

The iDEM trial (intervention for Dengue Epidemiology in Malaysia) to measure the effectiveness of integrated vector management with targeted outdoor residual spraying and autodissemination devices on the incidence of dengue in urban Malaysia: Study protocol for a cluster randomized controlled trial

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Study protocol

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Abstract

Background: In common with many South East Asian countries, Malaysia is endemic for dengue. Dengue control in Malaysia is currently based on reactive vector management within 24 hours of a dengue case being reported. Preventive rather than reactive vector control approaches, with combined interventions, are expected to improve the cost-effectiveness of dengue control programs. The principal objective of this cluster randomized controlled trial is to quantify the effectiveness of a preventive integrated vector management (IVM) strategy on the incidence of dengue as compared to routine vector control efforts.

Methods: The trial is conducted in randomly allocated low and medium cost clusters located in the Federal Territory of Kuala Lumpur and Putrajaya. The IVM approach combines: targeted outdoor residual spraying with K-Othrine Polyzone, deployment of mosquito traps as auto-dissemination devices, and community engagement activities. The trial includes 280 clusters randomly allocated in a 1:1 ratio. The clusters receive either the preventive IVM in addition to the routine vector control activities, or the routine vector control activities only. Epidemiological data from monthly confirmed dengue cases during the study period will be obtained from the Vector Borne Disease Sector, Malaysian Ministry of Health e-Dengue surveillance system. Entomological surveillance data will be collected in 12 clusters randomly selected from each arm.

To measure the effectiveness of the IVM approach on dengue incidence, a negative binomial regression model will be used to compare the incidence between control and intervention clusters. To quantify the effect of the interventions on the main entomological outcome, ovitrap index, a modified ordinary least squares regression model using a robust standard error estimator will be used.

Discussion: Considering the ongoing expansion of dengue burden in Malaysia, setting up proactive control strategies is critical. Despite some limitations of the trial such as the use of passive surveillance to identify cases the results will be informative for a better understanding of effectiveness of proactive IVM approach in the control of dengue. Evidence from this trial may help justify investment in preventive IVM approaches as preferred to reactive case management strategies.

Trial registration: ISRCTN, ISRCTN81915073, retrospectively registered on 17 April 2020 (<https://doi.org/10.1186/ISRCTN81915073>)

Background

Dengue fever, caused by one of the four dengue virus serotypes (DENV1-DENV4), is a mosquito-borne disease of public health importance due to its increasing burden worldwide [1]. With 390 million estimated infections and 10,000 deaths per year, dengue is the predominant mosquito-borne virus in humans [2]. In the absence of progress in dengue prevention, 2.25 billion more people will be at risk in 2080, putting 60% of the world's population at risk [1].

In Malaysia, *Aedes aegypti*, and *Aedes albopictus* are the primary and secondary mosquito species responsible for dengue transmission [3]. The incidence of dengue in Malaysia increased from 31.6 reported cases per 100,000 inhabitants in the year 2000 to 159.7 in 2010 [4] and to 396.4 per 100,000 inhabitants in 2015 [5]. In 2010, the country spent an estimated US\$73.5 million (95% confidence interval CI = US\$62.0–US\$86.3 million) on the national dengue vector control program [6]. The control of dengue in Malaysia is based on rapid reactive vector management within 24 hours of a dengue case being reported. The program consists of space spray, source reduction and larviciding. Space spray has however a relatively short life span compared to residual spraying [7, 8], and repeated use in a non-targeted application increases the potential of mosquitoes to develop resistance.

Vector control has been shown to be highly effective in reducing the density of *Aedes* populations [9], but there is limited evidence of its efficacy in reducing the incidence of *Aedes*-borne diseases [10, 11]. Designing more effective and preventive rather than reactive vector control programs with combined interventions could be a key solution in dengue prevention and control. In the absence of an effective dengue vaccine, the World Health Organization (WHO) calls for locally adapted and sustainable integrated vector management (IVM) [12, 13].

The study protocol presented here is a cluster randomized controlled trial (cRCT) based on proactive IVM that combines three components: 1) targeted outdoor residual spraying (TORS) with K-Othrine Polyzone (Bayer SAS), 2) deployment of mosquito traps as auto-dissemination devices (ADDs, In2Care[®] Mosquito Traps, In2Care B.V., The Netherlands), and 3) community engagement (CE) activities.

K-Othrine[®] Polyzone, with deltamethrin as its active ingredient (62.5 g/L), is a polymer enhanced suspension concentrate aqueous formulation which has low odour and high level of safety. The polymer technology used in this formulation protects the active ingredients from weather conditions, rainfall and mechanical abrasion and ensures they remain available on treated surfaces for up to three months, thereby reducing environmental contamination. The product has been approved for use by the WHO [14] and prequalified as vector control product (PQT-VC reference 008-004).

Auto-dissemination devices are black plastic containers that mimic breeding sites for female mosquitoes. The container is filled with 5 litres of water with a floating device on top of the water that holds a statically charged netting with a biocide powder mixture [15]. ADDs use In2Mix powder that contain two active ingredients. The first is technical grade pyriproxyfen (PPF) at doses of 0.375 gram per refill sachet. PPF is an insect growth regulator (IGR) that prevents mosquito larvae from developing into adults. The second active ingredient is spores of the insect specific fungus *Beauveria bassiana* GHA strain at 0.05 gram per refill sachet. Adult female mosquitoes that visit the ADD pick up the In2Mix powder solution from the statically charged netting. The fungus slowly kills the mosquitoes, while the PPF is transferred by the mosquito to surrounding breeding sites (i.e. auto-dissemination), thereby reducing *Ae. aegypti* populations by decreasing the emergence of new adult mosquitoes [15]. These user-friendly control devices target cryptic, hard to find breeding sites and facilitate precision-targeted larval control and continuous breeding suppression of vector populations [15, 16].

Previous field evaluation of TORS and ADDs showed effective reduction of the mosquito population [3, 17]. In addition, the combination of insecticides with different modes of action in the same program is likely to limit insecticide resistance in the local *Aedes* vector population. However, to the best of our knowledge, the combination of these components has not yet been tested in a large trial with dengue incidence as the primary endpoint.

Local communities are key actors in vector control programs and their engagement is essential for success and sustainability of such programs [18-20]. We expect that community engagement by proactive communication and information-sharing with the population of the study areas will increase their adherence to operational procedures, thus enhancing the efficacy of the proposed combined vector control tools to promote favourable outcomes with respect to decreased dengue incidence.

Objectives

The principal objective of this cRCT trial is to quantify the effectiveness of a preventive IVM strategy combining TORS, deployment of ADDs, and CE activities on the incidence of dengue in randomly selected areas in the Federal Territory of Kuala Lumpur and Putrajaya (Malaysia) as compared to routine vector control efforts.

The secondary objective is to evaluate the effectiveness of the IVM approach on the population density of *Aedes* mosquitoes.

Trial design

The study is a non-blinded cRCT conducted in selected areas of the Federal Territory of Kuala Lumpur and Putrajaya, Malaysia. The trial protocol was registered with the ISRCTN registry on 17 April 2020 as ISRCTN81915073.

For the purpose of the trial, a cluster is defined as a locality i.e. one residential area composed of neighbouring buildings that share the same facilities such as parking lots, food court, groceries store, playground, and community halls.

The trial includes 280 clusters which were randomly allocated in a 1:1 ratio, to receive either the proactive IVM in addition to the routine vector control activities or the routine vector control activities only (see interventions section for more details).

Methods

Study setting

The trial is conducted in selected low and medium cost buildings located in the Federal Territory of Kuala Lumpur and Putrajaya, Malaysia (Figure 1). Kuala Lumpur covers 243km² (94 square miles) and had an

estimated population of 1.77 million in 2020. Putrajaya covers 49 km² (18.9 square miles) and had an estimated population of 0.10 million in 2020.

Figure 1: Map of the study areas: Federal Territory of Kuala Lumpur and Putrajaya

Localities (clusters) in the Federal Territory of Kuala Lumpur and Putrajaya with recurrent dengue outbreaks reported from 2015 to 2018 were eligible (n=895). We excluded localities with missing data on the population (n=102), localities within a 1 km radius of an ongoing study with *Wolbachia* (n=3), high cost localities (n=246), and localities with less than 1000, or more than 8000 inhabitants (n=215). Of the remaining 329 localities, 280 with the highest incidence rates in 2015-2016 were retained for the trial. Eligibility criteria for dengue cases follow the flowchart of the e-Dengue surveillance system (Additional file 1). Only confirmed dengue cases will be included (see “Outcomes” below).

Interventions

The study flowchart for selection and randomization of eligible clusters is shown in Figure 2. Clusters (localities) randomly selected to the intervention arm will receive the proactive IVM strategy i.e. TORS, deployment of ADDs (performed by the staff of a pest control organisation hired for the purpose of the trial), and active community engagement. Existing dengue prevention and control measures will continue in both control and intervention clusters as per Standard Operational Procedures and guidelines given by the Malaysian Ministry of Health. This program consists of health education, space spray (thermal fogging and ultra-low volume space spray), source reduction, and larviciding within 24 hours a dengue case is being reported. All information on the insecticide and technical information (date, extent of activity, place, etc.) is recorded and described in trial reports.

The trial will run for 2 years and the time schedule of enrolment, intervention and data monitoring activities is shown in Table 1.

Figure 2: Flowchart of cluster selection and allocation, Federal Territory of Kuala Lumpur and Putrajaya

Targeted outdoor residual spraying

The insecticide dosage is 25 mg/m² and is applied by trained operators using a residual hand compression sprayer (Semco). The target spray areas are outer walls covered or partly covered (corridors, staircase, parking, lobby or any corner liable to become a resting spot for mosquitoes) in all floors. We exclude sections of wall close to power plugs, elevator buttons, walls covered with wall paper, oil-based painted walls oil painted walls or tiles. Residents are requested to remove edible plants, hanging clothes, pet cages (e.g. of cats, dogs and birds), and to cover aquaria during the day of the intervention. Parts of corridors with such objects are not sprayed. Based on our findings on the residual effects of K-Othrine® Polyzone [17, 21], TORS is conducted every four months in all intervention areas.

To control the quality of TORS spraying, each spray pump is equipped with a control flow valve. The latter is a device that fits next to the nozzle, and ensures that output remains constant as the pressure in the

spray tank decreases. The spray pumps are washed after each use to avoid clotting of the insecticide in the nozzles. All spray cans are washed thoroughly, checked and calibrated once a week. During spraying activities, a quality control officer observes the spraying procedure and technique. Pictures and video recorded randomly by the officer will be used for the quality control evaluation, future reference or records. Training and evaluation of spray and calibration is provided to all operators every two months.

Deployment of auto-dissemination devices

The strategy for ADD deployment in these urban settings was adapted from In2Care standard recommendation (1 trap/400m²), based on results from our pilot study in Johor Bahru, Malaysia [21] and another study evaluating the vertical infestation of *Aedes* in high-rise building [22]. Smart deployment of ADDs only on floors with higher expected mosquito density was found to be sufficient to reduce the mosquito population in the whole building.

The exact number of ADDs and suitable location is determined at the start of the study (Additional file 2). ADDs are distributed over each floor as evenly as possible and placed according to the manufacturer's recommendations in semi – indoor environment such as corridors, multipurpose halls, lobby, prayer hall, shops and parking (i.e. in shaded sites).

Community engagement

Community engagement (CE) starts at the time of baseline data collection and is maintained throughout the study period by a team specially trained in CE activities. The main objectives of this activity are to create public awareness and trust, and to increase their participation during and following the deployment of the intervention. The first step of CE activities consists of an appointment with the community leaders in each cluster through phone calls using a standard script in order to schedule a face-to-face meeting. The purpose, objectives, procedure and timelines of the iDEM trial are presented to community leaders followed by a question and answer session. Information on demographic and architectural characteristics such as number of buildings, number of floors per building, number of units per floor, total number of occupants, parking lots, and public facilities (gymnasium, kindergarten, shops, restaurants, swimming pool, and playground) of the cluster are recorded after the briefing. A second meeting involving the residents is organized upon the community leader request. Information, education and communication material such as banners, posters (Additional file 3), and brochures are distributed to explain the objectives of the study and the role of the community during and following the deployment of the intervention. Softcopy of these materials is sent to the building managers through email or WhatsApp message and subsequently disseminated among the community. A notification is sent to the building manager one week before the deployment of the intervention to remind them of the date and details about the intervention.

Outcomes

The primary outcome measure is the estimated dengue incidence rate in both control and treatment clusters. The number of confirmed dengue cases is provided by the e-Dengue system (<http://spwd.arasm.gov.my>).

Dengue is a notifiable disease in Malaysia. All clinically suspected or confirmed dengue cases are notified by the medical officer to the nearest District Health Office within 24 hours (Additional file 1). Currently, dengue confirmation is based on dengue rapid combo test for 1) qualitative detection and differentiation of specific IgG and IgM antibodies to dengue virus in human serum and plasma, and 2) dengue rapid NS1 test for qualitative detection of dengue NS1 antigen in human whole blood, serum and plasma [23]. Further laboratory tests are performed if necessary (under clinical management not determined by the current trial). The e-Dengue case definition is based on the international classification of diseases ICD (10: A90, A91) and positive diagnostic results of either NS1, IgM or IgG or any combination of test results [24]. For the purpose of this trial, only confirmed dengue cases will be used for statistical analyses.

Secondary outcomes measures include:

1. Ovitrap index (OI): number of positive ovitraps divided by the total number of recovered ovitraps.
2. Larval index (LI): number of larvae identified divided by the number of ovitraps recovered.
3. Container index (CI): proportion of water-holding surveyed containers infested with *Aedes* larvae or pupae.
4. Adult survey: mean number of female adults *Aedes* collected in sticky ovitraps.
5. Resistance to deltamethrin: measurement of knock-down and mortality rates in wild strain adult mosquitoes population
6. Residual activity of deltamethrin measured by wall deposit bioassays [25]

Sample size

The sample size determination was adapted from the bootstrap approach of Kleinman & Huang [26] for cluster randomized trials. To take into account the regression to the mean due to the selection of the localities with the highest incidence rates in 2015-2016, sample size determination was based on the incidence rates of the years 2017-2018. For the purpose of sample size calculation, the estimated number of inhabitants in each cluster was obtained as follows: number of blocks (buildings) x number of floors x number of apartments in each floor x 4.9 (i.e. estimated number of individuals in each apartment is based on National Health and Morbidity Survey 2019 and Department of Statistics). The mean of the cluster-level incidence rates was 0.34% per year (range: 0 to 4% per year). The power calculation used resampling with replacement to generate plausible incidence distributions during the future study period, in each arm. The resampling was repeated 5000 times. Each time, the resampled data from the clusters underwent a simulated randomization to the intervention or control arm. For those in the control arm, the inferred dengue incidence rates were calculated from the confirmed dengue cases observed during the years 2017-2018. For clusters in the intervention arm, the inferred incidence rates were calculated from the observed incidence rates multiplied by the expected effect of the intervention. The effect of the

intervention was estimated from this simulated dataset using a negative binomial regression model. Finally, the power was estimated as the proportion of analyses with a p-value ≤ 0.05 (two-sided). If we assume that the minimum effect that we are able to detect is a reduction in the incidence by 33% in the intervention areas (0.34% and 0.23% estimated mean incidence rates per year in the control and intervention arms, respectively), the inclusion of 280 eligible clusters (140 per arm) achieves a power of 85%. This means that, if the rates are as assumed, the study has 85% probability of establishing a benefit of the intervention, using a two-sided significance level of 5%. This power was considered acceptable and this sample size was used for the trial.

Allocation

The random allocation of the clusters to the control or the intervention arm (1:1 ratio) was carried out using the PLAN procedure of SAS software, version 9.4 (Copyright (c) 2002-2012 by SAS Institute Inc., Cary, NC, USA), and was stratified on the economic level of the clusters (low or medium) because this is associated with dengue incidence.

In each stratum, clusters were randomly numbered and block randomization were carried out with block sizes of 10 and 16 in order to allocate the clusters to the intervention or control arm. Randomization was carried out after the protocol was approved by the IMR Research Committee.

This trial is not blinded. All clusters were allocated at the start of the trial so an allocation concealment mechanism is not required. The allocation sequence has been generated by a statistician of the Service of Biostatistics of Hospices Civils de Lyon, France. The code written by this first statistician was double-checked by a second statistician in regard to the conformity to the randomization protocol. The list of clusters in each arm was saved in a text file and transmitted to the investigators (IMR-Malaysia).

Data collection

Epidemiological data

No active recruitment of dengue cases is planned, as the information on the incidence of dengue during the overall study period in both control and intervention clusters is extracted from the national e-Dengue surveillance database. For each confirmed dengue case, demographic data, signs and symptoms at first medical visit, date of diagnosis, results of the rapid dengue COMBO test, hospitalization and vital status at discharge are extracted.

Entomological data

Entomological data for the assessment of secondary outcomes are collected in 12 clusters randomly selected from each arm (24 in total) and stratified by socio-economic status (medium versus low cost).

Climatic parameters

Common climatic parameters (rainfall, temperature, and humidity) have been reported to influence the intensity and magnitude of dengue incidence [27-29]. These parameters will be requested from the Malaysian Meteorological Department, Ministry of Energy, Science, Technology, Environment and Climate Change for two points: Petaling Jaya and Kuala Lumpur International Airport.

Monitoring *Aedes* population densities

The density of *Aedes aegypti* and *Ae. albopictus* populations will be monitored using larval and adult collection methods in a sample of 12 clusters per arm. Larval surveys are carried out using a minimum of 40 ovitraps per cluster. Each ovitrap consists of a 250 ml cylindrical, black plastic container (7.5cm diameter, 9.0cm height, 300ml volume) filled with tap water to a depth of 5.5 cm and is equipped with a removable oviposition paddle made from a thin strip of brown hardboard (10 cm x 2.5 cm x 0.3 cm). Larval surveys start five weeks before the start of the intervention to measure the baseline density. Ovitrap are positioned randomly in spaces with minimum human, physical, and environmental disturbance; a) in the semi-indoor environment – defined as the area outside of the housing units but still sheltered by the roof (e.g. shared corridor and stairway); b) in partially or totally shaded outdoor areas (e.g. near bushes, small plants, and temporary structures); c) indoor (under the sofa, dining table, sink, bed, working / study desk) only in the apartments of those who volunteer to have them.

To determine the OI, all ovitraps are collected once a week and replaced by new ones with fresh tap water and egg-free oviposition paddles. The collected ovitraps are brought back to the IMR laboratory (Kuala Lumpur) for further processing. Ovitrap contents and oviposition paddles are transferred into plastic containers and labelled with the name of the cluster and date of collection. Larvae emerged from the eggs laid in the ovitraps undergo species identification [30, 31] using a compound microscope (Nikon Eclipse® E100, Japan).

To determine the CI, a minimum of 20 suspected mosquito breeding containers within 50 m radius of the study cluster are examined once a month in each cluster. Larval samples from the positive containers are collected in a sample bottle and brought back for identification. The larvae are allowed to emerge into adults for morphological identification.

To monitor adult mosquito densities, monthly surveys are carried out with sticky ovitraps, i.e. a modification of conventional ovitraps as described by Lau et al [32]. The inner wall of the ovitrap is lined with a 5.5 cm x 24 cm transparent plastic sheet. The inner plastic sheet is covered with insect glue (Neopeace, ACM, Malaysia) attached to the container using adhesive tape on both sides of the top of the container. A hole is drilled about 3cm above the bottom to avoid flooding of the trap with rain water. A total of 50 – 200 sticky ovitraps are deployed in the study area, in every alternate floor with emphasis on dark and humid areas such as garbage rooms, emergency exits, shoe racks and vegetation considered as *Aedes* resting places. The adult samples are isolated in a single vial and send for identification.

Monitoring mosquito resistance

Adult Bioassay Test

We will assess measure susceptibility levels to deltamethrin in the field population. The test will follow the WHO protocol [33]. Pyrethroid resistance monitoring is conducted on sugar-fed adult female mosquitoes aged three to five days against a diagnostic concentration (0.03% deltamethrin, 0.25% permethrin, 0.15% cyfluthrin, and 0.03% lambda-cyhalothrin), which is used to impregnate paper at the recommended exposure time. Five replicates of 20 adult field strain mosquitoes from the 24 clusters selected for entomological endpoints are prepared. Two replicates of susceptible mosquitoes from the insectary laboratory of IMR are used as control strains. The cumulative knockdown counts are recorded every minute within the exposure period of one hour, or until 90% knockdown is observed. After the exposure period, all the tested mosquitoes (live and knocked down) are transferred into holding tubes for a 24-hour recovery period and supplied with 10% sugar solution in cotton balls. Adult mortality is then recorded. If the mortality rate in the control group is between 5% and 20%, the percentage of mortality in the exposed mosquito is corrected by using Abbot's formula [33]. Surviving mosquitoes are transferred into Eppendorf tubes and kept at -80°C for enzyme micro-assay.

Enzyme Micro-assay

Three enzyme assays, namely mixed function oxidase [34, 35], esterase [35, 36], and insensitive acetylcholinesterase [35] are conducted in sugar-fed female mosquitoes, aged 3–5 days, to determine the enzyme activity. The female mosquitoes are raised from eggs collected in the field. The same homogenate of an individual mosquito is used to assess all three enzymes assays.

Monitoring quality of TORS and ADDs post-application

To assess post-application quality of K-Othrine® Polyzone spray, wall deposit bioassays are conducted 48 hours after spraying and repeated every three weeks for a period of four months [25]. Standard WHO bioassay cones are firmly positioned onto the TORS treated wall in a perpendicular position using masking tape. Ten adult female mosquitoes (sucrose-fed, 3–5 days old, non-blood fed) are introduced into the bioassay cone for 30 minutes through the aperture using a battery-operated aspirator, then collected back and observed for 24 hours mortality. Three biological replicates of 10 mosquitoes are prepared for laboratory and field strains of *Aedes aegypti*. Residual effect of K-Othrine® Polyzone is determined by evaluating the knock down time and mortality rate. Knock down is observed for 30 min at 1-minute intervals. After exposure, the mosquitoes are aspirated out, transferred to paper cups, held at $27 \pm 2^\circ\text{C}$ with $75 \pm 10\%$ relative humidity and sugar-fed. The mortality is recorded 24h after testing. The level of resistance is evaluated using the WHO criteria [33].

To assess ADD quality post-application, the presence/absence and estimated quantities of mosquito larvae in the water inside the ADDs are recorded at each service. Every month, 250 ml water samples are taken from three randomly selected deployed ADDs that are positive for *Aedes* larvae and brought back to the laboratory to confirm the presence and activity of the pyriproxyfen larvicide. In each 250 ml ADD water sample, 25 third instar (L_3) larvae from the IMR rearing colony of *Aedes aegypti* are added [33].

Emergence of mosquito adults is monitored every day until all larvae have emerged or died, or all larvae from control emerge into adult. An emergence rate of adult mosquito less than 10% is indicative of adequate pyriproxyfen quality in the ADD water sample. The samples are collected at the same time as the Container Index sampling and are used for quality control of the ADD and as positive control for the auto-dissemination testing.

To assess auto-dissemination efficacy of the ADDs, water samples of nearby containers are assessed for pyriproxyfen content. The *Aedes*-positive samples from natural containers collected for the CI (as explained above) are used to monitor larval development. Per 10ml water sample collected, one susceptible strain larvae (L₃, *Aedes aegypti*) is added to the water. Emergence of adults is monitored every day until all larvae have emerged or died. Significant reductions in adult mosquito emergence rates (i.e. high rates of larval/pupal mortality) in the intervention cluster samples compared to the control cluster samples is indicative of pyriproxyfen autodissemination from the ADDs.

Datamanagement

Epidemiological data are extracted from the e-Dengue system by the staff of the Vector Borne Disease Sector, Disease Control Division, Ministry of Health Malaysia. Confirmed dengue patients are assigned a unique anonymous code. Entomological data are reported on paper-based forms and subsequently entered into an Excel file by data managers at IMR. A second data manager checks the accuracy of the Excel file by comparing the paper-based forms and data entered in the Excel file. Access to the data is password protected and restricted to authorized study investigators and data management staff.

Both epidemiological and entomological data are sent to the department of Biostatistics in Lyon-France on a monthly basis. Data are controlled for consistency by the team in Lyon. The list of inconsistent or erroneous values (i.e. data queries) is sent to the data managers in IMR for verification. A database in SAS software is prepared for final statistical analyses. A common identifier for clusters is used in order to link the entomological and epidemiological databases.

The original paper-based entomological forms are stored in secured locked cabinets at IMR. As for epidemiological data, access to the data is password protected and restricted to only authorized study investigators and data management staff. The anonymized databases sent to the service of Biostatistics of Hospices Civils de Lyon are stored in the study folder on a secured computer server. Only the biostatisticians in charge of the analyses have access to the data. A daily backup of the study folder is made automatically.

No human samples will be collected for, or held by, this study. Entomology specimens will be stored at IMR. This study will be carried out in compliance with the Malaysian Personal Data Protection Act 2010, stipulating full protection of an individual's personal information.

All data, documents, the database, and the intervention procedure will be available for audits by regulatory and independent authorities appointed by funders or IMR.

Plans to promote participant retention and complete follow-up

Our previous study showed that regular contacts between the study population and the field workers creates public trust [21]. To maintain adherence to the interventions, a liaison officer assigned to each cluster contacts (via phone call or messaging) the building manager regularly to collect all complaints and feedback from the population. The pest control organisation in charge of TORS and ADDs deployment provides also regularly feed back to the CE team on any concerns faced in the field. These are discussed and taken into consideration in order to improve adherence to the intervention.

Statistical methods

Statistical analysis will be performed using the statistical analysis software SAS® version 9.4. or higher. Missing data will be reported and their impact on the outcomes may be explored. No interim analyses are planned.

Primary outcome

The primary endpoint is to measure the effectiveness of the IVM approach on dengue incidence. The average of the cluster-level incidence rates will be compared between the control and intervention arms. In each cluster, the incidence rate will be estimated as the ratio of the number of dengue cases registered during the trial (starting from the first cycle of spraying and ending three months after the last spraying cycle), divided by the number of person-years. The number of inhabitants per cluster, as obtained for the sample size calculation, is checked and corrected if needed during the site visits for the purpose of calculation of incidence rates.

A negative binomial regression model will be used to compare the incidence between control and intervention clusters. This will yield the rate ratio and its 95% confidence interval, taking into account overdispersion. The response variable for this analysis will be the number of cases, and the logarithm of the person-years will be included as an offset. Stratification (low versus medium income) will be included as a covariate.

Secondary outcome

The main secondary outcome will be the OI. The latter will be estimated over the baseline and intervention period in the 24 clusters selected for entomological monitoring. To quantify the effect of the interventions on the proportion of positive ovitraps, a modified ordinary least squares regression model using a robust standard error estimator will be implemented [21]. Using this model, the effect of the interventions will be quantified by the estimation of a difference of risk with its 95% confidence interval. This analysis will be carried out for both species overall and for each of the two species of mosquito separately.

Larval count per ovitrap (LI) will be estimated in the 24 clusters selected for entomological monitoring over the baseline and intervention period. A negative binomial regression will be used with the number of

larvae as the response variable. The analysis will be adjusted by the baseline measurement of the outcome. Using this model, the effect of the interventions will be quantified by the estimation of a ratio of means with its 95% confidence interval. This analysis will be carried out for both species overall and for each of the two species of mosquito separately if possible.

The adult population will be estimated by counting the number of adult female *Aedes* mosquitoes collected with sticky ovitraps during the study. The total number of adult females will be divided by the number of sticky ovitraps used to obtain the mean number of adult females.

The emergence rate, defined as the proportion of larvae that emerge as adult mosquitoes, will be estimated in each arm and compared between the two arms using the chi-squared test or the Fisher exact test. This analysis will be carried out for both mosquito species combined, and for each species separately.

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data

The main analysis will be by intention to treat. The clusters will be analysed in their randomisation arm. As explained above, the primary outcome is provided by the e-Dengue system and will be consequently available for the overall included clusters during the study period. A secondary per protocol analysis will be carried out excluding the clusters with any major deviations to the protocol. Major deviations will be defined by the technical committee (trial governance members) during the data review.

Trial governance

Implementation of the field work is coordinated by IMR (Malaysia). The Malaysian Ministry of Health provide technical expertise and is in charge of dengue data extraction from the e-Dengue database. The Departments of Epidemiology and Public Health, and of Biostatistics (Claude Bernard University and Hospices Civils de Lyon-France) host the project leader and are responsible for statistical analyses. The trial is governed by a technical committee composed of academic/private partnership with skills and knowledge in the epidemiology of infectious disease in particular vector-borne diseases, entomology, new vector control technologies, policy makers, and statistics. The committee defines the study design, review available data, and make decisions on trial conduct.

Adverse event reporting and harms

The product for spraying outdoor walls is already registered in Malaysia and has been in use for vector control management. Both bio-actives of ADDs have short half-lives and are classified as low risk for non-target organisms. However, instructions about precautionary measures to be taken before and after the intervention are provided in the posters (supplementary material appendix 2) and during community engagement meetings. In addition, a dedicated phone number is provided by the research team to be contacted in the case of an event which might be linked to the interventions. We do not plan to set up a data monitoring committee for the purpose of this cRCT. The trial interventions do not include drugs, and

no interim analysis is planned. On ethical review in Malaysia, the study was assessed as no more than minimal risk.

Plans for communicating important protocol amendments to relevant parties

Any modification in the protocol that significantly affect the scope or the scientific quality of the investigation will be submitted to the ethical committee with an amendment containing a verbatim description of the changes.

Dissemination plans

The results of the trial will be disseminated through international scientific conferences and submitted to highly ranked peer-reviewed journals for publication. The findings will also be shared with regional, national, and international stakeholders and partners. Reporting will follow the guidelines in the Consolidated Standards of Reporting Trials (CONSORT) Statement [37]. Authorship will follow guidelines established by the International Committee of Medical Journal Editors (<http://www.icmje.org/>) which require substantive contributions to the design, conduct, interpretation, and reporting of a trial.

Discussion

In the absence of vaccines and therapeutics, efficient, sustainable and cost-effective vector control programs are considered the principal method of protection against vector-borne diseases. New vector control technologies, prototypes or products such as spatial repellents, ADDs, sterile insect techniques, and *Wolbachia* are currently under investigation by the Vector Control Advisory Group VCAG-WHO [38]. However, the epidemiological evidence of their impact on the incidence of vector-borne diseases is limited [39].

The iDEM trial aims to address this gap by measuring the effectiveness of a preventive IVM approach on the incidence of dengue. The well-known spatial heterogeneity of dengue incidence that leads to increased baseline inter-cluster variance and the inter-annual fluctuation of dengue epidemic have been taken into consideration during the study power calculation and by conducting the study over two years respectively. The results of the trial will provide valuable insights that could be of interest to national and international institutions involved in the control of dengue.

The vector control tools of the proposed IVM are relatively new and have been scientifically proven to be effective against vectors [3, 21, 40]. High bio-efficacy for K-Othrine® Polyzone used in semi-indoor areas with > 80% mortality for a period of almost four months has been reported in two studies carried out in Malaysia [21, 41]. These results suggest that TORS can potentially reduce the frequency of current insecticide applications, in particular in densely populated urban districts where coverage of indoor preventive measures is low. ADDs are currently registered and used in over 45 countries worldwide and were shown to be effective as auto-dissemination device under field conditions [21, 40].

The choice of RCT was based on the WHO guideline that considers this type of trial as level 1 of the hierarchy of study designs that is sufficiently robust to generate evidence on the efficacy and public health value of vector control products [12]. The cluster rather than individual-based RCT is well adapted to assess organizational and behavioural interventions, health promotion programs, or interventions that are usually implemented at the level of health organizational units or geographical areas. Although such design requires large sample size, it reduces contamination between those assigned to the intervention or control arm. The large scale cRCT described in this protocol is unique in its approach. It combines two relatively new control tools that complement each other by targeting both larval and adult stages of the mosquito's life cycle. Using this combination, the TORS method will potentially reduce the infective adult mosquito while the ADDs will help to sustain the mosquito population below the threshold as described in the Malaysian Ministry of Health guideline [42]. This is further complemented by extensive public engagement activities to make the public better aware of the problems and solutions, encouraging a behavioural change. This could potentially increase the efficacy of vector control programs and decrease dengue incidence.

The study area is suitable for the proposed trial because Malaysia is one of the Asian countries hardest hit by dengue, with all its current 32.6 million inhabitants at risk. The country has appropriate infrastructure (expertise in vector control management, strong social mobilization capacities, and existence of surveillance systems), that facilitates the successful implementation of such large-scale trials. The WHO-IVM approach [13] underlines the importance of strengthening communication among different healthcare bodies such as policy-makers and vector-borne disease programme managers for the implementation of mosquito control strategies. The trial is, therefore, carried out in close collaboration with the Malaysian Ministry of Health, enabling local support and continuity of the proposed IVM approach if promising results are observed.

The trial is designed to measure the reduction in the incidence of dengue in the intervention relative to the control areas using incidence data extracted from the national e-Dengue surveillance system. Although passive dengue surveillance in endemic countries is essential for timely detection and containment of outbreaks, it is subject to under-ascertainment (cases not being diagnosed) and under-reporting (cases being diagnosed but not included in surveillance data). In Malaysia, failure to report leads to penalties for medical doctors. This is therefore likely to reduce the under-reporting, in particular for symptomatic dengue cases. Imperfection of the dengue diagnosis tests is another concern [43]. The use of combined IgM and NS1 detection into one test in Malaysia allows for high sensitivity during both the early (NS1) and late (IgM) phases of the disease, thus improving the overall test performance. Only cases with positive RDT test and fulfilling the international clinical criteria (ICD10: A90, A91) are registered in the e-Dengue surveillance system, thereby improving the specificity of the system [23].

One possible source of bias is that due to the nature of the intervention, blinding was not feasible. A feeling of protection provided by the intervention could potentially affect health-seeking behaviour in case of a febrile episode, or to a lesser extent to the use of other preventive measures. The latter reasoning has been suggested as an explanation in a recent study that evaluated the impact of insecticide treated

curtains on dengue virus transmission [44]. Another concern is the continued compliance of participating clusters and their collaboration with the field workers during the intervention cycles to remove bulky objects from the corridors at the time of TORS spraying. Public trust in the Malaysian Ministry of Health, responsible for routine dengue prevention and control, and representing the main coordinator of the present intervention together with continuous dialogue with the community by providing information about the program is expected to alleviate this issue. The routine vector control activities, which clearly are not being interrupted, will be monitored. However, these interventions would cause only an underestimation of the true effect of the proposed IVM strategy, relative to no action.

Even if efficient in the prevention and control of dengue, particularly for difficult to treat urban setting, a vector control program such as the preventive IVM approach presented here can be scaled-up as a national program if it is cost effective. Analysis of the cost-effectiveness of the proposed IVM strategy compared to the routine vector control activities is therefore a key parameter for its future adaptation as a national vector control program. Efforts are ongoing to secure the budget for the economic evaluation of our trial that will be conducted as an ancillary study of the present protocol.

In conclusion, a long-term residual effect of K-Othrine® Polyzone [21, 41], proven efficacy of ADDs [15, 16, 40] and relatively simple maintenance of both vector control methods would enable a sustainable preventative strategy instead of a responsive vector control. We expect that co-application of these vector control tools together with public cooperation could prove an invaluable vector control approach to reduce the burden of dengue. By regular communication with the residents, we hope to create local capabilities by transferring responsibilities (e.g. adding water to dried ADDs) and promote their participation (e.g. destruction of breeding sites) in national efforts to reduce the burden of dengue.

Considering the ongoing expansion of dengue burden in Malaysia, setting up proactive vector control strategies are critical. The results of this trial will be informative for a better understanding of IVM in the control of dengue. The results will be informative for a better understanding of effectiveness of proactive IVM approach in the control of dengue. Evidence from this trial may help justify investment in preventive IVM approaches as preferred to reactive case management strategies. It is conceivable that the result of this trial could be used in other locations with similar ecology or adapted to other *Aedes* borne diseases with same vector and process of transmission.

Abbreviations

ADD: Auto-dissemination device; CE: Community engagement; cRCT: Cluster Randomized Controlled Trials; CI: Container Index; ICD: International classification of diseases; IgG: Immunoglobulin G; IgM: Immunoglobulin M, IGR: Insect Growth Factor; iDEM: Intervention for Dengue Epidemiology in Malaysia; IMR: Institute for Medical Research; IVM: Integrated Vector Management; LI: Larval Index; OI: Ovitrap Index; PPF: Pyriproxyfen; TORS: Targeted Outdoor Residual Spray; WHO: World Health Organisation

Declarations

Trial status

At the time of submission of this manuscript the trial has completed the baseline data collections. The intervention began on February 10th 2020 and is expected to be completed by February 2022. As of October 16, 2020, community engagement activities were completed in all intervention clusters and consent form obtained. The first intervention cycle is now completed and the second cycle started on October 9th.

Ethics approval and consent to participate

The study protocol was submitted and approved by the IMR Research Management Committee and registered within the National Medical Research Register (NMRR-18-636-39710). The protocol has been also approved by the Medical Ethics research committee (MREC) on Dec 2019 (KKM/NIHSEC/P19 – 2362-7).

In Malaysia, each locality (cluster) has its own management team. Before the start of the intervention, meetings with the head of management are organized to explain the purposes of the trial. Written informed consent (supplementary material, appendix 5) to carry out the vector control activities is sought after the briefing.

Collection of epidemiological data, i.e. dengue incidence data will not seek consent at individual level because the data are from existing surveillance and under the 2017 the WHO Guidelines on Ethical Issues in Public Health Surveillance specifically guideline number 12, informed consent is not invariably necessary for surveillance*. All personal data concerning dengue cases will remain the property of the Malaysian Ministry of Health and kept strictly confidential. Only anonymized data will be shared with the department of Statistics in Lyon-France. The study will not provide medical care during or after the trial. The primary endpoint of the trial comes from the existing dengue surveillance system.

*WHO guidelines on ethical issues in public health surveillance. Geneva: World Health Organization; 2017. Licence: CC BY-NC-SA 3.0 IGO. ISBN 978-92-4-151265-7

Consent for publication

We do not intend to publish images of people, or identifiable data, in manuscripts deriving from the trial.

Availability of data and materials

Source documents as well as entomological specimens will be stored at the Medical Entomology Unit, Infectious Diseases Research Centre, Institute for Medical Research (Kuala Lumpur-Malaysia). The final dataset generated for the final analysis will be stored in the department of Biostatistics, Hospices Civils de Lyon-France. The database that will support the findings of the study will be available from the authors upon reasonable request and with permission of the Malaysian Ministry of Health. The full

protocol and statistical code will be accessible upon request. Participant level-data are not expected to be available.

Competing interests

RS and TM are remunerated by and hold shares in In2Care BV. FB and FS are employee of Bayer. Other authors do not have any competing interest to declare.

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Authors' contributions

MSE and NAH conceived the study. NAH manages the implementation of the interventions. MSE oversees the coordination of the study. NAH, RS, JR, and TM contributed to design of entomology work. NA, MR, CLJ and MSE contributed to study design and statistical analysis sections of the manuscript. MSE wrote the first draft of the manuscript. All authors critically reviewed and approved the final manuscript.

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Tables

Table 1: Time schedule of intervention, data collection and analysis

Year	2019			2020												2021											
Project Activities	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
COMMUNITY ENGAGEMENT																											
Site Visit & Site Scouting																											
Public Engagement																											
ENTOMOLOGY ENDPOINT																											
Ovitraping (once a month)																											
Identification of the larvae (once a month)																											
Mosquito colonization																											
Larval Survey (once every month)																											
Adult Survey (once every month)																											
Determination of Resistance (every cycle)																											
WHO Wall Deposit Bioassay																											
VECTOR CONTROL ACTIVITIES																											
TORS Cycle 1																											
TORS Cycle 2																											
TORS Cycle 3																											
TORS Cycle 4																											
Distribution of ADDs																											
ADDs Servicing and Monitoring - 1																											
ADDs Servicing and Monitoring - 2																											
ADDs Servicing and Monitoring - 3																											
ADDs Servicing and Monitoring - 4																											
ADDs Servicing and Monitoring - 5																											
ADDs Servicing and Monitoring - 6																											
ADDs Servicing and Monitoring - 7																											
Collection of ADTs																											
EPIDEMIOLOGY ENDPOINT																											
Collection of Dengue Cases Reported																											
Data Analysis																											

Figures

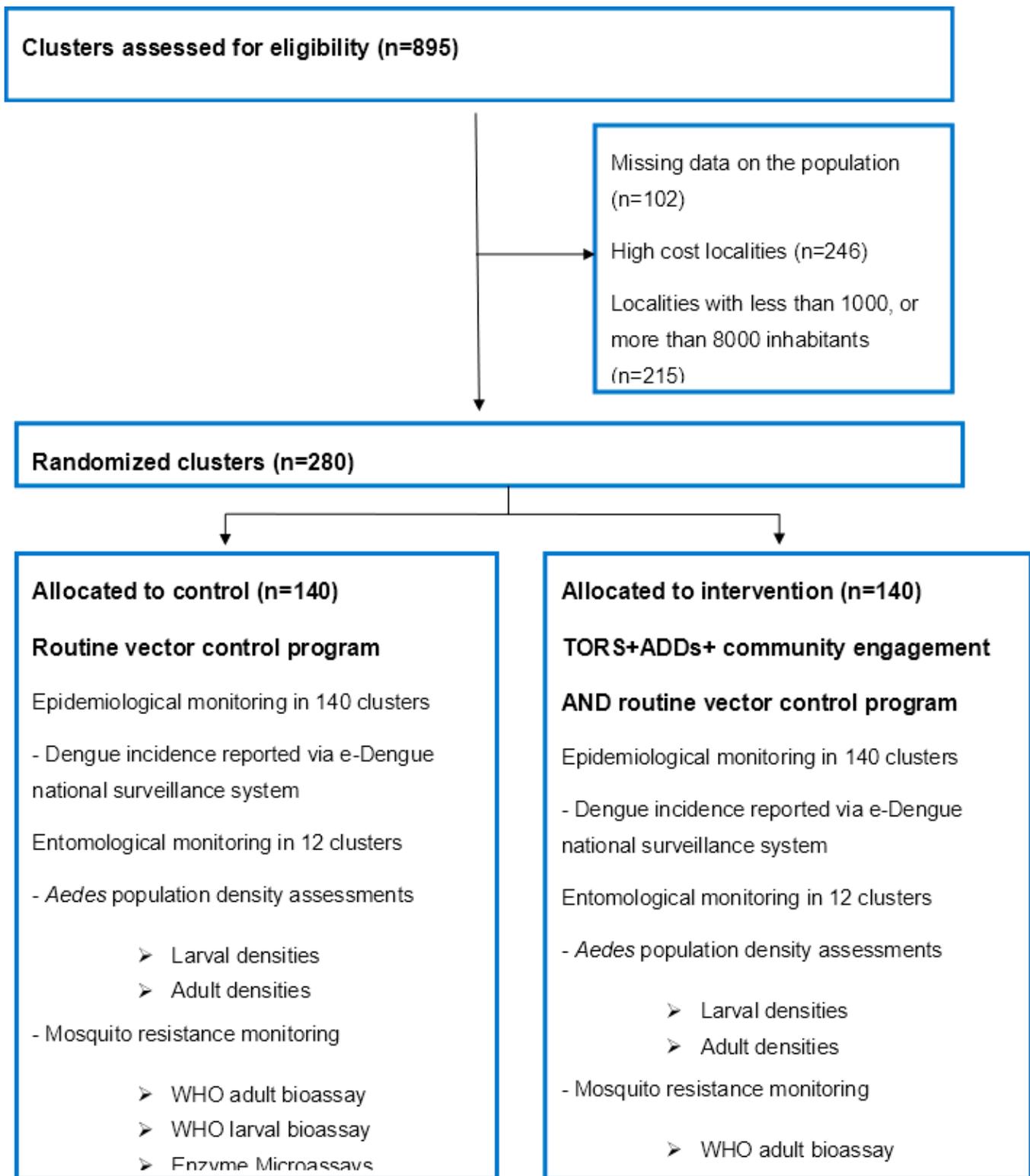


Figure 2

Flowchart of cluster selection and allocation, Federal Territory of Kuala Lumpur and Putrajaya

Supplementary Files

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- [Additionalfile2ADDstrategy30.10.2020.docx](#)
- [Additionalfile3poster30.10.2020.docx](#)
- [Additionalfile4SPIRITChecklist30.10.2020.docx](#)
- [Additionalfile5consentform.pdf](#)