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Targeted outdoor residual spraying, autodissemination devices and their combination against Aedes mosquitoes: Field implementation in a Malaysian urban setting

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Abstract:	Background Currently, dengue control relies largely on reactive vector control programs. Proactive vector-control using a rational, well-balanced Integrated Vector Management (IVM) approach may prove more successful for dengue control. Methodology As part of the development of a cluster randomized controlled epidemiological trial, a

study was conducted in Johor Bahru, Malaysia. The study included one control site (3 buildings) and three intervention sites to be treated with targeted outdoor residual spraying only (TORS site, 2 buildings), deployment of autodissemination devices only (ADD site, 4 buildings) and combination of outdoor residual spraying and deployment of autodissemination devices (TORS+ADD site, 3 buildings). The primary entomological measurement was percent of positive ovitraps—ovitrap index (OI). The effect of each intervention on OI was analysed by a modified ordinary least squares regression model.

Principal findings

Relative to the control site, the TORS and ADD sites showed reduction in the Aedes ovitrap index (-6.5%, p=0.04 and -8.3%, p=0.10 respectively). Analysis by species showed that as compared to the control site, Ae. aegypti density was lower in ADD (-8.9%, p=0.03) and TORS (-10.4%, p=0.02). No such effect was evident in the TORS+ADD site.

Conclusions/significance

the present study provides insights on the methods to be used for the main trial. The combination of multiple insecticides with different modes of action in one package is innovative, although we could not demonstrate the additive effect of TORS+ADD. Further work is required to strengthen our understanding of how these interventions impact dengue vector populations and dengue transmission.

Targeted outdoor residual spraying, autodissemination devices and their combination against *Aedes* mosquitoes: Field implementation in a Malaysian urban setting

Short title: Outdoor Residual spraying and autodissemination devices against *Aedes* in Malaysia

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Abstract

Background: Currently, dengue control relies largely on reactive vector control programs. Proactive vector-control using a rational, well-balanced Integrated Vector Management (IVM) approach may prove more successful for dengue control.

Methodology: As part of the development of a cluster randomized controlled epidemiological trial, a study was conducted in Johor Bahru, Malaysia. The study included one control site (3 buildings) and three intervention sites to be treated with targeted outdoor residual spraying only (TORS site, 2 buildings), deployment of autodissemination devices only (ADD site, 4 buildings) and combination of outdoor residual spraying and deployment of autodissemination devices (TORS+ADD site, 3 buildings). The primary entomological measurement was percent of positive ovitraps—ovitrap index (OI). The effect of each intervention on OI was analysed by a modified ordinary least squares regression model.

Principal findings: Relative to the control site, the TORS and ADD sites showed reduction in the *Aedes* ovitrap index (-6.5%, p=0.04 and -8.3%, p=0.10 respectively). Analysis by species showed that as compared to the control site, *Ae. aegypti* density was lower in ADD (-8.9%, p=0.03) and TORS (-10.4%, p=0.02). No such effect was evident in the TORS+ADD site.

Conclusions/significance: the present study provides insights on the methods to be used for the main trial. The combination of multiple insecticides with different modes of action in one package is innovative, although we could not demonstrate the additive effect of TORS+ADD. Further work is required to strengthen our understanding of how these interventions impact dengue vector populations and dengue transmission.

Key words

Malaysia; *Aedes*; vector control; integrated vector management; dengue

Introduction

Aedes mosquitos, primarily *Aedes aegypti* and to a lesser extent *Aedes albopictus* are responsible for the transmission of several viruses which cause dengue fever and dengue haemorrhagic fever, yellow fever, Zika viral disease and chikungunya fever. Over 3.5 billion people are estimated to be at risk in more than 120 countries with 390 million estimated infections per year. Of these infections, approximately 500,000 patients present with severe dengue requiring hospitalization, and an estimated 2.5% result in fatality [Bhatt et al., 2013; Gyawali et al., 2016].

In South-East Asia, the annual average of dengue illness was estimated to be about 2.9 million cases and 5,906 deaths, for a total a cost of approximately US$1 billion, almost half (US$451 million) being direct costs [Shepard et al., 2013].

Dengue is endemic in Malaysia, putting all 27.5 million inhabitants at permanent risk of infection. The annual incidence of dengue in Malaysia varied between 69.9 to 93.4 per 1000 population from 2001 to 2013 [Woon et al., 2018]. In 2009, the direct costs of dengue (medical costs and productivity loss) were over US$102 million. In addition, the Malaysian government spent US$73.5 million (0.03% of its GDP or 1.2% of its Health Care budget) on its National Dengue Vector Control Program. This amounts to US$1,591 per reported dengue case. Such expenditure on dengue vector control is not unique. Surrounding countries spend similar amounts: as an example, the yearly cost of dengue management in Singapore was US$50 million (0.02% GDP) [Carrasco et al., 2011].

The efficacy of vector control in reducing the density of *Aedes* population is well established [Schliessmann et al., 1974; PAHO, 1997; Kourí et al., 1998], but evidence of impact on *Aedes*-borne disease incidence is lacking [Bowman et al., 2014; Andersson et al., 2015].

Consequently, there is no consensus regarding the most cost-effective vector control tools to reduce their incidence [Achee et al., 2015]. The World Health Organization (WHO) recommends implementing cost-effective, sustainable and ecological sound integrated vector management (IVM), adapted to the local situation and using local resources and existing systems [WHO, 2012; WHO, 2017].

In Malaysia, dengue control relies mainly on reactive vector control such as space spray method (fogging), larviciding using temephos and Bti and source reduction. Proactive year-round vector-control using a rational, well balanced IVM strategy could have a greater impact on dengue fever incidence and may prove more cost-effective than the currently used reactive approach.

We plan to set-up a cluster randomized controlled trial (cRCT) to evaluate the effectiveness of a proactive IVM strategy on the incidence of dengue in Malaysia. The IVM strategy will combine targeted outdoor residual spraying (TORS) by K-Othrine Polyzone, deployment of auto-dissemination devices (ADDs) and extensive public engagement activities.

The active ingredient of the TORS, K-Othrine Polyzone, has been prequalified by the WHO for use in vector control activities [WHO, 2018]. K-Othrine Polyzone indoor residual spraying (IRS) application has been proven to reduce adult and immature *Aedes* populations (Paredes-Esquivel et al., 2016). K-Othrine Polyzone kills host-seeking and resting adult mosquitoes landing on the treated substrate, thereby lowering the number of the adult mosquitoes in the area [Dunford et al., 2018]. Its use in TORS can potentially reduce the frequency of current insecticide applications for *Aedes* control due to its longer residual effect (Hamid et al., 2019).

ADDs (In2Care®) attract and kill *Aedes* mosquitoes via a combination of a slow killing adulticide, the entomopathogenic fungus *Beauveria bassiana* strain GHA, and the juvenile hormone analogue pyriproxyfen (PPF), a larvicide that can be auto disseminated to surrounding breeding sites (Buckner et al., 2017). ADDs rely on mosquito behaviour to distribute the pesticide to cryptic, hard to find breeding sites and can potentially offer precision-targeted larval control and sustained breeding suppression of vector populations (Farenhorst et al., 2009; Snetselaar et al., 2014). Gravid female mosquitoes enter the trap searching for a place to lay their eggs. When landing on the floater the females contact gauze contaminated with PPF and *B.* The latter can take 7-14 days to develop and then kill exposed mosquitoes, providing the opportunity to transfer PPF from the ADDs to other surrounding larval habitats [Snetselaar et al., 2014].

The results of a field implementation study carried out to evaluate the feasibility and to provide guidance to optimize the methods and procedures for the set-up and conduct of the cRCT are presented here.

Methods

Setting

The study was carried out from February to June 2018 (3 weeks pre-treatment and 10 weeks intervention) in Johor Bahru, Malaysia. The study included one control site and three intervention sites to be treated with a) targeted outdoor residual spraying only (TORS site), b) deployment of autodissemination devices only (ADD site) and c) combination of outdoor residual spraying and deployment of autodissemination devices (TORS+ADD site). The study sites were located within 10Km radius with each other. TORS+ADD and ADD sites were 3Km apart (Figure 1). The control site comprised three buildings of 17 floors each. TORS site was composed of two buildings of 14 floors each. The number of buildings for ADD and TORS+ADD sites were respectively four (9 floors per building) and three (4 floors per building). This research was approved by the Malaysian Ministry of Health's Medical Research and Ethics Committee (17 Oct 2017).

Insecticide and treatments

Following the collection of pre-treatment data for a period of three weeks, outdoor space spraying was conducted (Ministry of Health Malaysia 2009) in all study sites for a quick and short-term reduction of the *Aedes* population. TORS was applied in TORS and TORS+ADD sites at week five and consisted of spraying semi-indoor and outdoor perimeter concrete walls with K-Othrine Polyzone. The latter contains deltamethrin as its active ingredient (62.5 g/l). The insecticide dosage was 25 mg/m^2 and was applied by using a compression sprayer. ADDs were deployed in two sites (ADD and TORS+ADD).

According to manufacturing specification, one ADD is necessary for every 400m^2. A logical distribution of ADDs generating a similar efficacy would be to treat every floor. But the key element of ADDs being the autodissemination effect, three strategies were evaluated in the intervention sites ADD and ADD+TORS to find a more economical distribution pattern : A) two ADDs on each floor (Strategy A, two buildings in ADD site and one in TORS+ADD site), B) two ADDs every second floor excluding the top floor(Strategy B, one in each site), and C) two ADDs on each of the first 2 floors, and 2 on the top floor (Strategy C, one in each site). Strategy C stems from the concept that most breeding sites are found at ground level, but high-rise buildings often have water reservoirs and potential breeding sites on the roof (Wan-Norafikah et al., 2010; Lau et al., 2013; Zainon et al., 2016).

Monitoring of *Aedes* population

A total of 87 outdoor (near bushes and small plants) and 136 semi-indoor (along the corridor, e.g. near shoe racks and flower pots) ovitraps were placed in the study areas to monitor mosquito density. Semi-indoor was defined as not being completely enclosed by walls (e.g. corridors open on one side) but covered and protected from sunlight and heavy rainfall. Entomological data were collected during the pre-treatment and for 10 weeks following the intervention.

An ovitrap consists of a 300 ml black plastic container with 6.5 cm of diameter and 9.0 cm in height. Fresh water was added to a level of 5.5 cm and an oviposition paddle (10 cm x 2.5 cm x 0.3 cm) made from hardboard was placed in the water with the rough surface upwards in each ovitrap. The ovitraps were collected and taken back to the laboratory every 7 days. All the larvae were counted and identified under a compound microscope (NIKON ECLIPSE E100, Japan). Evaluation of the adult *Aedes* population was based on the analysis of ovitraps (Lee et al., 1992) recommended by the Malaysian Ministry of Health.

Population-based survey and community engagement

We conducted a survey by interviewing 10% of the study population (head of households or any available adult) to evaluate their socio-economic status and to identify the most suitable communication strategy for the main trial.

Community engagement was conducted by meeting with head of localities and COMBI volunteers prior to the start of the study to explain the purpose of the study and to secure their cooperation and good will.

Statistical analyses

The primary entomological outcome was the weekly ovitrap index (OI), which is the percentage of positive ovitraps (i.e. those with evidence of larvae in the trap). This was calculated as the number of positive ovitraps divided by the total number of recovered ovitraps in each site at the end of each week. We also calculated the number of larvae per ovitrap (the larvae index, or LI) expressed as the total number of *Aedes* sp. larvae in each recovered ovitrap at the end of each week. To quantify the effect of each intervention on OI in comparison to control, a modified ordinary least squares regression model using a robust standard error estimator was implemented (Cheung et al., 2007). The mean LI during the pre-treatment (baseline) period of each site and the ovitrap location (semi-outdoor vs outdoor) were included in the regression model, as well as the intervention applied. The same analysis strategy was applied to quantify the intervention effect on LI using a negative binomial regression model.

Knowing the slow killing effect of ADDs due to targeting the next generation of mosquitoes, we also evaluated the effect of the interventions overtime by dividing the intervention period in two: weeks 1-5 and weeks 6-10. The analysis of each outcome (OI and LI) included an interaction between the intervention periods and the intervention sites.

Identification of the most suitable strategy for the deployment of ADDs was based on the above-mentioned modified ordinary least squares regression model for the OI and a negative binomial model for the LI. All analyses were carried out with SAS® software using the procedures proc surveyreg for the OI analysis and proc genmod for the LI analysis (version 9.4, SAS® Institute Inc., Cary, NC, USA). The regression coefficients were tested using Wald test. Statistical significance (two-sided) was set at $p \leq 0.05$.

Results

During the surveys, between 80 and 100% of the semi-indoor and outdoor ovitraps were recovered after seven days. Of the total of 65,118 larvae examined, 39,070 (60.0%) were *Ae. aegypti*, and 25,982 (39.9%) were *Ae. albopictus*. During the pre-treatment, the highest mean OI (56.5%) were found in TORS+ADD site while the lowest values were observed in the ADD site (mean: 19.0%) (Figure 2). Following the intervention, we observed an increase in the overall OI in all study sites, although there was weekly variation in both control and intervention areas. The overall OI and mean larvea index was in general higher in outdoor as compared to semi-indoor areas (Supp.Table S1). Analysis by species showed higher OI and LI for *Ae. aegypti* in semi-indoor areas (Supp.Table S1). As for OI, the mean LI overall was higher in all study sites during the intervention period as compared to the pre-treatment period (Supp. Figure S1).

The results of the effect of the intervention on OI are summarized in Table 1. As compared to the control site, the overall outdoor and semi-indoor OI was lower in the intervention sites ADD (-8.3%, p=0.04) and TORS (-6.5%, p=0.10) and slightly higher in TORS+ADD (+1.8, p=0.63). The difference reached statistical significance only in the ADD site. Relative to the control site, the outdoor and semi-indoor OI for *Ae. aegypti* was lower in ADD (-8.9%, p=0.03) and TORS (-10.4%, p=0.02) and slightly higher in TORS+ADD (+4.9%, p=0.29). Regarding *Ae. albopictus*, relative to the control site, outdoor and semi-indoor OI was slightly lower in ADD (-4.2%, p=0.19) and TORS+ADD (-3.4%, p=0.34) and slightly higher in TORS (+4.5%, p=0.18) but none reached statistical significance.

The analysis of the interaction with the period showed a greater effect of the intervention on OI during weeks 6-10 as compared to weeks 0-5 in TORS (-13.1% vs -0.66%, p=0.02) and ADD (-12.3% vs 4.7%, p=0.03) but the interaction did not reach statistical significance in TORS+ADD (-4.8% vs +7.9%, p=0.11) (Supp. Table S2).

The relative difference in mean number of larvae per ovitrap in ADD, TORS and TORS+ADD in comparison to the control site was estimated to be -35.4% (95% confidence interval (CI): -48.7, -18.7; p=0.004), -31.3% (95% CI: -46.8, -11.4; 0.0002) and +3.6% (95% CI: -22.9, +39.3; p=0.81) respectively (Table 2). Similar trends were observed for *Ae. aegypti* but the difference reached statistical significance only in ADD (-37.6%; p=0.002). Regarding *Ae. albopictus*, as compared to the control site, the mean number of larvae per ovitrap was lower in all intervention sites but none reached statistical significance. As for OI, the LI showed a greater effect of the intervention during weeks 6-10 compared to weeks 0-5 in TORS ($p<0.0001$) and ADD ($p<0.0001$) (Supp. Table S3).

Distribution of ADDs deployment strategies

Regarding the best strategy for the deployment of ADDs, the OI was significantly higher for strategy A (ADDs on all floors) (+10.9%; 95% CI: +0.02, +21.8, p=0.05) and strategy B (ADDs on every other floor excluding the top floor) (+18.2%; 95% CI: +7.4, +29.0;, p=0.001) as compared to strategy C (ADDs on the first 2 floors and on the top floor) (Supp. Table S4).

Population-based survey and community engagement

Baseline characteristics of the 732 individuals that completed the survey are presented in supplementary material (Supp. Table 5). Income categories were based on the report of Household Income and Basic Amenities 2016-Malaysia as follows: top 20% (T20: >US$1440/month), middle 40% (M40: US$720-1440/month), and bottom 40% (B40: <US$720/month) (Department of Statistics Malaysia; 2017). The highest percentage of individuals with primary school education and low income was observed in the TORS+ADT site. This site had also the highest rate of unemployed individuals. Television and radio were identified as the preferred source of information about dengue (71.5%), followed by internet (31%) and relatives (28.2%). COMBI volunteers were available in all study sites but did not participate to the study in the TORS+ADT site. Lower education level in this site might explain the observed lack of participation of COMBI volunteers.

Discussion

As part of the development of a cRCT, the present study provides insights on the methods to be used and some preliminary results on the effect of different vector control approaches on *Aedes* mosquito density in Johor Bahru-Malaysia.

As in other surveillance studies in Malaysia (Wan Norafikah et al., 2009; Lim et al., 2010; Norzahira et al., 2011), both *Aedes* vector species were present, though *Ae. aegypti* was the dominant species, representing 60.0% of the mosquito population.

We observed an increase in mosquito density, measured by OI and total larvae, following the intervention. It is reasonable to assume that the observed overall increase could be due to heavy rainfall. In a study carried out in Malaysia, the amount of rainfall was positively associated with OI after a one-month lag time, i.e. the time between hatching of eggs and first oviposition [Wee et al., 2013].

Relative to the control site, and even though hampered by sudden major rains, both interventions sites TORS and ADD showed a trend toward reduction in the *Aedes* populations, although the magnitude of these effects could not be expected to substantially reduce transmission. These preliminary results show that outdoor vector control strategies could be used for *Aedes* control in densely populated urban districts where coverage of indoor preventive measures is very low.

As reported in other investigations (Lee et al., 2015; Hamid et al., 2019) and in agreement with our results, TORS or ADDs effectively reduced the mosquito population. It can therefore be expected that co-application of these techniques together with public cooperation would further enhance the vector control efficacy. The lack of an observed additive effect of the combined TORS+ADD on the mosquito population may be related to socio-economic, waste management measures and architectural differences of the TORS+ADD site compared to the other intervention sites. Frequent presence of objects such as pet cages, fish aquarium, furniture and edible plants in the semi-indoor areas in this site led to TORS coverage of 50% as compared to 100% in the TORS site. An average coverage of 70% of walls is requested for an effective action of TORS. More discarded, often plastic, waste was also observed in the TORS+ADD site that could slow down the autodissemination effect of the ADDs. As plastic waste forms breeding sites, fewer females choose the ADD as primary breeding site. In addition, for those females that did choose the ADD first, the more breeding sites are available on leaving the ADD, the smaller is the initial effect. Some larger breeding sites need more than one mosquito visit to reach the appropriate threshold for killing over 80% of the pupae. Finally, 26% of ADDs were subject to vandalism in this site as compared to only 3% in the site with ADDs alone. The lower education level of the population and the lack of COMBI activities in TORS+ADT site could have contributed to higher vandalism, and presence of bird cages and aquaria at the time of TORS spraying, thus leading to the lack of effect. Moreover, the architecture of the buildings in TORS+ADD site make the semi-indoor walls more subject to rainfall and hence, plausibly, quicker wash-off of K-Othrine Polyzone during the heavy rainfall that occurred after the introduction of the intervention.

The observed greater effect of the intervention on the mosquito population overtime in the ADD site fit well with the slow killing effect of this device. ADD is designed to attract mosquitoes and then contaminate the adults which then carry pyriproxyfen to other sites before dying from the exposure to the *Beauveria* within approximately 10 days. The PPF targets the next generations; it prevents the pupae from transforming to the adult stage and tarsal contact with pyriproxyfen has been shown to suppress egg production and hatchability in adult females (Ohba et al., 2013). Thus, we do not expect to see much effect of PPF within the first 2 weeks. Over time accumulation of PPF occurs in surrounding breeding sites increasing the effectiveness of the ADDs. Depending on the size of the breeding site, a single contaminated mosquito might not be enough to kill the larvae in this breeding sites. Multiple visits might be necessary to reach this threshold, which again will delay the effect. A trend towards a lower proportion of positive ovitraps in the TORS+ADD area was observed although it is was not statistically significant. We do not have a specific explanation for the observed greater effect of TORS during weeks 6-10. An efficacy lag of one month on 24h mortality rates of *Anopheles gambiae* on wood panels treated with K-Othrine Polyzone was also reported by Dunford and collaborators (Dunford et al., 2018). The attempt to evaluate three ADD deployment strategies, including potentially suboptimal one, may have led to the effect of ADD being underestimated. However, the main objective of this study was to obtain information on the optimization of the intervention procedures for the cRCT, rather than obtaining a precise estimate of the intervention effect. Despite the reduced power resulting from multiple ADD deployment strategies across limited numbers of buildings, the results did give some insight as to optimal deployment. We found that strategy C (ADDs on the first 2 floors and on the top floor) seems to be a valid alternative to reduce the number of ADD needed while keeping the quality of the expected results. Strategy A with ADDs in every floor did not perform better than strategy C. Factors such as different overall population levels between buildings within a site or different distributions over the floors have been reported in the past (Lau et al., 2013) and could explain the observed results. The better result of strategy C compared to strategy B, even though more ADDs were deployed under strategy B, could be due to better/smarter distribution as strategy B did not include the second and the top floor. These floors have been reported as sometimes having a higher infestation than other floors (Wan-Norafikah et al., 2010; Zainon et al., 2016). If we were to draw a conclusion from these results, it would be that, in buildings up to 9 floors, reducing the ADD coverage from every floor to the first two and top floors seems to be possible without necessarily lowering the impact.

The data extracted from the National dengue surveillance system (eDengue) reported 11 dengue cases in the control area as compared to one, three and zero dengue cases in the TORS, ADD and TORS+ADD sites respectively during the study period. However, the study was not designed to test the impact of the interventions on dengue incidence.

Conclusions and lessons learned

The combination of multiple insecticides with different modes of action in one package is innovative, although we could not demonstrate the additive effect of TORS+ADD. Higher education level in TORS and ADT sites suggests better health literacy and could explain tangible results in these sites. Health education of the public will be the first step in community engagement for the planned cRCT epidemiological trial. Active public engagement will start before the intervention and will be maintained throughout the study period. Banners, posters, and announcement brochures will be distributed to explain the objectives of the study. Random allocation of eligible sites for the planned cRCT will be stratified on socio-economic status. The use of indoor ovitraps was not initially planned due to reluctance of the study population. However, regular contact between the study population and the field workers during the collection of baseline data created public trust and some flat owners accepted the ovitraps to be deployed in their homes (results not shown). For the cRCT, it is planned to place indoor ovitraps in volunteers' flats.

Offering a better understanding of a proactive IVM approach on *Aedes*-related diseases by conducting large scale randomized controlled trial is key to further reduce their incidence and improve global health. Successful implementation of such large-scale studies requires the existence of appropriate infrastructure (expertise in vector control management, strong social mobilization capacities, existence of surveillance systems) and high dengue endemicity. Furthermore, the Ministry of Health has an epidemiological and entomological surveillance system specifically for the *Aedes*-borne diseases: dengue, Zika and chikungunya. This system also records post-outbreak vector control activities and dengue virus serotypes. These are the main reasons for carrying out the planned trial in Malaysia. We believe that the planned cRCT will allow us to further expand upon and validate the entomological evidence generated here, to evaluate the impact of the proposed IVM approach on dengue incidence and to help shift the conception of policies to handle *Aedes*-borne diseases from treatment to prevention, thus saving public funding.

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Declaration of interest

FB and FS are employees of Bayer. RS is employee of In2Care. Other authors declare no conflict of interest.

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Table 1: Outdoor and semi-indoor Ovitrap index (overall and per species) in study sites during pre-treatment and intervention period and estimate of the ovitrap index differences in comparison to the control site (results of the modified ordinary least squares regression model)

	Pre-treatment		Intervention			
Study area	**N**	**OI (%)**	**N**	**OI (%)**	**Difference in OI relative to control* (95% CI)**	**p-value**
Overall						
Control	179	26.3	598	61.0	-	-
TORS	141	36.9	471	59.7	-6.5% (-14.4, +1.4)	0.10
ADD	142	19.0	484	52.9	-8.3% (-16.2, -0.3)	0.04
TORS+ADD	138	56.5	469	68.7	1.8% (-5.7, +9.4)	0.63
Ae. aegypti						
Control	179	18.4	598	47.2	-	-
TORS	141	12.1	471	31.0	-10.4% (-18.8, -2.0)	0.03
ADD	142	11.3	484	37.4	-8.9% (-16.9, -0.9)	0.01
TORS+ADD	138	26.8	469	47.3	4.9% (-4.2, +14.1)	0.29
Ae. albopictus						
Control	179	10.1	598	24.1	-	-
TORS	141	29.1	471	41.4	4.5% (-2.1, +11.1)	0.18
ADD	142	10.6	484	20.2	-4.2% (-10.5, +2.2)	0.19
TORS+ADD	138	41.3	469	34.9	-3.4% (-10.5, +3.6)	0.34

N: Total number of ovitraps recovered; OI: Ovitrap index; 95%CI: 95% confidence interval

*Adjusted for baseline and for ovitrap location

The number of oviposition sites was the same during the pre-treatment and intervention periods, but the positivity of the ovitraps was measured every week for 10 weeks during the intervention as compared to 3 weeks for the pre-treatment period.

Table 2: Outdoor and semi-indoor mean larval index (overall and per species) in study sites during pre-treatment and intervention period and estimate of the mean larvae index relative differences in comparison to the control site (results of the negative binomial model)

Larvae Index						
	Pre-treatment		**Intervention**			
Study area	**N**	**Mean (SD)**	**N**	**Mean (SD)**	**Relative difference* (95% CI)**	**p-value**
Overall						
Control	179	5.8 (16.1)	598	25.6 (36.4)	-	-
TORS	141	6.4 (14.7)	471	23.7 (37.7)	-31.3% (-46.8, -11.4)	0.0002
ADD	142	2.0 (7.0)	484	16.1 (27.8)	-35.4% (-48.7, -18.7)	0.004
TORS+ADD	138	15.3 (25.4)	469	30.2 (44.6)	3.63% (-22.9, +39.3)	0.81
Ae. aegypti						
Control	179	4.3 (14.4)	598	16.9 (30.8)	-	-
TORS	141	1.1 (5.1)	471	9.8 (26.6)	-24.9% (-51.8, +16.8)	0.20
ADD	142	0.9 (4.6)	484	10.4 (21.8)	-37.6% (-53.6, -15.9)	0.002
TORS+ADD	138	3.7 (11.1)	469	20.7 (41.3)	35.6% (-8.2, +100.4)	0.13
Ae. albopictus						
Control	179	1.5 (7.1)	598	8.6 (25.4)		
TORS	141	5.3 (13.9)	471	13.9 (26.9)	-26.39% (-48.9, +5.9)	0.09
ADD	142	1.1 (5.1)	484	5.7 (19.9)	-20.8% (-51.8, +30.2)	0.36
TORS+ADD	138	11.6 (22.6)	469	9.5 (21.9)	-12.5% (-44.4, +37.5)	0.56

N: Total number of ovitraps recovered; SD: standard deviation; 95% CI: 95% confidence interval

*Adjusted for baseline and for ovitrap location

Targeted outdoor residual spraying, autodissemination devices and their combination against *Aedes* mosquitoes: Field implementation in a Malaysian urban setting~~: A pilot study~~

Short title: Outdoor Residual spraying and autodissemination devices against *Aedes* in Malaysia

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Abstract

Background: ~~Dengue is endemic in Malaysia, putting all 27.5 million inhabitants at permanent risk of infection.~~ Currently, dengue control relies largely on reactive vector control programs. Proactive vector-control using a rational, well-balanced Integrated Vector Management (IVM) approach may prove more successful for dengue control.

Methodology: As part of the development of a cluster randomized controlled epidemiological trial, a ~~pilot~~ study was conducted in Johor Bahru, Malaysia. The study included one control site (3 buildings) and three intervention sites to be treated with targeted outdoor residual spraying only (TORS site, 2 buildings), deployment of autodissemination devices only (ADD site, 4 buildings) and combination of outdoor residual spraying and deployment of autodissemination devices (TORS+ADD site, 3 buildings). ~~The IVM approach combined space spraying, targeted outdoor residual spraying (TORS), larviciding and adulticiding using autodissemination devices (ADDs) and community engagement. The study included four sites with the following treatments: control, TORS, ADD, and TORS+ADD.~~ The primary entomological measurement was percent of positive ovitraps—ovitrap index (OI). The effect of each intervention on OI was analysed by a modified ordinary least squares regression model.

Principal findings: Relative to the control site, the TORS and ADD sites showed reduction in the *Aedes* ovitrap index (-6.5%, p=0.04 and -8.3%, p=0.10 respectively). Analysis by species showed that as compared to the control site, *Ae. aegypti* density was lower in ADD (-8.9%, p=0.03) and TORS (-10.4%, p=0.02). No such effect was evident in the TORS+ADD site.

Conclusions/significance: the present study provides insights on the methods to be used for the main trial. The combination of multiple insecticides with different modes of action in one package is innovative, although we could not demonstrate the additive effect of TORS+ADD.

~~Although we could not demonstrate the additive effect of TORS+ADD, the combination of both methods in one package is an innovative vector control intervention.~~ Further work is required to strengthen our understanding of how these interventions impact dengue vector populations and dengue transmission.

Key words

Malaysia; *Aedes*; vector control; integrated vector management; dengue

Introduction

Aedes mosquitos, primarily *Aedes aegypti* and to a lesser extent *Aedes albopictus* are responsible for the transmission of several viruses which cause dengue fever and dengue haemorrhagic fever, yellow fever, Zika viral disease and chikungunya fever. Over 3.5 billion people are estimated to be at risk in more than 120 countries with 390 million estimated infections per year. Of these infections, approximately 500,000 patients present with severe dengue requiring hospitalization, and an estimated 2.5% result in fatality [Bhatt et al., 2013; Gyawali et al., 2016].

In South-East Asia, the annual average of dengue illness was estimated to be about 2.9 million cases and 5,906 deaths, for a total a cost of approximately US$1 billion, almost half (US$451 million) being direct costs [Shepard et al., 2013].

Dengue is endemic in Malaysia, putting all 27.5 million inhabitants at permanent risk of infection. The annual incidence of dengue in Malaysia varied between 69.9 to 93.4 per 1000 population from 2001 to 2013 [Woon et al., 2018]. In 2009, the direct costs of dengue (medical costs and productivity loss) were over US$102 million. In addition, the Malaysian government spent US$73.5 million (0.03% of its GDP or 1.2% of its Health Care budget) on its National Dengue Vector Control Program. This amounts to US$1,591 per reported dengue case. Such expenditure on dengue vector control is not unique. Surrounding countries spend similar amounts: as an example, the yearly cost of dengue management in Singapore was US$50 million (0.02% GDP) [Carrasco et al., 2011].

The efficacy of vector control in reducing the density of *Aedes* population is well established [Schliessmann et al., 1974; PAHO, 1997; Kourí et al., 1998], but evidence of impact on *Aedes*-borne disease incidence is lacking [Bowman et al., 2014; Andersson et al., 2015].

Consequently, there is no consensus regarding the most cost-effective vector control tools to reduce their incidence [Achee et al., 2015]. The World Health Organization (WHO) recommends implementing cost-effective, sustainable and ecological sound integrated vector management (IVM), adapted to the local situation and using local resources and existing systems [WHO, 2012; WHO, 2017].

In Malaysia, dengue control relies mainly on reactive vector control such as space spray method ~~including~~ (fogging), larviciding using temephos and Bti and source reduction ~~after a dengue case is detected~~. Proactive year-round vector-control using a rational, well balanced IVM strategy could have a greater impact on dengue fever incidence and may prove more cost-effective than the currently used reactive approach.

We plan to set-up a cluster randomized controlled trial (cRCT) to evaluate the effectiveness of a proactive IVM strategy on the incidence of dengue in Malaysia. The IVM strategy will combine targeted outdoor residual spraying (TORS) by K-Othrine Polyzone, deployment of auto-dissemination devices (ADDs) and extensive public engagement activities.

The active ingredient of the TORS, K-Othrine Polyzone, has been prequalified by the WHO for use in vector control activities [WHO, 2018]. K-Othrine Polyzone indoor residual spraying (IRS) application has been proven to reduce adult and immature *Aedes* populations (Paredes-Esquivel et al., 2016). K-Othrine Polyzone kills host-seeking and resting adult mosquitoes landing on the treated substrate, thereby lowering the number of the adult mosquitoes in the area [Dunford et al., 2018]. Its use in TORS can potentially reduce the frequency of current insecticide applications for *Aedes* control due to its longer residual effect (Hamid et al., 2019).

ADDs (In2Care®) attract and kill *Aedes* mosquitoes via a combination of a slow killing adulticide, the entomopathogenic fungus *Beauveria bassiana* strain GHA, and the juvenile hormone analogue pyriproxyfen (PPF), a larvicide that can be auto disseminated to surrounding breeding sites (Buckner et al., 2017). ADDs rely on mosquito behaviour to distribute the pesticide to cryptic, hard to find breeding sites and can potentially offer precision-targeted larval control and sustained breeding suppression of vector populations (Farenhorst et al., 2009; Snetselaar et al., 2014). Gravid female mosquitoes enter the trap searching for a place to lay their eggs. When landing on the floater the females contact gauze contaminated with PPF and *B.* The latter can take 7-14 days to develop and then kill exposed mosquitoes, providing the opportunity to transfer PPF from the ADDs to other surrounding larval habitats [Snetselaar et al., 2014].

The results of a field implementation study carried out to evaluate the feasibility and to provide guidance to optimize the methods and procedures for the set-up and conduct of the cRCT are presented here. ~~The present study reports the results of a pilot study that was carried out to optimize the methods and procedures to be used for the main trial.~~

Methods

Setting

The ~~pilot~~ study was carried out from February to ~~May~~ June 2018 (3 weeks pre-treatment and 10 weeks intervention) in Johor Bahru, Malaysia. The study included one control site and three intervention sites to be treated with a) targeted outdoor residual spraying only (TORS site), b) deployment of autodissemination devices only (ADD site) and c) combination of outdoor residual spraying and deployment of autodissemination devices (TORS+ADD site).

The study sites were located within 10Km radius with each other. TORS+ADD and ADD sites were 3Km apart (Figure 1). The control site comprised three buildings of 17 floors each. TORS site was composed of two buildings of 14 floors each. The number of buildings for ADD and TORS+ADD sites were respectively four (9 floors per building) and three (4 floors per building). This research was approved by the Malaysian Ministry of Health's Medical Research and Ethics Committee (17 Oct 2017).

Insecticide and treatments

Following the collection of pre-treatment data for a period of three weeks, outdoor space spraying was conducted (Ministry of Health Malaysia 2009) in all study sites for a quick and short-term reduction of the *Aedes* population. TORS was applied in TORS and TORS+ADD sites at week five and consisted of spraying semi-indoor and outdoor perimeter concrete walls with K-Othrine Polyzone. The latter contains deltamethrin as its active ingredient (62.5 g/l). The insecticide dosage was 25 mg/m^2 and was applied by using a compression sprayer. ADDs were deployed in two sites (ADD and TORS+ADD).

According to manufacturing specification, one ADD is necessary for every 400m^2. A logical distribution of ADDs generating a similar efficacy would be to treat every floor. But the key element of ADDs being the autodissemination effect, three strategies were evaluated in the intervention sites ADD and ADD+TORS to find a more economical distribution pattern : A) two ADDs on each floor (Strategy A, two buildings in ADD site and one in TORS+ADD site), B) two ADDs every second floor excluding the top floor(Strategy B, one in each site), and C) two ADDs on each of the first 2 floors, and 2 on the top floor (Strategy C, one in each site). Strategy C stems from the concept that most breeding sites are found at ground level, but high-rise buildings often have water reservoirs and potential breeding sites on the roof (Wan-Norafikah et al., 2010; Lau et al., 2013; Zainon et al., 2016).

Monitoring of *Aedes* population

A total of 87 outdoor (near bushes and small plants) and 136 semi-indoor (along the corridor, e.g. near shoe racks and flower pots) ovitraps were placed in the study areas to monitor mosquito density. Semi-indoor was defined as not being completely enclosed by walls (e.g. corridors open on one side) but covered and protected from sunlight and heavy rainfall. Entomological data were collected during the pre-treatment and for 10 weeks following the intervention.

An ovitrap consists of a 300 ml black plastic container with 6.5 cm of diameter and 9.0 cm in height. Fresh water was added to a level of 5.5 cm and an oviposition paddle (10 cm x 2.5 cm x 0.3 cm) made from hardboard was placed in the water with the rough surface upwards in each ovitrap. The ovitraps were collected and taken back to the laboratory every 7 days. All the larvae were counted and identified under a compound microscope (NIKON ECLIPSE E100, Japan). Evaluation of the adult *Aedes* population was based on the analysis of ovitraps (Lee et al., 1992) recommended by the Malaysian Ministry of Health.

Population-based survey and community engagement

We conducted a survey by interviewing 10% of the study population (head of households or any available adult) to evaluate their socio-economic status and to identify the most suitable communication strategy for the main trial.

Community engagement was conducted by meeting with head of localities and COMBI volunteers prior to the start of the study to explain the purpose of the study and to secure their cooperation and good will.

Statistical analyses

The primary entomological outcome was the weekly ovitrap index (OI), ~~i.e. the number~~ which is the percentage of positive ovitraps (i.e. those with evidence of larvae in the trap). This was calculated as the number of positive ovitraps divided by the total number of recovered ovitraps in each site at the end of each week~~expressed as a percentage~~. We also ~~analysed the weekly larval~~ calculated the number of larvae ~~number~~ per ovitrap (the larvae index, or LI) expressed as the total number of *Aedes* sp. larvae ~~per~~ in each recovered ovitrap at the end of each week. To quantify the effect of each intervention on OI in comparison to control, a modified ordinary least squares regression model using a robust standard error estimator was implemented (Cheung et al., 2007). The mean LI during the pre-treatment (baseline) period of each site and the ovitrap location (semi-outdoor vs outdoor) were included in the regression model, as well as the intervention applied. ~~The analysis was adjusted on the LI at pre-treatment and the ovitrap location (semi-indoor vs outdoor).~~ The same analysis strategy was applied to quantify the intervention effect on LI using a negative binomial regression model.

Knowing the slow killing effect of ADDs due to targeting the next generation of mosquitoes, we also evaluated the effect of the interventions overtime by dividing the intervention period in two: weeks 1-5 and weeks 6-10. The analysis of each outcome (OI and LI) included an interaction between the intervention periods and the intervention sites.

Identification of the most suitable strategy for the deployment of ADDs was based on ~~a~~ the above-mentioned modified ordinary least squares regression model for the OI and a negative binomial model for the LI. ~~The analyses were adjusted on the pre-treatment LI and the intervention sites (ADD or TORS+ADD).~~ All analyses were carried out with SAS® software using the procedures proc surveyreg for the OI analysis and proc genmod for the LI analysis (version 9.4, SAS® Institute Inc., Cary, NC, USA). The regression coefficients were tested using Wald test. Statistical significance (two-sided) was set at p ≤0.05.

Results

During the surveys, between 80 and 100% of the semi-indoor and outdoor ovitraps were recovered after seven days. Of the total of 65,118 larvae examined, 39,070 (60.0%) were *Ae. aegypti*, and 25,982 (39.9%) were *Ae. albopictus*. During the pre-treatment, the highest mean OI (56.5%) were found in TORS+ADD site while the lowest values were observed in the

ADD site (mean: 19.0%) (Figure 2). Following the intervention, we observed an increase in the overall OI in all study sites, although there was weekly variation in both control and intervention areas. The overall OI and mean larvea index was in general higher in outdoor as compared to semi-indoor areas (Supp.Table S1). Analysis by species showed higher OI and LI for *Ae. aegypti* in semi-indoor areas (Supp.Table S1). As for OI, the mean LI overall was higher in all study sites during the intervention period as compared to the pre-treatment period (Supp. Figure S1).

The results of the effect of the intervention on OI are summarized in Table 1. As compared to the control site, the overall outdoor and semi-indoor OI was lower in the intervention sites ADD (-8.3%, p=0.04) and TORS (-6.5%, p=0.10) and slightly higher in TORS+ADD (+1.8, p=0.63). The difference reached statistical significance only in the ADD site. Relative to the control site, the outdoor and semi-indoor OI for *Ae. aegypti* was lower in ADD (-8.9%, p=0.03) and TORS (-10.4%, p=0.02) and slightly higher in TORS+ADD (+4.9%, p=0.29). Regarding *Ae. albopictus*, relative to the control site, outdoor and semi-indoor OI was slightly lower in ADD (-4.2%, p=0.19) and TORS+ADD (-3.4%, p=0.34) and slightly higher in TORS (+4.5%, p=0.18) but none reached statistical significance.

The analysis of the interaction with the period showed a greater effect of the intervention on OI during weeks 6-10 as compared to weeks 0-5 in TORS (-13.1% vs -0.66%, p=0.02) and ADD (-12.3% vs 4.7%, p=0.03) but the interaction did not reach statistical significance in TORS+ADD (-4.8% vs +7.9%, p=0.11) (Supp. Table S2).

The relative difference in mean number of larvae per ovitrap in ADD, TORS and TORS+ADD in comparison to the control site was estimated to be -35.4% (95% confidence interval (CI): -48.7, -18.7; p=0.004), -31.3% (95% CI: -46.8, -11.4; 0.0002) and +3.6% (95% CI: -22.9, +39.3; p=0.81) respectively (Table 2). Similar trends were observed for *Ae. aegypti* but the difference reached statistical significance only in ADD (-37.6%; p=0.002). Regarding *Ae. albopictus*, as compared to the control site, the mean number of larvae per ovitrap was lower in all intervention sites but none reached statistical significance. As for OI, the LI showed a greater effect of the intervention during weeks 6-10 compared to weeks 0-5 in TORS (p<0.0001) and ADD (p<0.0001) (Supp. Table S3).

Distribution of ADDs deployment strategies

Regarding the best strategy for the deployment of ADDs, the OI was significantly higher for strategy A (ADDs on all floors) (+10.9%; 95% CI: +0.02, +21.8, p=0.05) and strategy B (ADDs on every other floor excluding the top floor) (+18.2%; 95% CI: +7.4, +29.0;, p=0.001) as compared to strategy C (ADDs on the first 2 floors and on the top floor) (Supp. Table S4).

Population-based survey and community engagement

Baseline characteristics of the 732 individuals that completed the survey are presented in supplementary material (Supp. Table 5). Income categories were based on the report of Household Income and Basic Amenities 2016-Malaysia as follows: top 20% (T20: >US$1440/month), middle 40% (M40: US$720-1440/month), and bottom 40% (B40: <US$720/month) (Department of Statistics Malaysia; 2017). The highest percentage of individuals with primary school education and low income was observed in the TORS+ADT site. This site had also the highest rate of unemployed individuals. Television and radio were identified as the preferred source of information about dengue (71.5%), followed by internet (31%) and relatives (28.2%). COMBI volunteers were available in all study sites but did not participate to the study in the TORS+ADT site. Lower education level in this site might explain the observed lack of participation of COMBI volunteers.

Discussion

As part of the development of a cRCT, the present study provides insights on the methods to be used and some preliminary results on the effect of ~~an IVM approach~~different vector control approaches on *Aedes* mosquito density in Johor Bahru-Malaysia. ~~To our knowledge, this is the first study investigating a proactive approach combining two quite new vector control methods to manage both larval and adult stages of *Aedes* populations.~~

As in other surveillance studies in Malaysia (Wan Norafikah et al., 2009; Lim et al., 2010; Norzahira et al., 2011), both *Aedes* vector species were present, though *Ae. aegypti* was the dominant species, representing 60.0% of the mosquito population.

~~vector control methods used in the study i.e. TORS and ADDs were evaluated separately and showed to effectively reduce the mosquito population (Lee et al., 2015). The active ingredient of the TORS, i.e. K-Othrine Polyzone, has been prequalified by the WHO for use in vector control activities [WHO, 2018]. This insecticide kills host-seeking and resting adult mosquitoes landing on the treated substrate, thereby lowering the number of the adult mosquitoes in the area [Dunford et al., 2018]. Its use in TORS can potentially reduce the frequency of current insecticide applications for *Aedes* control due to its longer residual effect (Hamid et al., 2019).~~

~~ADDs rely on mosquito's behavior to distribute the pesticide in cryptic, hard to find breeding sites and can potentially offer precision-targeted larval control and sustained breeding suppression of vector populations (Farenhorst et al., 2009; Snetselaar et al., 2014). Gravid female mosquitoes enter the trap searching for a place to lay their eggs. When landing on the floater the females contact gauze contaminated with PPF and *B. bassiana* spores. *Beauveria bassiana* spores can take 7-14 days to kill exposed mosquitoes providing the~~

~~opportunity to transfer PPF from the In2Care mosquito trap to other surrounding larval habitats before dying [Snetselaar et al., 2014].~~

We observed an increase in mosquito density, measured by OI and total larvae, following the intervention. It is reasonable to assume that the observed overall increase could be due to heavy rainfall. In a study carried out in Malaysia, the amount of rainfall was positively associated with OI after a one-month lag time, i.e. the time between hatching of eggs and first oviposition [Wee et al., 2013].

Relative to the control site, and even though hampered by sudden major rains, both interventions sites TORS and ADD showed a trend toward reduction in the *Aedes* populations~~.~~, although the magnitude of these effects could not be expected to substantially reduce transmission. These preliminary results show that outdoor vector control strategies could be used for *Aedes* control in densely populated urban districts where coverage of indoor preventive measures is very low.

As reported in other investigations (Lee et al., 2015; Hamid et al., 2019) and in agreement with our results, TORS or ADDs effectively reduced the mosquito population. It can therefore be expected that co-application of these techniques together with public cooperation would further enhance the vector control efficacy. The lack of an observed additive effect of the combined TORS+ADD on the mosquito population may be related to socio-economic, waste management measures and architectural differences of the TORS+ADD site compared to the other intervention sites. ~~the reduced TORS coverage due to frequent~~ Frequent presence of objects such as pet cages, fish aquarium, furniture and edible plants ~~a~~ in the semi-indoor areas in this site led to TORS coverage of 50% as compared to 100% in the TORS site. An average coverage of 70% of walls is requested for an effective action of TORS. More discarded, often plastic, waste was also observed in the TORS+ADD site that could slow down the autodissemination effect of the ADDs. As plastic waste forms breeding sites, fewer females choose the ADD as primary breeding site. In addition, for those females that did choose the ADD first, the more breeding sites are available on leaving the ADD, the smaller is the initial effect. Some larger breeding sites need more than one mosquito visit to reach the appropriate threshold for killing over 80% of the pupae. Finally, 26% of ADDs were subject to vandalism in this site as compared to only 3% in the site with ADDs alone. The lower education level of the population and the lack of COMBI activities in TORS+ADT site could have contributed to higher vandalism, and presence of bird cages and aquaria at the time of TORS spraying, thus leading to the lack of effect. Moreover, the architecture of the buildings in TORS+ADD site make the semi-indoor walls more subject to rainfall and hence, plausibly, quicker wash-off of K-Othrine Polyzone during the heavy rainfall that occurred after the introduction of the intervention.

~~Extensive involvement of the community is a key parameter of the IVM approach and has been shown to enhance the effectiveness of vector control programs [Andersson et al., 2015]. Community engagement was conducted by meeting with local officials, head of COMBI committees and local COMBI volunteers prior to the start of the study to explain its purpose and secure their cooperation and good will. COMBI teams were available in all study sites but were actively involved in the study only in TORS and ADD sites. The lack of COMBI activities in the TORS+ADD site could have contributed to higher vandalism, and presence of bird cages and aquaria at the time of TORS spraying, thus leading to the lack of effect.~~

The observed greater effect of the intervention on the mosquito population overtime in the ADD site fit well with the slow killing effect of this device. ADD is designed to attract mosquitoes and then contaminate the adults which then carry pyriproxyfen to other sites before dying from the exposure to the *Beauveria* within approximately 10 days. The PPF targets the next generations; it prevents the pupae from transforming to the adult stage and tarsal contact with pyriproxyfen has been shown to suppress egg production and hatchability in adult females (Ohba et al., 2013). Thus, we do not expect to see much effect of PPF within the first 2 weeks. Over time accumulation of PPF occurs in surrounding breeding sites increasing the effectiveness of the ADDs.- Depending on the size of the breeding site, a single contaminated mosquito might not be enough to kill the larvae in this breeding sites. Multiple visits might be necessary to reach this threshold, which again will delay the effect. A trend towards a lower proportion of positive ovitraps in the TORS+ADD area was observed although it is was not statistically significant. We do not have a specific explanation for the observed greater effect of TORS during weeks 6-10. An efficacy lag of one month on 24h mortality rates of *Anopheles gambiae* on wood panels treated with K-Othrine Polyzone was also reported by Dunford and collaborators (Dunford et al., 2018). The attempt to evaluate three ADD deployment strategies, including potentially suboptimal one, may have led to the effect of ADD being underestimated. However, the main objective of this study was to obtain information on the optimization of the intervention procedures for the cRCT, rather than obtaining a precise estimate of the intervention effect. Despite the reduced power resulting from multiple ADD deployment strategies across limited numbers of buildings, the results did give some insight as to optimal deployment. We found that strategy C (ADDs on the first 2 floors and on the top floor) seems to be a valid alternative to reduce the number of ADD needed while keeping the quality of the expected results. Strategy A with ADDs in every floor did not perform better than strategy C. Factors such as different overall population levels between buildings within a site or different distributions over the floors have been reported in the past (Lau et al., 2013) and could explain the observed results. The better result of strategy C compared to strategy B, even though more

ADDs were deployed under strategy B, could be due to better/smarter distribution as strategy B did not include the second and the top floor. These floors have been reported as sometimes having a higher infestation than other floors (Wan-Norafikah et al., 2010; Zainon et al., 2016). If we were to draw a conclusion from these results, it would be that, in buildings up to 9 floors, reducing the ADD coverage from every floor to the first two and top floors seems to be possible without necessarily lowering the impact.

The data extracted from the National dengue surveillance system (eDengue) reported 11 dengue cases in the control area as compared to one, three and zero dengue cases in the TORS, ADD and TORS+ADD sites respectively during the study period. However, the study was not designed ~~powered sufficiently~~ to test the impact of the interventions on dengue incidence.

Conclusions and lessons learned

The combination of multiple insecticides with different modes of action in one package is innovative, although ~~Although~~ we could not demonstrate the additive effect of TORS+ADD. ~~, the combination of both methods in one package is an innovative vector control intervention.~~

Higher education level in TORS and ADT sites suggests better health literacy and could explain tangible results in these sites. Health education of the public will be the first step in community engagement for the planned cRCT epidemiological trial. Active public engagement will start before the intervention and will be maintained throughout the study period. Banners, posters, and announcement brochures will be distributed to explain the objectives of the study. Random allocation of eligible sites for the planned cRCT will be stratified on socio-economic status. The use of indoor ovitraps was not initially planned due to reluctance of the study population. However, regular contact between the study population and the field workers during the collection of baseline data created public trust and some flat owners accepted the ovitraps to be deployed in their homes (results not shown). For the cRCT, it is planned to place indoor ovitraps in volunteers' flats.

~~Moreover, using a combination of several active ingredients (chemical and biological with different modes of action) in the same program is expected to have significant benefits for insecticide resistance management. Many national programs are using combinations of methods but evidence on best practices and the most cost-effective integrated approaches is needed.~~

Offering a better understanding of a proactive IVM approach on *Aedes*-related diseases by conducting ~~the planned~~ large scale randomized controlled trial is key to further reduce their incidence and improve global health. Successful implementation of such large-scale studies requires the existence of appropriate infrastructure (expertise in vector control management, strong social mobilization capacities, existence of surveillance systems) and high dengue endemicity. Furthermore, the Ministry of Health has an epidemiological and entomological surveillance system specifically for the *Aedes*-borne diseases: dengue, Zika and chikungunya. This system also records post-outbreak vector control activities and dengue virus serotypes. These are the main reasons for carrying out the planned trial in Malaysia. We believe that the planned cRCT will allow us to further expand upon and validate the entomological evidence generated here, to evaluate the impact of the proposed IVM approach on dengue incidence and to help shift the conception of policies to handle *Aedes*-borne diseases from treatment to prevention, thus saving public funding.

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Declaration of interest

FB and FS are employees of Bayer. RS is employee of In2Care. Other authors declare no conflict of interest.

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Table 1: Outdoor and semi-indoor Ovitrap index (overall and per species) in study sites during pre-treatment and intervention period and estimate of the ovitrap index differences in comparison to the control site (results of the modified ordinary least squares regression model)

	Pre-treatment		**Intervention**			
Study area	**N**	**OI (%)**	**N**	**OI (%)**	**Difference in OI relative to control* (95% CI)**	**p-value**
Overall						
Control	179	26.3	598	61.0	-	-
TORS	141	36.9	471	59.7	-6.5% (-14.4, +1.4)	0.10
ADD	142	19.0	484	52.9	-8.3% (-16.2, -0.3)	0.04
TORS+ADD	138	56.5	469	68.7	1.8% (-5.7, +9.4)	0.63
Ae. aegypti						
Control	179	18.4	598	47.2	-	-
TORS	141	12.1	471	31.0	-10.4% (-18.8, -2.0)	0.03
ADD	142	11.3	484	37.4	-8.9% (-16.9, -0.9)	0.01
TORS+ADD	138	26.8	469	47.3	4.9% (-4.2, +14.1)	0.29
Ae. albopictus						
Control	179	10.1	598	24.1	-	-
TORS	141	29.1	471	41.4	4.5% (-2.1, +11.1)	0.18
ADD	142	10.6	484	20.2	-4.2% (-10.5, +2.2)	0.19
TORS+ADD	138	41.3	469	34.9	-3.4% (-10.5, +3.6)	0.34

N: Total number of ovitraps recovered; OI: Ovitrap index; 95%CI: 95% confidence interval

*Adjusted for baseline and for ovitrap location

The number of oviposition sites was the same during the pre-treatment and intervention periods, but the positivity of the ovitraps was measured every week for 10 weeks during the intervention as compared to 3 weeks for the pre-treatment period.

549 **Table 2**: Outdoor and semi-indoor mean larval index (overall and per species) in study sites during pre-treatment and intervention period and estimate of the mean larvae index relative differences in comparison to the control site (results of the negative binomial model)

Larvae Index						
	Pre-treatment		**Intervention**			
Study area	**N**	**Mean (SD)**	**N**	**Mean (SD)**	**Relative difference* (95% CI)**	**p-value**
Overall						
Control	179	5.8 (16.1)	598	25.6 (36.4)	-	-
TORS	141	6.4 (14.7)	471	23.7 (37.7)	-31.3% (-46.8, -11.4)	0.0002
ADD	142	2.0 (7.0)	484	16.1 (27.8)	-35.4% (-48.7, -18.7)	0.004
TORS+ADD	138	15.3 (25.4)	469	30.2 (44.6)	3.63% (-22.9, +39.3)	0.81
Ae. aegypti						
Control	179	4.3 (14.4)	598	16.9 (30.8)	-	-
TORS	141	1.1 (5.1)	471	9.8 (26.6)	-24.9% (-51.8, +16.8)	0.20
ADD	142	0.9 (4.6)	484	10.4 (21.8)	-37.6% (-53.6, -15.9)	0.002
TORS+ADD	138	3.7 (11.1)	469	20.7 (41.3)	35.6% (-8.2, +100.4)	0.13
Ae. albopictus						
Control	179	1.5 (7.1)	598	8.6 (25.4)		
TORS	141	5.3 (13.9)	471	13.9 (26.9)	-26.39% (-48.9, +5.9)	0.09
ADD	142	1.1 (5.1)	484	5.7 (19.9)	-20.8% (-51.8, +30.2)	0.36
TORS+ADD	138	11.6 (22.6)	469	9.5 (21.9)	-12.5% (-44.4, +37.5)	0.56

552 N: Total number of ovitraps recovered; SD: standard deviation; 95% CI: 95% confidence interval

*Adjusted for baseline and for ovitrap location

Figure 1

Figure 1: Geographical localisation of the study sites

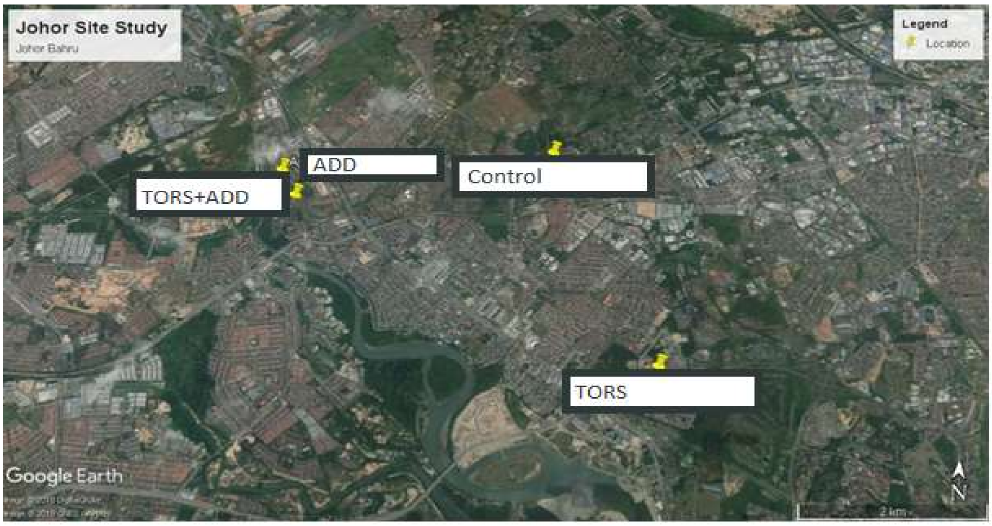
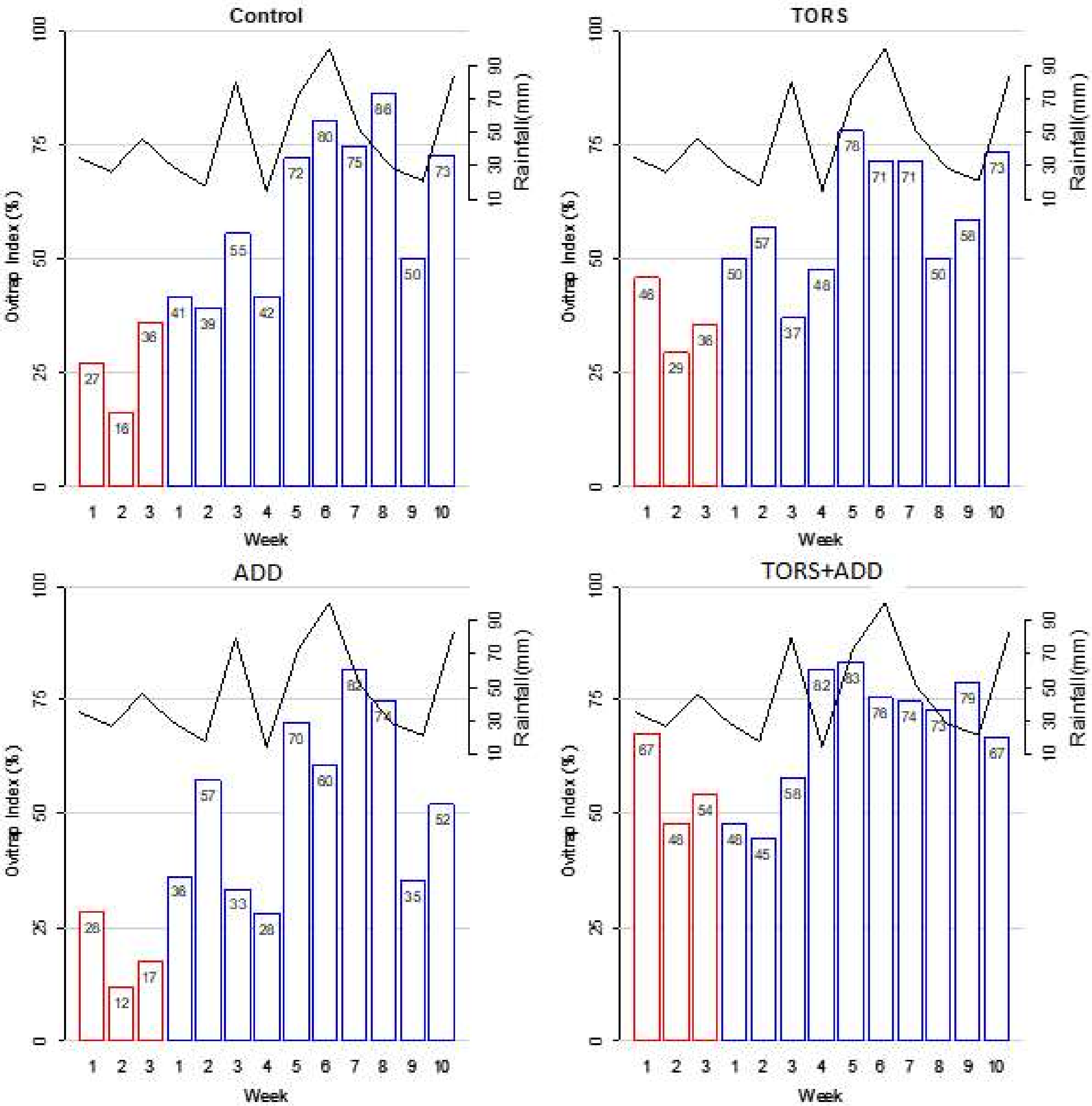


Figure 2



Supplementary material

Table S1: Ovitrap index and mean larvae per recovered ovitrap overall and by species according to location following intervention in the study areas

Study area	Ovitrap index (%)			Mean larvae Index (SD)		
	Overall	*Ae. aegypti*	*Ae. albopictus*	Overall	*Ae. aegypti*	*Ae. albopictus*
Control						
Semi-indoor	55.1	52.2	10.3	21.4(33.5)	19.7(32.6)	1.6(6.5)
Outdoor	78.7	32.0	65.3	38.3(48.2)	8.7(23.1)	29.6(43.3)
TORS						
Semi-indoor	39.4	34.4	9.5	8.9(17.9)	8.1(17.2)	0.7(3.1)
Outdoor	77.6	28.0	69.6	36.8(45.2)	11.2(32.8)	25.6(32.7)
ADT						
Semi-indoor	51.0	45.9	10.8	14.2(24.0)	12.8(23.6)	1.4(5.8)
Outdoor	57.9	15.0	45.1	21.2(35.6)	4.1(14.5)	17.2(34.5)
TORS+ADT						
Semi-indoor	65.5	60.2	15.0	30.2(45.2)	27.8(43.6)	2.4(9.6)
Outdoor	71.6	35.4	53.5	30.3(44.1)	14.2(37.9)	16.1(27.5)

Figure S1: Larvea index and rainfall (mm) per week during the baseline (red) and the intervention period (blue) in study sites

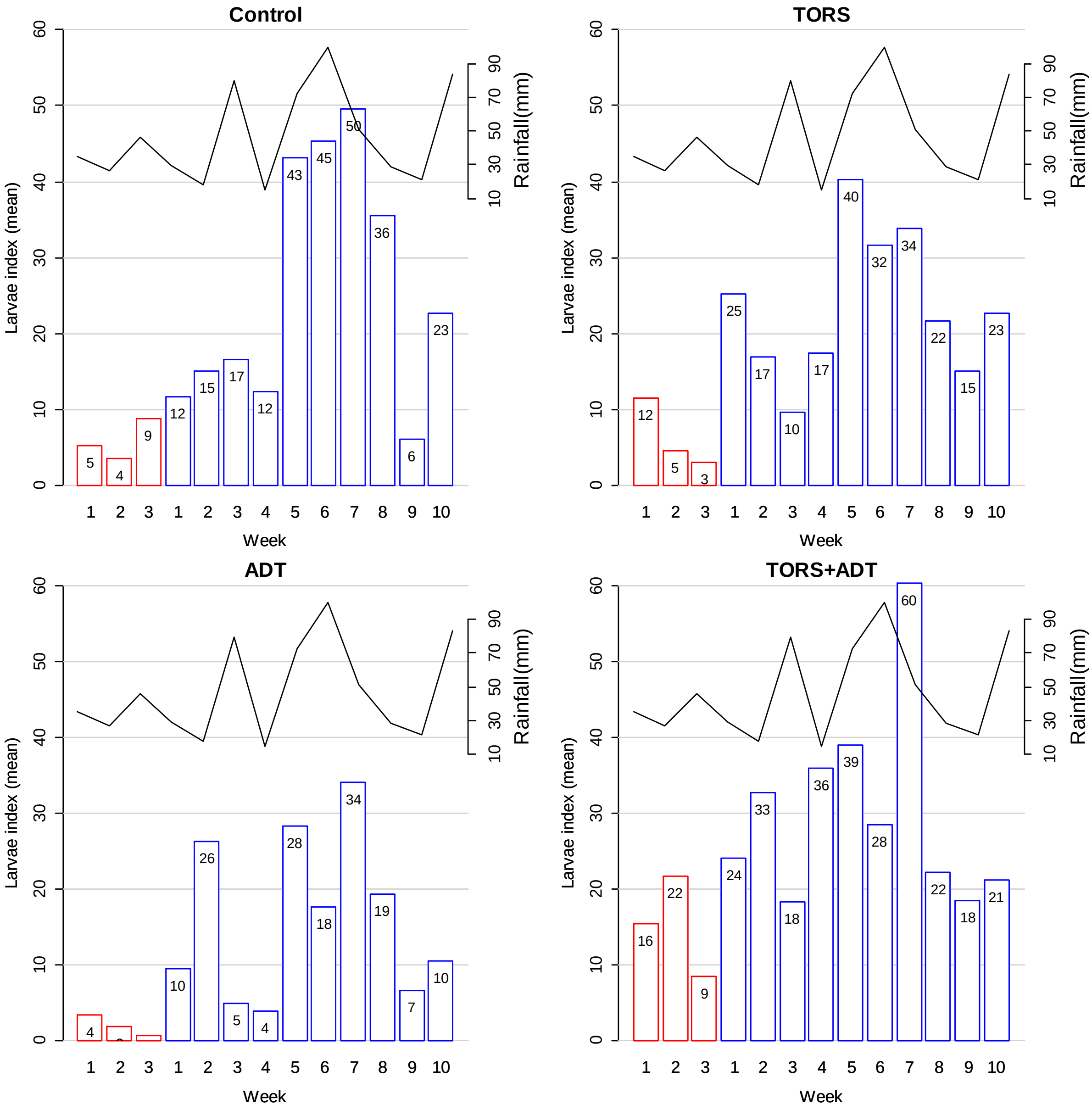


Table S2: Estimate of outdoor and semi-indoor (overall and by species) ovitrap index (OI) differences in comparison to the control site according to the intervention period

Study area	Period 1		Period 2	
	Difference in OI (%) relative to control* (95% CI)	**p-value**	**Difference in OI (%) relative to control* (95% CI)**	**p-value**
Overall				
TORS	-0.66 (-10.5, 9.2)	0.89	-13.1 (-21.9, +4.2)	0.004
ADT	-4.7 (-13.9, +4.7)	0.32	-12.3 (-21.5, +3.2)	0.008
TORS+ADT	+7.9 (-1.4, +17.3)	0.09	-4.8 (-14.3, +4.7)	0.32
Ae. aegypti				
TORS	-8.9 (-18.2, +0.3)	0.06	-12.6 (-23.2, -1.9)	0.02
ADT	-5.9 (-15.1, +3.2)	0.20	-12.4 (-21.9, -2.7)	0.01
TORS+ADT	+10.1 (-0.3, +20.5)	0.06	-0.8 (-11.8, +10.3)	0.89
Ae. albopictus				
TORS	+10.8 (+2.8, +18.8)	0.008	-2.0 (-10.0, +5.9)	0.62
ADT	-0.2 (-7.3, +6.9)	0.95	-8.4 (-16.1, -0.6)	0.03
TORS+ADT	+3.6 (-4.5, +11.7)	0.38	-10.7 (-19.-, -1.7)	0.02

Period 1: The first 5 weeks of intervention; Period 2: the second five weeks of intervention

* Adjusted for baseline larvae index and for ovitrap location

Table S3: Estimate of outdoor and semi-indoor (overall and by species) mean larvae index relative differences in comparison to the control site according to the intervention period

Study area	Period 1		Period 2	
	Relative difference (%)* (95% CI)	**p-value**	**Relative difference (%)* (95% CI)**	**p-value**
Overall				
TORS	-17.9 (-41.9, +15.8)	0.26	-42.2 (-56.0, -24.1)	<0.0001
ADT	-22.1 (-43.7, +7.9)	0.13	-43.2 (-55.9, -26.6)	<0.0001
TORS+ADT	+33.6 (-9.1, +96.4)	0.14	-15.7 (-39.2, +16.9)	0.31
Ae. aegypti				
TORS	-32.6 (-63.4, +24.1)	0.21	-24.8 (-53.1, +20.6)	0.24
ADT	-19.9 (-47.4, +21.9)	0.30	-45.9 (-60.7, -25.6)	0.0002
TORS+ADT	+78.6(+8.7, +193.5)	0.02	+7.8 (-28.1, +61.5)	0.72
Ae. albopictus				
TORS	-12.0 (-50.7, +57.0)	0.66	-39.0 (-61.8, -2.8)	0.04
ADT	23.4 (-39.3, 150.9)	0.57	-48.5 (-69.9, -11.8)	0.02
TORS+ADT	-3.4 (-51.6, 92.9)	0.92	-19.8 (-57.6, +51.6)	0.49

Period 1: The first 5 weeks of intervention; Period 2: the second five weeks of intervention

*Adjusted for baseline larvae index and for ovitrap location

Table S4: Estimated difference in ovitrap index (OI) according to the location strategy of autodissemination traps (ADT), taking strategy C as reference (results of the modified ordinary least squares regression model)*

Location strategy	Difference in OI relative to strategy C	95% CI (%)	p-value
TORS+ADT and ADT sites*			
Strategy A	+10.6%	+0.02, +21.8	0.05
Strategy B	+18.2%	+7.4, +29.0	0.001
ADT site**			
Strategy A	+16.1%	+1.95, +30.3	0.03
Strategy B	+16.5%	+0.38, +32.6	0.04
TORS+ADT site**			
Strategy A	+2.5%	-11.5, +16.6	0.72
Strategy B	+19.4%	+ 7.2, +31.6	0.002

OI: ovitrap index, CI: confidence interval

*adjusted for baseline larvae index and site

**adjusted for baseline larvae index

Table S5: Characteristics individuals that completed the survey

Site	Control	TORS	ADT	TORS+ADT
Mean age	38.5	39.9	43.2	42.7
Male %	43.2	33.3	31.2	32.6
Ethnicity (%)				
Malay	96.7	76.0	3.3	94.9
Chinese	0.5	0.0	45.9	0.0
Indian	1.4	19.9	45.9	1.0
Others	0.7	3.5	4.9	4.1
missing	0.7	0.6	0	
Education level (%)				
Primary school	9.5	18.3	11.5	28.6
Secondary school	73.2	49.1	57.4	53.1
Vocational/technical	4.7	2.3	3.3	3.1
College/university	7.4	26.3	22.9	9.2
Others	2.4	0.6	1.6	4.1
Missing	2.8	3.5	3.3	2.0
Occupation (%)				
Unemployed	50.8	46.2	47.5	62.2
Government sector	2.4	7.0	0	1.0
Private sector	40.1	42.1	45.9	32.6
Retired	3.6	3.5	6.6	3.1
Missing	3.1	1.2	0.0	1.0
Monthly household income				
B40: below US$720	76.0	68.9	50.8	83.7
M40: US$720-1440	9.7	23.9	42.7	9.2
T20: >US$1440	0.5	1.2	3.3	2.0
Missing	13.8	5.8	3.3	5.1
Status of housing (%)				
Own	10.4	47.9	68.8	70.4
Rent	85.9	50.9	31.2	29.6
Missing	3.6	1.2	0.0	0.0
Total family number (%)				
2 and below	7.4	11.7	22.9	15.3
3-4	38.0	41.5	39.3	34.7
5 and above	50.4	46.2	37.7	50.0
Missing	4.3	0.6	0.0	0.0

Point-by-point answer to reviewers

Manuscript number: BER-D-19-00236

Reviewer #1

1. The evaluation of vector control tools is complicated by the heavy rains which drove up the population density during the intervention period, as well described by the authors, but even in comparison to the control buildings only a very small effect size was seen by either tools, and no control was demonstrated by the two combined. It would be useful to put the ~8% reduction in context in the Discussion in terms of the effect it might have on transmission, in a larger treatment area

Authors: The discussion was edited to take into consideration the reviewer's suggestion. In particular, we discuss the small size of the observed effect. Please see the revised version lines 301-302.

2. The conclusion of the article seems to be 'we haven't shown any advantage of this IVM approach, but an innovative vector control tool would be beneficial'. This is back to front - the authors see the benefit of a new IVM tool, and so run this pilot trial to optimise the methods before a RCT, but were not able to show efficacy. To strengthen the conclusion, they could then give a hypothesis of why the IVM may not have been effective in this case, and how they would optimise the pilot trial, should they decide to go ahead with one given this weak result. It is very unfortunate that the conditions in the TORS+ADD buildings were so different to those in the other treatment buildings, which seem to contribute to the poor performance of the combined tools. It makes it very difficult to evaluate the IVM approach - these limitations are discussed, but it would be good to see the authors describe how the method would be optimised for the RCT to be confident that a fair comparison can be made, for example better matching the control and treatment sites for relevant characteristics

Authors: We extensively modified the conclusion section, including the addition of lessons learned, and steps to optimise the methods to optimize the cluster-randomised trial (cRCT).

3. It is a shame that ADD distribution strategy C didn't include placing ADDs on the second and top floor, as well as other floors, to allow better comparison between the approaches.

Authors: We believe the reviewer refers to Strategy B as Strategy C did have traps on the first 2 floors and the top floor. The logic behind the three strategies was to compare complete floor coverage (strategy A), with coverage every other floor (Strategy B) or strategic coverage Strategy C (only the first 2 floors and the top floor as these floors were reported in literature to account for the highest number of breeding sites. However, we do agree that the number of sites limited out capacity to compare these strategies, i.e. it may have been better not to have used multiple strategies, and we now acknowledge this in the discussion. Please see also our response to point 9.

4. The Introduction is concise and relevant, though I would suggest perhaps moving the description of TORS and ADD here from the Methods.

Authors: We moved the description of TORS and DD from the method to the introduction as per reviewer's suggestion. Please see the revised version lines 112-133.

5. How far apart were the buildings included for each treatment? A map might be helpful here, to give an idea about the expected migration between sites during the study, and citations to previous

studies which show the importance of vertical v horizontal movement of mosquitoes in this sort of setting.

Authors: The distance is reported in the revised version lines 145-146 and a map is added as per reviewer's suggestion (please refer to Figure 1 of the revised manuscript).

6. I would like more description about the placement of ADDs, particularly whether they were in similar sites to the ovitraps and thus potentially competing as oviposition sites and biasing the results. Some comment on the impact of not placing any ADDs or ovitraps indoors would also be informative.

Authors: As per the company recommendations, ADDs were placed in shaded areas where mosquitos like to breed. The number in each site was based on the length of the corridor. When both ovitrap and ADDS were deployed in the same floor, the distance between them were about 3 to 5 metres. ADDs were not in similar sites to the ovitrap.

We did not place ADDS indoor because they are approved for outdoor use only. The use of indoor ovitraps was not initially planned due to reluctance of the study population. However, regular contact between the study population and the field workers during the collection of baseline data created public trust and some flat owners accepted the ovitraps to be deployed in their homes. We did not present the results because the baseline data are missing. For the cRCT, it is planned to place indoor ovitraps in volunteers' flats. We added this point in the revised version of the conclusions and lessons learned.

7. Please expand on the sentence 'The analysis was adjusted on the LI at pre-treatment and the ovitraps location' as it is not clear to me what was done.

Authors: We clarified what was done in the text. Please see the revised version lines 199-204.

8. As well as giving the SAS procedures used please also describe the statistical tests which they perform.

Authors: The two outcomes were not directly compared between the different sites or the different strategies using statistical tests. The effect of the interventions was estimated and tested using two regression models (the modified ordinary least squares regression model and the negative binomial regression model). That was described in the section "Statistical analysis" of the methods.

9. The statistical power of the experiment is reduced by the fact that the ADD and TORS+ADD treatment sites were divided into 3 distribution strategies. Only one building is thus included in each of these treatments. These data sets cannot really be combined and used to fairly evaluate the efficacy of these treatments relative to the TORS only and control sites, because they are different treatments.

Authors: We now acknowledge this limitation in the discussion. Please see the revised version lines 352-357. However, we disagree that buildings cannot be combined within each site. The ADD and TORS+ADD arms had the same strategies which maintains their comparability. In general, planned variation of an intervention within a unit, as in a split plot design, does not rule out comparisons between the units. We agree that the combination of three strategies for the deployment of ADDs, including potentially suboptimal strategies, may have led to underestimate the effect of the ADD intervention in comparison to TORS alone or control. However, the objective of this study was mainly to obtain information allowing in particular to optimize the intervention

procedures that will be used in the randomized trial, and not to obtain a precise estimate of the intervention effect. Please also see our response to point 3.

10. It would be nice to comment on the proportion of Aedes collected by outdoor v semi-indoor ovitraps.

Authors: We provide now data on the *Aedes* population according to the semi-indoor vs outdoor location. Please see the revised version lines 223-225 and supplementary material Table 1.

11. I am not sure that we would expect ADDs to be much slower acting than TORS, since exposed females are prevented by PPF exposure from laying further eggs. Isn't the increase in efficacy over time simply a cumulative effect of female killing and sterilisation over time.

Authors: The expectation of slower action is based on the target differences between treatments and the accumulation of larvicide over time. TORS is meant to kill adults immediately upon contact. Adults entering the ADDs leave the traps alive to spread the larvicide to other breeding sites before succumbing to the fungus infection. But this takes 7-10 days. The pyriproxyfen targets the next generation of adults. Thus, we do not expect to see much effect of PPF within the first 2 weeks. Depending on the size of the breeding site, a single contaminated mosquito might not be enough to kill the larvae in this breeding sites. Multiple visits might be necessary to reach this threshold. This will create a delay in the effect as well. Results from field experiments in the Florida Keys indicated that after 6 weeks, the ADDs achieved a larvicidal effect of about 87% in surrounding breeding sites. After 12 weeks the percentage had increase to 94%.

Though sterilization of females is reported in other studies, In2Care has never specifically looked at this for female Aedes contaminated in their traps as the fungus component also should kill the females over time. It could certainly be that the contact and pickup in the ADD is sufficient for a sterilization effect which could add to the cumulative effect if the fungus has not killed the female before completing the next oviposition cycle.

12. A bias could have been introduced into the results by the increase in numbers from the baseline data collection to the intervention period, though I would expect it to reduce the OI due to there being more oviposition sites for the same number of adult females, whereas the opposite was seen in this case.

Authors: The number of ovitraps was greater during the intervention period than during the pre-treatment period (see Table 1). This difference is due to the fact that the length of the intervention period was 10 weeks vs 3 weeks for the pre-treatment period. The number of oviposition sites was the same during the two periods but the positivity of the ovitraps was measured every week. That explains the increase of the number of ovitraps during the intervention period. We added this information in footnote of the Table 1. Please see the revised version lines 546-548.

13. Please explain how plastic waste slows the autodissemination effect of the ADDs - I guess that the more oviposition sites there are the more thinly the treatment is spread and the more female adults need to be exposed to treat all sites?

Authors: The reviewer is correct in its assessment. Plastic waste is the number one breeding site creator in most of these areas. The more waste there is the fewer the actual number of females choosing for the ADD as primary breeding site. In addition, for those females that did choose the trap as the first stop, the more breeding sites she can choose from after leaving the ADD the smaller the effect is in the beginning. Some larger breeding sites need more than one visit to reach

the appropriate threshold for killing over 80% of the pupae. We provided some clarification in the revised version lines 315-320.

14. The community engagement efforts should be included in the Methods section, if they are so critical to the success of the interventions. Give that it is so important, why were no COMBI activities performed in the TORS+ADD site?

Authors: We added methods and results of the community engagement. Please see the revised version lines?? And supplementary material Table 5. Our hypotheses on the reasons of absence of effect in TORS+ADT site are provided in discussion (lines 322-325).

15. I don't think that the results of this study are strong enough to draw any conclusion about the best distribution strategy of ADDs, though it does seem like this is worth investigating further to maximise the efficacy of this tool.

Authors: We partly agree with the reviewer. Even though the results did not show a clear division between the 3 strategies, the fact that there is an effect and that this effect seems to be present at the strategically placed ADDs, indicates that this strategy has an effect. Combined with a much better economic outlook, this strategy does present the most interesting strategy to continue with. We fully agree that a second experiment to prove the efficacy of this strategy is a sensible next step.

16. The purpose of this study was apparently to inform the methods to be used for the proposed RCT. The Discussion lists several limitations of the study which mean that no substantial effect of either control tool and especially not the combined approach was observed. It would be good to include the lessons learned in the Conclusion.

Authors: Lessons learned were included in the conclusion as per reviewer's request. Please see the conclusion section of the revised version.

Reviewer #2

1. My impression is that the authors were overly ambitious in their goals. It may have been easier to focus on comparing just one or two treatments, and/or to rotate these two treatments between sites over time. This may not have been practical to do. Furthermore, I found it hard to understand why they wanted to test different ADD strategies as part of this experiment.

Authors: We agree with the reviewer that we were overly in our goals. Please see also our response to point 1 raised by reviewer 1. The early purpose of this pilot was to gain a better feel for the products on an operational level and to determine the best number of ADDs per block as this has a significant impact on the overall costs of the intervention both in material as in servicing costs.

2. It may have been good to consider alternative outcome measures, such as mosquito age-grading. Perhaps the interventions changed the age profile of the populations, which would have a significant impact on disease transmission if they were able to demonstrate that.

Authors: We agree that mosquito-age-grading could be a good alternative to assess the impact of the intervention on disease transmission. However, assessing the impact of the intervention on disease transmission on a small number of clusters and for a short period of time was not the objective of this study. The suggested outcome measure is planned for the upcoming cRCT.

3. To re-cast the work in a different light may not be possible, so trying to see these results published would be a good outcome. It is recommended that the authors confront the limitations of the experimental design more fully and focus more strongly on the TORS and ADD only sites results where there WERE significant reductions in mosquitoes.

Authors: We carefully revised the discussion section to answer the reviewer's request, in particular by describing the limitations more fully.