control and elimination: a review of projects published between 2008 and 2013.** *Malar J.* 2014; **13**: 473.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

15. Who GMP: **Planning meeting for operational research on malaria elimination.** 2013.
Reference Source

16. South A: **Burma's Longest War: anatomy of the Karen conflict.** Institute. Burma Cent. Netherlands. Amsterdam. 2011.
Reference Source

17. Perandin F, Manca N, Calderaro A, *et al.*: **Development of a real-time PCR assay for detection of *Plasmodium falciparum*, *Plasmodium vivax*, and *Plasmodium ovale* for routine clinical diagnosis.** *J Clin Microbiol.* 2004; **42**(3): 1214–9.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

18. Ashley EA, Dhorda M, Fairhurst RM, *et al.*: **Spread of artemisinin resistance in *Plasmodium falciparum* malaria.** *N Engl J Med.* 2014; **371**(5): 411–23, [cited 2014 Jul 31].
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

19. Bentley DR, Balasubramanian S, Swerdlow HP, *et al.*: **Accurate whole human genome sequencing using reversible terminator chemistry.** *Nature.* 2008; **456**(7218): 53–9.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

20. Manske M, Miotto O, Campino S, *et al.*: **Analysis of *Plasmodium falciparum* diversity in natural infections by deep sequencing.** *Nature.* 2012; **487**(7407): 375–9.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

21. Amato R, Lim P, Miotto O, *et al.*: **Genetic markers associated with dihydroartemisinin-piperaquine failure in *Plasmodium falciparum* malaria in Cambodia: a genotype-phenotype association study.** *Lancet Infect Dis.* 2017; **17**(2): 164–173.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

22. Miles A, Iqbal Z, Vauterin P, *et al.*: **Indels, structural variation, and recombination drive genomic diversity in *Plasmodium falciparum*.** *Genome Res.* 2016; **26**(9): 1288–99.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

23. Pearson RD, Amato R, Auburn S, *et al.*: **Genomic analysis of local variation and recent evolution in *Plasmodium vivax*.** *Nat Genet.* 2016; **48**(8): 959–64.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

24. Srimuang K, Miotto O, Lim P, *et al.*: **Analysis of anti-malarial resistance markers in *pfmdr1* and *pfcrt* across Southeast Asia in the Tracking Resistance to Artemisinin Collaboration.** *Malar J.* BioMed Central; 2016; **15**(1): 541.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

25. Kulldorff M: **A spatial scan statistic.** *Communications in Statistics: Theory and Methods.* 1997; **26**(6): 1481–1496.
Publisher Full Text

26. Das S, Jang IK, Barney B, *et al.*: **Performance of a High-Sensitivity Rapid Diagnostic Test for *Plasmodium falciparum* Malaria in Asymptomatic Individuals from Uganda and Myanmar and Naive Human Challenge Infections.** *Am J Trop Med Hyg.* 2017; **97**(5): 1540–1550.
PubMed Abstract | **Publisher Full Text**

Open Peer Review

Current Referee Status: ✔ ✔

Version 1

Referee Report 22 November 2017

✔ **Christopher V. Plowe**

Division of Malaria Research, Institute for Global Health, University of Maryland School of Medicine, Baltimore, MD, USA

This is a well written and thorough description of a hybrid research/programmatic activity aiming to evaluate and scale up surveillance and mass drug administration to eliminate malaria from a large area of Myanmar's Karen State encompassing hundreds of villages. The approaches and procedures are well described, with the exception that the actual sample sizes for prevalence surveys are not provided along with the description of the procedure for determining sample size. The supplemental materials may be helpful to other groups undertaking malaria elimination interventions in other areas not covered by strong health systems.

Is the rationale for, and objectives of, the study clearly described?
Yes

Is the study design appropriate for the research question?
Yes

Are sufficient details of the methods provided to allow replication by others?
Yes

Are the datasets clearly presented in a useable and accessible format?
Yes

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

> Author Response 18 Dec 2017
>
> **Daniel Parker**, University of California, Irvine, USA
>
> We thank the reviewer for their comments and suggestions.
>
> The reviewer is correct that this information is missing and that it could be valuable for readers. We have now included the tables (Supplementary File 4; Supplementary File 5) that were constructed

to estimate survey sample sizes necessary for a given village size, based on power analyses and operational constraints (these surveys were operationally challenging because of the need for a cold chain and transporting the samples to the laboratory within 24 hours). The sample sizes for baseline surveys were smaller than follow up survey sample sizes. Follow up surveys that were done > 12 months after MDA had larger sample sizes in order to have greater confidence (and smaller confidence intervals) post-MDA.

Our goal in writing this protocol was to describe in detail (with no word limits) how the operational components of the project were set up. A subsequent manuscript describing the results of the project, including operational results, will soon follow. We would prefer to not include any specific operational or research results at this time.

Competing Interests: No competing interests were disclosed.

Referee Report 23 October 2017

doi:10.21956/wellcomeopenres.13804.r26885

Philip Bejon

Wellcome Trust Research Programme, Kenyan Medical Research Institute (KEMRI), Kilifi, Kenya

Drug-resistant falciparum parasites are a major global emergency, hence elimination in the Myanmar/Thai border as well as the Cambodia/Thai border is highly desirable, and the project described here is a logical and important progression of the small-scale pilots carried out earlier. Making this protocol openly available is a useful step, and the protocol is well described. I have a number of minor comments and questions for clarification.

The abstract discussion is written in terms of a project that has illustrated an outcome and that relied on strong rapport. The results or evaluation of the project aren't presented here, so perhaps this should be rephrased in terms of intention to demonstrate or likely outcomes in future tense rather than past tense.

The introduction could provide useful additional context if it mentioned what is being done on the Thai/Cambodia border, and also the recent findings of spread of resistant parasite clones in Vietnam.

The methods mention GPS and GLONASS. Why were both used? And what was done when there were discrepant readings?

Under Community Engagement "MPs" is first used without spelling out (defined next paragraph as Malaria Posts).

What was done when the initial CE team contact suggested concerns or frank resistance? Were there pre-prepared FAQ sheets and other sensitization materials ready? Were any materials translated/backtranslated?

Were there processes established for monitoring completeness of data collection in real time and sending queries for out-of-range values?

Malaria Prevalence Surveys: What was the software and algorithm used for the randomization process?

How was randomization applied to villagers? Were they all enumerated first and then randomly selected to receive a return visit or was the process more "on the ground". Were whole households sampled (convenient) or were villagers sampled across households?

For the PCR how were the standards produced and quality controlled? (Presumably from known high parasitaemia cultures diluted through uninfected blood, but this isn't stated).

Antimalarial resistance monitoring was done at MPs but not in community surveys. Is it possible a reservoir of resistance among low density parasitaemias in the community would be missed like this?

Under statistical analysis could consider using SATScan, and perhaps looking at prevalence of resistance for spatial clustering as well as prevalence of infection? For positive vs negative results it may be appropriate to look at Ripley's K function for cases/controls rather than Moran's I which is for continuous data, since the binned prevalences won't consider sample size in each location, and the prevalences are unlikely to be normally distributed.

Data: The shape files and locations of villages could be considered study data, and would be useful to those who subsequently work in the field. However the data may well be too sensitive to post open access, in which case a note should be made regarding a data governance system via which applications for data can be made.

Discussion: The second paragraph limitations would be readily addressed by using SATScan, which would take the arbitrary definition away from the protocol and address neighbouring villages for hotspots.

The evaluation of effectiveness of this protocol when put into practice on the ground, and the epidemiological data generated will be very interesting and important. This is a really major and very important undertaking.

Is the rationale for, and objectives of, the study clearly described?
Yes

Is the study design appropriate for the research question?
Yes

Are sufficient details of the methods provided to allow replication by others?
Yes

Are the datasets clearly presented in a useable and accessible format?
Yes

Competing Interests: No competing interests were disclosed.

Referee Expertise: Clinical Epidemiology

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 18 Dec 2017

Daniel Parker, University of California, Irvine, USA

Drug-resistant falciparum parasites are a major global emergency, hence elimination in the Myanmar/Thai border as well as the Cambodia/Thai border is highly desirable, and the project described here is a logical and important progression of the small-scale pilots carried out earlier. Making this protocol openly available is a useful step, and the protocol is well described. I have a number of minor comments and questions for clarification.

The abstract discussion is written in terms of a project that has illustrated an outcome and that relied on strong rapport. The results or evaluation of the project aren't presented here, so perhaps this should be rephrased in terms of intention to demonstrate or likely outcomes in future tense rather than past tense.

Agreed. Our primary goal in this protocol paper was to describe the establishment of the project, which is still ongoing. We have therefore changed the wording in the abstract to indicate that the establishment is what was done in the past but that the project is ongoing.

The introduction could provide useful additional context if it mentioned what is being done on the Thai/Cambodia border, and also the recent findings of spread of resistant parasite clones in Vietnam.

Agreed. We have now added text mentioning that there are targeted MDA studies being done in Cambodia, Laos and Vietnam. We have also added a statement and reference about the spread of resistant clones throughout the Greater Mekong Subregion.

The methods mention GPS and GLONASS. Why were both used? And what was done when there were discrepant readings?

The satellite-enable geo-referencing devices used for this project are both GPS and GLONASS capable. We enabled GLONASS capability solely to increase the accuracy of our readings (at the expense of the loss of a bit of battery power). From the reviewers comment we see that our wording made it appear that we were using multiple devices simultaneously when in reality we were using one device with both GLONASS and GPS capabilities. We have now changed the wording in an attempt to make this clear:

"In order to understand the settlement demography and geography of the region, the area was systematically mapped using field teams and satellite-enabled geo-referencing devices. The devices simultaneously used GPS (global position system) and GLONAS (Globalnaya navigatsionnaya sputnikovaya sistema) satellites to increase accuracy of geographical coordinates (latitude and longitude)."

Under Community Engagement "MPs" is first used without spelling out (defined next paragraph as Malaria Posts).

Thank you, we have now corrected this.

What was done when the initial CE team contact suggested concerns or frank resistance?

We have now added the following statement to the CE section of the manuscript:
"In situations where villagers presented concerns or resistance to either surveys or MDA there were further discussions with important community, village and township leaders. A few villagers voiced concerns with regard to loss of blood that were alleviated when medical experts explained that the amount of blood being taken was minimal and would not have an effect on villagers. There were also political concerns since the project signed a memorandum of understanding with the central government and since many of these communities have been involved in a longstanding civil conflict with the military. Through meetings between the CE team and villagers, the villagers came to understand that this program was not from a central government organization, but rather an outside organization that works under the auspices of all locally and nationally relevant organizations. While participation varied in some communities, ultimately no communities completely refused to participate in MDA. Some surveys (15) could not be conducted because of a lack of willing participants. In this case, clinical incidence and consultation rates were closely monitored to ensure that population continued to trust and consult the MP in case of fever."

Were there pre-prepared FAQ sheets and other sensitization materials ready?

Yes. We have now added the following statement to the manuscript:

"The CE team created and distributed community engagement materials in order to sensitize and explain the project to villagers. Materials included posters and audio announcements (statements that were created by the CE team but given to village headmen to announce in periodic village announcements). METF posters, in S'kaw Karen and Burmese languages, that encourage people to visit malaria posts when they feel sick were placed in malaria posts throughout the target area."

Were any materials translated/backtranslated?

Yes. We have now added the following statement to the ethics statement in the manuscript:

"The project used written informed consent, translated into S'kaw Karen – the most commonly spoken native language in the target area. The forms were back-translated by native speakers at SMRU and corrected when necessary prior to use in the field. All CE team members are fluent in the language and were able to explain the forms and the project in S'kaw Karen to villagers."

Were there processes established for monitoring completeness of data collection in real time and sending queries for out-of-range values?

Yes. We have now added the following statement to the manuscript:

"A data evaluation algorithm was established in order to monitor data quality. Missing reports, abnormal data reporting, spikes in clinical malaria cases, or potential problems in stock inventories were checked weekly. After potential data entry errors were excluded, the malaria post supervisors in charge of any problematic MPs were contacted to assess

the situation and the required response.”
Malaria Prevalence Surveys: What was the software and algorithm used for the randomization process?

We have now added the following statement to the manuscript:

“Villages within each grid cell were then randomly selected using sampling functions in STATA v14.1 (“sample”) and R v3.4.0 (“sample()”).”

How was randomization applied to villagers? Were they all enumerated first and then randomly selected to receive a return visit or was the process more “on the ground”. Were whole households sampled (convenient) or were villagers sampled across households?

We now attempt to better explain this process. Participant selection was done in the field, frequently in villages with no villager list. Every attempt was made to select a representative sample but in some small villages it was necessary to select multiple people from the same houses. We have expanded this comment as follows:

“Following CE, survey teams approached village headmen to aid in selecting villagers for possible participation in a survey. In most cases no village census was available. Survey teams took samples from adults who agreed to participate, attempting to balance samples across sex and broad age groups, until reaching the sample size needed based on the full village population (Supplementary File 4 and Supplementary File 5). This sample size represented a significant proportion of the village population. Assuming that 50% of inhabitants were older than 18, which was verified in complete census obtained during MDA interventions, the sample size often comprised between 30 and 50% (baseline) and 50-80% (M12) of adult village population (Supplementary File 5). In follow up surveys (> M12) and in very small villages (e.g. 20 houses) it was necessary to sample multiple people from the same household.”

For the PCR how were the standards produced and quality controlled? (Presumably from known high parasitaemia cultures diluted through uninfected blood, but this isn't stated).

The qPCR followed the methods described by Imwong et al (Journal of Clinical Microbiology, 2014) including the preparation of control standards using *P. falciparum* 3D7 ring-stage synchronized cultured parasites, in which the precise number of infected red blood cells per tube was obtained using fluorescence-activated cell sorting (FACS), following the protocol described by Malleret et al. (Sci Rep, 2011)

Antimalarial resistance monitoring was done at MPs but not in community surveys. Is it possible a reservoir of resistance among low density parasitaemias in the community would be missed like this?

During surveys, participants providing a venous blood sample and other village inhabitants who accepted it received a standard RDT. A significant proportion also presented symptoms and would have consulted at the MP in the coming days. DBS were collected from *P. falciparum* positive cases, and treatment was administered. This represented ~30% of all carriers detected by qPCR in the surveys, but a small fraction of

the total number of clinical cases recorded across the target area.
The analysis is ongoing, but preliminary results suggest that strains infecting clinical cases and asymptomatic carriers do not differ significantly in terms of resistance markers.

Under statistical analysis could consider using SATScan, and perhaps looking at prevalence of resistance for spatial clustering as well as prevalence of infection?

We have now added a statement about the use of scan statistics (SatScan) for spatial analysis of prevalence, incidence, and prevalence of resistance markers.

For positive vs negative results it may be appropriate to look at Ripley's K function for cases/controls rather than Moran's I which is for continuous data, since the binned prevalences won't consider sample size in each location, and the prevalences are unlikely to be normally distributed.

The reviewer brings up a good point. The original version of this manuscript was vague in explaining the spatial analyses that were used. In reality, we incorporated many more spatial analytic tools than Moran's I or LISA statistics when exploring the data (including variography; a range of global, local and focal clustering statistics; and smoothed heatmaps).

Our primary goal in looking for clustering was to explore patterns in village level prevalence. From both an operational and research perspective, we wanted to know if villages with similar prevalence (as estimated from our qPCR surveys) clustered in space. We would not use Moran's I for unstandardized count data or for absence/presence (case/control) data. The prevalence estimates accounted for village size in their design (from S Table XX) and we looked for spatial autocorrelation in the resulting continuous variable.

It is true that these data are not normally distributed – they are right skewed, with many villages having very low prevalence and a few (hotspots) having high prevalence. Instead of relying on p-values and test statistics, we repeated the Moran's I calculation at multiple distance bands and examined their behavior as we compared village prevalences from villages that were farther and farther away. We also used pairwise relative semivariograms (which are known to handle skewed data well) and found the same general pattern that was exhibited by the Moran's I correlograms we constructed (with villages that are closest being most alike).

We have now amended the statement in the text as follows:
"Statistical clustering of both incidence and prevalence was assessed using a range of geostatistical and cartographic approaches. Analytic approaches for analyzing spatial autocorrelation of population standardized incidence and prevalence data included the global Moran's *I* statistic, local indicators of spatial autocorrelation, Kuldorf's scan statistic, variography and spatial correlograms. It was necessary to carefully interpret results and to look for congruity between approaches because of the skewed nature of the data (with many villages having low prevalence and few having high prevalence). Choropleth maps were created for incidence at the village tract level and point maps were

created and used for operational needs (e.g. to design stocking or survey strategies and to assess potential gaps in health care coverage)."

Data: The shape files and locations of villages could be considered study data, and would be useful to those who subsequently work in the field. However the data may well be too sensitive to post open access, in which case a note should be made regarding a data governance system via which applications for data can be made.

The shape files and point data for villages within the region are sensitive and ultimately belong to the several different organizations and institutions who comprise the METF. Village tract shapefiles are freely available from the Myanmar Information Mapping Unit (MIMU, http://themimu.info/gis-resources). The village tract level data are shared with the Myanmar National Malaria Program

Discussion: The second paragraph limitations would be readily addressed by using SATScan, which would take the arbitrary definition away from the protocol and address neighbouring villages for hotspots.

We agree that there are several other ways (including SatScan) to define hotspots. Our goal here was simply to describe what has been done. Further work will assess the size and predictability of hotspots using a range of tools and approaches. We have now added the following statement to the limitations section:

"Geostatistical approaches such as the use of Kulldorff's scan statistic may be useful for defining hotspots of infections and drug resistant strains."

The evaluation of effectiveness of this protocol when put into practice on the ground, and the epidemiological data generated will be very interesting and important. This is a really major and very important undertaking.

Thank you for your very helpful comments, critiques and suggestions.

Competing Interests: No competing interests were disclosed.