Study Protocol with SAP

Title:

Cluster Randomized Controlled Trial: Efficacy of Gravid Oviposition Sticky Trap (GOS) and Dengue NS1 Antigen Rapid Diagnostic Test for Early Surveillance of Dengue Among Adult Aedes Mosquitoes to Reduce Dengue Outbreaks in PJU10, Damansara Damai, Selangor, Malaysia

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Executive summary of study

Dengue, a serious vector-borne disease in Southeast Asian countries is fast emerging as a pandemic-prone disease globally. To add to the existing challenges, its mosquito vector, *Aedes aegypti* followed by *Aedes albopictus* are also vectors for other diseases such as chikungunya (outbreaks in 2008-2009) and Zika. In Malaysia, 120,000 cases of dengue were reported in 2015. In 2017 (until October), there were about 72,000 cases.

Surveillance are dependent on *Aedes* larval surveys and notifications of lab-confirmed human infections. However, there is no correlation between larval indices and cases of dengue. It is known that some asymptomatic people are infectious to mosquitoes. Therefore, the existing, reactive programme lacks sensitivity and is delayed, and has proven insufficient to stave off epidemics.

This current proposal unfolds a proactive and innovative paradigm shift. The creation of an in-house user-friendly technique to detect dengue virus in mosquitoes coupled with a community approach for early detection of dengue cases is an important and timely project. This proposed project will directly contribute to the Government's effort of reducing dengue as well as other vector borne diseases.

Traps will be set to collect *Aedes* mosquitoes weekly and virus will be detected in mosquitoes using dengue NS1 antigen test kit. When the positive mosquito is detected, the community will be alerted to conduct control measures (search & destroy). In the control areas, only ongoing dengue control and surveillance by the health authorities will be continued. KAP will be done before and after the intervention in intervention areas. If this study is successful, it has the potential to be a Model for Dengue Surveillance and Control and dossier to be submitted to WHO for adoption by Southeast Asian countries.

a) Research background

1. Problem Statement

Dengue is on the increase and the current surveillance and control measures are insufficient to control epidemics. The current method used is reactive rather than the proposed proactive measure.

2. Aim

This trial aims to demonstrate community effectiveness of new proactive paradigm in reducing dengue epidemics. The main hypotheses are 1) This new paradigm (GOS trap and NS1 kit) will reduce dengue epidemics compared to the usual activities carried out by the Ministry of Health (including larval control and information and education material dissemination during outbreaks) as assessed through entomology surveys. 2) Community will be more receptive to this new surveillance activity as they will receive information of infected mosquitoes before dengue cases are reported, thus will be able to take informed steps to prevent themselves from being infected.

3. Objectives

a) To determine new paradigm (GOS trap and NS1 kit) in reducing dengue epidemics compared to the usual activities carried out by the Ministry of Health (including larval control and information and education material dissemination during outbreaks) as assessed through entomology surveys.

b) To determine whether the community will be more receptive towards this new surveillance activity as they will receive information of infected mosquitoes before dengue cases are reported.

4. Research Questions

Will the combination of setting up traps to collect *Aedes* mosquitoes coupled with detection of virus in the mosquitoes help to reduce/prevent impending dengue epidemics? Will this method be able to cover more premises compared to house to house larval surveys? Will this method reduce the cost of dengue surveillance and control compared to the current ongoing method?

5. Literature Review

Dengue and chikungunya are major increasing public health problems across more than 150 countries (1). Dengue infection has major health impact in any person in the community including pregnant women. Study done in the University Malaya Medical Centre showed maternal dengue infection affected at least 2.5% of pregnancies in our hyperendemic setting. Vertical transmission, as defined by detection of dengue IgM in cord serum, was 1.6%. (2) Dengue infection was also found to be associated with increased risk of miscarriage. (3)
In 2015, Zika virus too has become a huge public health problem in the Americas and has the potential to spread across the globe (4,5). Vector control has been the hallmark of the dengue control programme in many countries in Southeast Asia (6), since there are no anti-dengue drugs available, and the most recent dengue vaccine is partly efficacious as it falls short of the levels of protection required for a standalone intervention (7).

House to house larval surveys, source reduction, larviciding, fogging, ULV (was introduced in the 1970s and still being used) for dengue prevention and control are no longer practical and need to be augmented by more targeted but less ambitious outbreak responses that focus on a few tools that might justify expense of deployment (8). However, according to recent reports these tools are not cost effective for dengue control (9,10). Novel techniques such as the release of genetically modified mosquitoes (RIDL) and the use of the bacteria *Wolbachia* to control the population of the *Ae. aegypti* are still under trial (12-15). Urgent effective strategies for control are required ahead of the evidence from these trials, which would also require a lengthy process to assess the environmental and ecological impact of these interventions. Public and community support are important elements for these interventions to be successful.

The lack of correlation between larval indices and dengue cases (16) and the development of resistance by *Aedes* to pyrethroids and temephos insecticides (17-20) pose a serious challenge not only towards dengue control but also towards chikungunya virus and Zika virus control programmes. In many countries, studies are being conducted on the use of sticky traps to lure and trap gravid *Aedes* female adults (21-24). These traps come in different designs and some contain insecticides or incorporate pyriproxyfen to kill the progeny (25, 26). In a randomised control trial using BG sentinel traps, trapping slightly reduced the density of *Aedes* in the experimental area compared to the control area (27). However, in a trial using CDC autocidal gravid trap (AGO) there was a significant reduction between 53 to 70% in the intervention area compared to the control area (28).

In our previous phase l study conducted in an urban area in peninsular Malaysia, we showed that the infected *Ae. aegypti* mosquito was obtained from sticky non-insecticidal traps- the in-house developed sticky trap [named GOS trap (Gravid Oviposition Sticky trap)] before the first case was reported (29). This preliminary trial has shown that the sticky trap is a cheap and effective way to collect *Aedes* mosquito. Dengue virus was detected in *Ae. aegypti* before the

epidemic. The NS1 antigen test kit, a simple tool was utilised by public health staff to demonstrate the presence of infected mosquitoes and thus preventive action was initiated before an epidemic occurs. It was highlighted that a shift to this toolkit would be more efficient for a control programme than the current practice of house-to-house larval surveys. The health staff involved in this study also found that it was easier to obtain the adult mosquitoes then to carry out labour intensive larval surveys.

Subsequent to the above-mentioned phase 1 study, a prospective longitudinal phase 2 study was initiated to evaluate the use of Gravid Ovipositing Sticky (GOS) trap and the NS1 testing system for surveillance of dengue virus transmission in urban Malaysia over a two-year period. The findings showed that dengue cases would occur lag one week after infected mosquitoes are obtained and will peak at lag of 2 to 3 weeks (30). These results showed that this proactive strategy is beneficial for dengue vector surveillance programme. The usage of the GOS trap which is simple to setup, cost effective (below USD 1 per trap) and environment friendly (i.e. use recyclable plastic materials) to capture *Ae. aegypti* followed by a rapid method of detecting of dengue virus using the NS1 dengue antigen kit has shown promising results. Control measures could be initiated when positive mosquitoes are detected, for example activity such as search and destroy breeding containers. This innovative usage of GOS trap coupled with NS1 detection in mosquito provides a comprehensive early warning and surveillance system that has the predictive capability for epidemic dengue.

6. Relevance to Government Policy, if any

This cluster randomised trial is expected to provide robust estimates of the intervention effects. A rigorous evaluation of these vector control interventions is vital to the development of an evidence-based control strategy and to help direct government resources in the right direction to reduce dengue epidemics.

7. Methodology

This is a community-based study assessing the knowledge, awareness and practice on dengue infection and its prevention. We choose PJU10 because it is the dengue 'hotspot' area according to local health council. We have identified a low-cost housing area with population of approximately 48700 people.

Sample size calculation:

Sample size for intervention trial:

To test for effectiveness of intervention in reducing dengue cases, on average, each housing unit will have 2% risk of dengue per year. A sample of 5710 unit (2855 per arm) will be required to achieve 80% power at alpha of 0.05 with the assumption of reduction in 50% of risk at the end of intervention. Eight apartments with 7979 residential units will be selected and randomised into intervention and control. Apartment will be the item of randomisation.

To test for effectiveness of intervention to improve knowledge, assuming the knowledge in control group is 50% and knowledge in intervention is 70%, total sample calculated to achieve 80% power at alpha of 0.05, is 208 (104 per arm). After inflation of 20% sample required is 250 (125 per arm).

Sample size calculation for survey/cross-sectional study:

For prevalence study (knowledge), assuming baseline knowledge in population is 50%, total sample calculated to achieve 80% power at alpha of 0.05, is 384. After inflation of 20% sample required is 460.

For sero-prevalence study (blood), assuming baseline dengue sero-prevalence in population is 60%, total sample calculated to achieve 80% power at alpha of 0.05, is 369. After inflation of 20% sample required is 443.

According to the sample size calculation, the final sample size will be 500. Therefore, 250 in intervention group and 250 in the non-intervention group (control).

Phase I: KAP Questionnaire survey and blood taking

Pre-tested questionnaires will be distributed to members of community who have given consent to participate in this study.

Inclusion criteria:

1) All men and women aged above 18 years, including pregnant women.
2) Able to give consent
3) Willing to participate in blood taking

Exclusion criteria:

1) Age below 18 years
2) Unable to give consent
3) Not willing to give blood

After the questionnaire survey, 3 ml of venous blood will be taken to analyse for seroprevalence of dengue infection (dengue IgG and IgM will be carried out).

Phase II: Placement of GOS mosquito trap in the intervention group

All clusters in intervention arm will receive the GOS traps. A maximum of 21 traps will be set per apartment block, on every third floor. The traps will be monitored weekly and the abdomen of *Aedes* obtained will be tested for dengue virus using the NS1 antigen test kit. If positive search and destroy measures will be conducted by the community under the supervision of community leader and the health staff. To identify the mosquito which carries the virus, dengue NS1 antigen tests will be carried out from the head and thorax of the individual mosquitoes from positive pool of mosquitoes. For the control clusters, routine dengue control and surveillance will be carried out as per the current Ministry of Health control activity, where no traps will be set, and dengue control measure will be initiated when human cases are reported from the control group. Data collection will be conducted for 1 year and 6 months.
When a positive mosquito is obtained, flyers and banners will be distributed and hung to inform the residents of the presence of dengue transmission in the apartment block/apartment. Alternatively, the residents may also be approached house-to-house or an educational booth set up at strategic locations in the apartment to warn and educate the residents.

Phase III: Repeat of KAP questionnaire

The KAP questionnaire survey will be repeated 15 months after intervention in the intervention arm.

Phase IV: Statistical Analyses

Data of serologically confirmed dengue cases (by NS1 or IgM/IgG) from the eight residential blocks will be obtained from the Selangor State Health Department, Malaysia. It is mandatory for all hospitals and private practitioners to report cases to the Ministry of Health. The date of onset of case will be used for all data analyses.

For analysing the change in level of dengue KAP, the mean of the percentage score of the population for each domain (knowledge & attitude/practises) will be determined for the pre- and post-questionnaire. Then, paired t-test will be performed to determine the presence of significant changes in both means. A p-value of at most 0.05 indicates a significant change/ improvement in the mean score (An increase in score percentage indicate a better outcome). Additionally, an individual percentage score of 80% and above indicates good knowledge/attitude/practice. Accordingly, chi-squared test will be used to determine the extent to which good knowledge is related to good attitudes/practises

All statistical analyses will be done using weekly data and R programming language for statistical analysis (version 3.2.4) (30). The family of distributed lag non-linear models (DLNM), (*DLNM* package version 2.20), which can simultaneously analyse non-linear factor-response dependencies and delayed effects, and provides an estimate of the overall effect in the presence of delayed contributions will be utilised. For dengue cases and its relation to dengue-positive *Aedes* pool, the model to be used will be: glm (case ~ cb.total_aedes + cb.ns1positive + ns(time, 3) + woy, family = quasipoisson, data) where woy = week of the year. Both the *Ae. aegypti* / *Ae. albopictus* trapped per week and cases per week at each apartment will be analysed by generalised linear mixed model (GLMM). Zero inflation and Poisson distribution were incorporated in the analysis. Differences in numbers of *Ae. aegypti* and cases between blocks and between floors were tested with Tukey's contrasts at $P = 0.05$.

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