Author's response to reviews

Title: Determining the efficacy of guppies and pyriproxyfen (Sumilarv® 2MR) combined with community engagement on dengue vectors in Cambodia: study protocol for a randomized controlled trial

Authors:

John Hustedt (john.hustedt@lshtm.ac.uk)
Dyna Doum (d.doum@malariaconsortium.org)
Vanney Keo (v.keo@malariaconsortium.org)
Sokha Ly (lysokha@yahoo.com)
Bunleng Sam (sambunleng@gmail.com)
Vibol Chan (chanv@who.int)
Neal Alexander (neal.alexander@lshtm.ac.uk)
John Bradley (john.bradley@lshtm.ac.uk)
Didot Prasetyo (didot.prasetyo.ctr@namru2.org.kh)
Agus Rachmat (agus.rachmat.ctr@namru2.org.kh)
Muhammad Shafique (m.shafique@malariaconsortium.org)
Sergio Lopes (s.lopes@malariaconsortium.org)
Rithea Leang (rithealeang@gmail.com)
Jeffrey Hii (j.hii@malariaconsortium.org)

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Author’s response to reviews:

Dear Editors,

Please see the revised manuscript based on your comments, and those of the reviewer, attached here. I have tried to respond to all these comments in the section below. Please let me know if you have any additional edits or comments. Look forward to hearing from you soon.
Reviewer reports:

Editor:

1. Please fully define all your outcomes following the framework described in Zarin NEJM 2011;364:852-60. Your outcome definition should include these 5 elements: the domain (name of the outcome), specific measurement, metric, method of aggregation, and time point.

JH: We have tried to include all 5 elements for each outcome. For example, in secondary outcome number 1 the domain is Minimum Infection Rate; the specific measurement is rate of dengue virus in adult female Aedes mosquitoes; the metric is the number of infected mosquitoes per 1,000 specimens tested; the method of aggregation is at the cluster level; and the time point is at the end of the study. The definitions were revised on page 14 line 1 as below.

Primary Outcome Measures

Population density of adult female Aedes: The outcome will be measured as the number of mosquitoes per unit of time spent aspirating by adult resting catches at 0,4,8, and 12 months post-intervention and will be aggregated at the cluster level.

Secondary Outcome Measures

Survey Indicators:

1. Minimum Infection Rate for dengue virus in adult female Aedes mosquitoes: The outcome is measured as the number of infected mosquitoes per 1,000 specimens tested at the end of the study and will be aggregated at the cluster level.

2. House index (HI): The outcome is measured as the proportion of houses surveyed positive for Aedes larvae and/or pupae in any water container during entomology surveys at 0,4,8, and 12 months post-intervention aggregated at the cluster level.

3. Container index (CI): The outcome is measured as the proportion of surveyed containers containing Aedes larvae and/or pupae during entomology surveys at 0,4,8, and 12 months post-intervention aggregated at the cluster level.
4. Breteau index (BI): The outcome is measured as the number of containers positive for Aedes larvae and/or pupae per 100 houses during entomology surveys at 0, 4, 8, and 12 months post-intervention aggregated at the cluster level.

5. Pupae Per House (PPH): The outcome is measured as the number of Aedes pupae per household during entomology surveys at 0, 4, 8, and 12 months post-intervention aggregated at the cluster level.

6. Pupae Per Person (PPP): The outcome is measured as the number of Aedes pupae per person during entomology surveys at 0, 4, 8, and 12 months post-intervention aggregated at the cluster level.

7. Percentage of respondents with knowledge about Aedes mosquitoes causing dengue: The outcome is measured as the number of respondents with knowledge that Aedes mosquitoes cause dengue dividing by those without in Knowledge, Attitudes, and Practice surveys carried out at baseline and endline aggregated at the cluster level.

Coverage/Adherence

8. Guppy fish coverage: The outcome is measured as the proportion of eligible water containers with ≥1 guppy fish from community health worker reports done monthly from 0 to 12 months post-intervention and aggregated at the cluster level.

9. Sumilarv® 2MR coverage: The outcome is measured as the proportion of eligible water containers with ≥1 MR from community health worker reports done monthly from 0 to 12 months’ post-intervention and aggregated at the cluster level.

2. Please write your methods and procedures using active voice.

JH: All sentences should now be in active voice.

Reviewer #1: Basic information on the scale of the trial should be evident in the abstract (i.e. number of clusters, sampling effort).

The statement of what is the primary outcome should mention how the mosquitoes are trapped (both in the summary and on p12 under 'Primary Outcome Measure'. From the rest of the paper I understand this to be adult resting collections, as described on p15.

JH: We added the text below in the abstract. We didn’t add more about the specifics of the collections because of the word limits in the abstract. However, more specifics of the collection can be found in the methods section on page 17 line 9.

In this cluster randomized, controlled superiority trial, 30 clusters will be sampled with each cluster comprised of one or more villages… The primary outcome will be the population density
of adult female Aedes mosquitoes (i.e. number per house) as assessed by adult resting collections...Entomology surveys sample 1200 households and Knowledge, Attitudes, and Practice sample 600 households at each time point.

On page 14, line 1 we added: The outcome will be measured as the number of mosquitoes per unit of time spent aspirating by adult resting catches at 0,4,8, and 12 months post-intervention and will be aggregated at the cluster level.

The introduction sections provide a very thorough review of the evidence on Aedes interventions in Cambodia, but very little data from elsewhere. It would be good to see more detail on the global evidence base for the interventions being tested, while the information on interventions that are not being tested could be made more concise.

JH: More detail has been added and some previous text has been deleted starting on page 2 line 5 as follows:

Ae. aegypti is highly anthropophilic (preference for human beings), endophilic (resting indoors), endophagic (preference for feeding indoors) [1]. This partially explains why previous studies showed that household water storage jars contained over 80% of Ae. aegypti larvae in Cambodia, and why these jars became the main target for dengue vector control activities [2]. The continued reliance on the larvicide temephos (distributed with brand name Abate®) as the primary means of vector control [19], despite resistance of Ae aegypti in urban Phnom Penh and incipient resistance in our study site, Kampung Cham [3] was criticized by Khrun & Manderson who concluded that “continued reliance on temephos creates financial and technical problems, while its inappropriate distribution raises the possibility of larvicide resistance.” [1]. It is apparent that local evidence-based decision making guided by operational research and entomological and epidemiological surveillance and evaluation, one of the guiding principles of IVM, is needed. Additionally, a recent systematic literature review on the community effectiveness of temephos for dengue vector control identified similar concerns, but observed that when applied as a single intervention temephos was effective at suppressing entomological indices in the absence of insecticide resistance [4]. However, when applied in combination with other interventions the effect was not observed, and regardless of how it was used no evidence suggested the use of temephos was associated with reductions in dengue transmission [4].

Following the success of community-based Mesocyclops (a genus of copepod crustaceans) programs in locally eliminating Aedes mosquitoes in Vietnam [5-7] along with evidence from Japan [8], Mexico [Gorrochotegui-Escalante et al 1993], Laos [Jennings et al 1995], Honduras [Marten GG et al 1994], USA [Marten GG et al 1990], the Cambodian NDCP implemented a two year Mesocyclops project in Kratie province from 2002-2004, searching for an alternative vector control option [9]. Initial results showed a reduction in the Aedes population in the intervention area, but by the end of the project larval densities in the intervention area had increased by 62% from baseline. This apparently lower effectiveness in Cambodia may be because Mesocyclops from the local water sources had various parasites, and colonizing them parasite-free requires special training beyond what is possible in most rural Cambodian villages. The environment could have played a role as Northern Vietnam (where programs were most successful) has dramatically different geography, weather, and flora and fauna.
The search for other vector control options continued with an evaluation of Bacillus thuringiensis israelensis (Bti), a Gram positive, soil dwelling bacterium used as a biological control agent [10]. The evaluation of Bti in Phnom Penh showed positive results with significant reductions in the number of pupae for at least 2 and 2.5 months in containers with river and well water, respectively [10]. A recent systematic review also found that Bti can be effective at reducing the number of immature Aedes in treated containers, but insufficient evidence to recommend the use of Bti as a single agent for long term control of dengue vectors and prevention of dengue fever [11]. More extensive usage and evaluation of Bti by the Cambodian government as suggested by Boyce et al. [11] is currently ongoing in Kandal and Kampong Thom Provinces (Personal communication, Bunleng Sam, 2015).

Jar covers with long-lasting insecticidal netting (LN) treated with deltamethrin tested in Cambodia were found to have significantly fewer pupae per house, with a threefold decline in Ae. aegypti adult females per house and adult mosquito survival [2]. However, the magnitude of the reduction diminished over time, due to a gradual reduction of insecticidal effect of the jar covers and a residual deltamethrin life of 22 weeks [2]. Interestingly, this is less than the average residual life of deltamethrin in treated bed nets [21]. Another cause may have been children not always keeping the jar covering on after extracting water, and using them as toys around the house (Personal communication with To Setha, 2015) as Khun et al. noted in Cambodia before [12]. Mixed results were obtained from the effectiveness studies of similar jar covers for household dengue vector control in Venezuela over eighteen months (no significant association with entomology indicators [13], India over 14 months (a substantial reduction in dengue vector density, Arunachalam et al 2012) and Vietnam (strong negative effect on the prevalence of immature Ae. aegypti, which persisted for at least 5 months after treatment, Tsunoda et al 2013). Improvements in textile engineering and design to prevent entry and egress of mosquitoes, especially when the container is used, and an increase the insecticidal effectiveness may be needed for jar covers to be cost-effective public health interventions [2].

The use of a larvivorous guppy fish (Poecilia reticulata) was evaluated in 14 Cambodian villages [14], and subsequently in a larger study of 28 Cambodian villages [15]. Results from the initial study done from 2006-2007 were extremely encouraging with guppies in 56% of eligible containers, and a 79% reduction in Aedes infestation compared to the control. Guppy fish are not able to colonize all potential Aedes breeding sites, especially those which are polluted or with a volume of less than 50L (To Setha, personal communication, 2015). However, despite not having guppies, the smaller or discarded containers in the intervention villages had 51% less infestation than those in the control, suggesting a community-wide protective effect [14]. This could partly be due to a spillover effect from treatment villages as no results of guppy coverage were reported in the paper. These results led the WHO and the Asian Development Bank (ADB) to fund a larger scale-up in 2010-2011 which included Communication for Behavioural Impact (COMBI) activities in Lao and Cambodia. Results showed 88% guppy fish coverage in eligible water containers and a Container Index or proportion of surveyed containers containing Ae. aegypti larvae/pupae and indoor resting adult females of near zero (while control had a CI of 30) at the end of the project in Cambodia [15]. Similarly, encouraging results were found in Laos as part of the same project. A recent review found that although there have been several evaluations of the effectiveness of larvivorous fish for dengue vector control around the world, considerable
limitations existed in all the studies which restricted any conclusions on community effectiveness [16].

Despite positive results from the use of guppies, additional tools beyond larvivorous fish are required to target additional miscellaneous breeding sites including containers too small for guppy survival. One such alternative evaluated in Cambodia is long-lasting pyriproxyfen (PPF) releasers, which were placed directly in household water containers by village health workers and replaced every six months [17, 18]. PPF is a juvenile hormone analogue that interferes with the metamorphosis of juvenile Aedes mosquitoes, preventing their development into adults [19]. The results of the first study in 2003 were so promising — at higher doses, inhibition of adult emergence (IE) was greater than 87% for six months — that a larger second study was designed [17]. This showed that a novel 5% controlled release formation led to IE above 90% for 20 weeks, and above 80% for 34 weeks [18]. A new controlled-release PPF matrix release formulation (Sumilarv® 2MR) based on these earlier prototypes has since been developed and is suitable for containers uninhabitable by guppy fish. Results from a systematic review recently submitted by Hustedt et al. (personal communication, John Hustedt, 2017) direct treatment with PPF granules (with the same active ingredient) results in near 100% IE for 90 days at higher concentrations, with a steady reduction with time post-treatment or with decreasing concentration of active ingredient. The added benefit of Sumilarv® 2MR is that it only requires one distribution every six months (the entirety of the rainy season) and cuts down on operational costs as compared to temephos or Bti which have residual efficacy of 2-3 months [10, 20].

P4 line 22: the description of PPF as an intervention needs to specify how this is deployed. Clearly the impact of any larviciding intervention is conditional on the coverage and the frequency of deployment.

JH: The description of how it was deployed were added on page 5, line 22 as below:

One such alternative that has been evaluated in Cambodia was long-lasting pyriproxyfen (PPF) releasers, which were placed directly in household water containers by village health workers and replaced every six months [17, 18].

P9 line 5: one hopes that the trial will 'test' or 'estimate' the community effectiveness of the interventions. 'Demonstration' of the effectiveness would assume that the result is already known.

JH: “Demonstration” was changed to “estimate” on page 7 line 23.

P9 line 5: hypothesis 1 should be explicit that the hypothesis relates to the combination of interventions (not the interventions separately).

JH: This relation was added on page 8 line 1: The combination of guppies, Sumilarv® 2MR and COMBI activities will reduce…

P7 line 9: there is a broken link to a reference.
JH: The reference to Annex 1 is fixed now.

P7 line 13 et seq.: how were cluster sizes and boundaries determined? The number of clusters is rather small, and the study would presumably be more powerful with more, smaller clusters.

JH: There are two main reasons why we chose to have fewer large clusters rather than more smaller clusters. The first is that although more small clusters do bring you closer to individual randomization and provide more statistical power, there is also less opportunity to evaluate the mass-effect of the intervention. The second is that, operationally, it is difficult to split up villages into smaller clusters or use only part of the village for your intervention. Doing so would tend to make the trial conditions less like those of a scaled-up public health programme. Additionally, the study was already adequately powered for our study outcomes. Therefore, we chose to include the entire village or villages naturally clustered together rather than increasing the number of clusters and reducing the number of households in each cluster.

The following text was added to explain this on page 9 line 4:

Although increasing the number of clusters and decreasing the sample size may be closer to individual randomization and provide more statistical power, it also provides less opportunity to evaluate the mass-effect of the intervention. Additionally, the study was already adequately powered for our study outcomes. Therefore, we chose to include the entire village or villages naturally clustered together rather than increasing the number of clusters and reducing the number of households in each cluster.

P7 line 20: how do the authors know that a 200m buffer will be sufficient to avoid spill-over effects? If the mosquitoes fly an average of 50-100m per generation, then one might anticipate spill-over effects over longer distances with multiple generations of mosquitoes. How will the investigators know whether such effects happen or not? Will the data all be geolocated, allowing investigation of edge effects?

JH: The text was changed to incorporate the answer to this comment on page 26 line 16. Additionally, in this study there was much more than a 200m buffer between most of the villages.

There is no way to completely rule out any spill-over effects from villages, for example some people may accidentally bring home mosquitoes inside goods purchased in the market or allow them to escape from inside transport passing through the village. This number is likely small and unavoidable. As the flight range for Aedes in this area is 50-100m, having a buffer distance of 200m should be sufficient as there are very few water jars or clean water in the space between the households. It is also unlikely there will be breeding in the rice fields which are dry most of the year. However, all our data is geolocated, and we can investigate edge effects at the end of the project.

P8 & p9: The omission of a guppy only, or COMBI only arm appears to mean that the incremental impact of the guppies will not be identifiable. This could lead to a package of
interventions being promoted, that includes a significant useless component. How will the investigators determine whether any effect is accounted for by COMBI, or guppies, or if both are required?

JH: As mentioned in the manuscript on page 10 line 9, “COMBI related activities have been shown to have a significant impact on coverage of interventions in Cambodia and elsewhere, therefore ideally it should be included with any community based intervention.”

A joint toolkit by WHO/FAO/UNICEF has described COMBI’s importance in this excerpt from their toolkit: “Huge amounts of money have been spent on communication campaigns to prevent and control diseases such as highly pathogenic avian influenza (HPAI). The impact of many of these communication programmes, however, has not been clearly demonstrated, usually because of the divide between the people who design outbreak control interventions (e.g. epidemiologists, veterinarians and public health specialists) and those ‘communicating’ and ‘mobilizing’ communities. Technical interventions must be understood and applied in their behavioural, cultural, economic, political and social context. It is these settings that determine the success of control and prevention measures.” COMBI is much more than simply designing behaviour change communication messages, and should try to guide the development of any intervention where possible. Additionally, this operational research was meant to determine the community effectiveness of interventions that could be implemented by the Ministry of Health. As we understood that the government would only consider options with COMBI, we decided not to include a guppy only arm.

An addition to the paper to reflect this section was amended on page 10 line 11:

COMBI related activities have been shown to have a significant impact on coverage of interventions in Cambodia and elsewhere [14, 15, 21]. COMBI is much more than simply designing behaviour change communication messages, and should try to guide the development of any intervention where possible. Additionally, this operational research was meant to determine the community effectiveness of interventions that could be implemented by the Ministry of Health. As the government and WHO preferred options with COMBI, we decided not to include a guppy only arm.

P12: the list of secondary outcome measures includes measures of adherence or coverage as well as of intervention effect. It would be helpful to see some indication of which fit into each category; it would also be helpful to see the rationale for each outcome measure, and how these data will be analysed.

JH: Please see these additions starting on pg.14 line 6.

Secondary Outcome Measures

The secondary outcomes for the trial include those in two main categories (Survey indicators, Coverage/Adherence):
P14 line 13. Has the allocation already been carried out, or is it still to take place? The statement about putting sheets of paper on the wall is in the past tense.

JH: The allocation has already taken place, but to be consistent with the tense in the rest of the protocol I have changed it to future tense. Although allocation has already finished at this point, it was not yet completed when the manuscript was first submitted. (See Page 16, line 21)

P18. The statistical methods section is rudimentary. It is not clear what comparative measures between the arms will be calculated. Presumably effectiveness will be estimated as proportionate reductions, relative to the control arm. How will confidence intervals and significance tests be carried out? How will the statistical analysis allow for clustering? Will there be any attempt to use process measures derived from the secondary outcomes to attribute causality?

JH: The additional information on statistical methods below has been added to manuscript on page 20 line 17

The effectiveness will be estimated through the use of density ratios relative to the control arm. Confidence intervals and significance tests will be obtained through negative binomial regression using the MASS package in R[22]. With one record per cluster there will be no within-cluster correlation. For the primary analysis, causality should reasonably be attributable to any between-arm differences as the clusters were randomized and there is a control group. Coverage measures will be used in additional exploratory analysis [23-25].

References:


