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Nurulhusna Ab Hamid, Neal Alexander, Remco Suer, Nazni Wasi Ahmed, Rose Nani Mudin, et al.. Targeted outdoor residual spraying, autodissemination devices and their combination against *Aedes* mosquitoes: field implementation in a Malaysian urban setting. *Bulletin of Entomological Research*, 2020, 110 (6), pp.700-707. 10.1017/S0007485320000188 . hal-04386999

HAL Id: hal-04386999

<https://hal.science/hal-04386999v1>

Submitted on 27 Oct 2024

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Bulletin of Entomological Research

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

Manuscript Number:	BER-D-19-00236R1
Full Title:	Targeted outdoor residual spraying, autodissemination devices and their combination against Aedes mosquitoes: Field implementation in a Malaysian urban setting
Article Type:	Research Paper
Corresponding Author:	Mitra SAADATIAN-ELAHI, PhD Hospices Civils de Lyon Lyon, FRANCE
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Hospices Civils de Lyon
Corresponding Author's Secondary Institution:	
First Author:	Mitra SAADATIAN-ELAHI, PhD
First Author Secondary Information:	
Order of Authors:	Mitra SAADATIAN-ELAHI, PhD Nurulhusna Ab Hamid Neal Alexander Remco Suer, PhD Nazni Wasi Ahmed Rose Nani Mudin Topek Omar Rahmat Dapari Shahrom Nor Azian Che Mat Din Roslinda Abdul Rahman Ropiah Jaraee Frederic Baur Frederic Schmitt Nick Hamon Jason H. Richardson Carole Langlois-Jacques Muriel Rabilloud
Order of Authors Secondary Information:	
Abstract:	<p>Background</p> <p>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.</p> <p>Methodology</p> <p>As part of the development of a cluster randomized controlled epidemiological trial, a</p>

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—ovitrapp 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* ovitrapp 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.

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

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

6

7 Nurulhusna Ab Hamid¹, Neal Alexander², Remco Suer³, Nazni Wasi Ahmed¹, Rose Nani
8 Mudin⁴, Topek Omar⁴, Rahmat Dapari⁴, Shahrom Nor Azian Che Mat Din⁵, Roslinda Abdul
9 Rahman⁵, Ropiah Jaraee⁶, Frederic Baur⁷, Frederic Schmitt⁷, Nick Hamon⁸, Jason H.
10 Richardson⁸, Carole Langlois-Jacques⁹, Muriel Rabilloud⁹, Mitra Saadatian-Elahi¹⁰

11

12 ¹Medical Entomology Unit, Institute for Medical Research, WHO Collaborating Centre,
13 Institute for Medical Research, Ministry of Health Malaysia, Jalan Pahang, 50588 Kuala
14 Lumpur

15 ²MRC Tropical Epidemiology Group, Department of Infectious Disease Epidemiology, London
16 School of Hygiene and Tropical Medicine, Keppel St, London, WC1E 7HT

17 ³In2Care B.V., Marijkeweg 22, 6871SE Wageningen, the Netherlands

18 ⁴ Vector Borne Disease Sector, Disease Control Division, Ministry of Health Malaysia, Level 4,
19 Block E10, Complex E, Federal Government Administrative Center, 62590 Putrajaya,
20 Malaysia

21 ⁵Public Health Division, Johor, Johor State Health Department, Ministry of Health Malaysia,
22 Jalan Persiaran Permai, 81200 Johor Bahru Johor.

23 ⁶ Entomology and Pest Unit Public Health Division, Johor, Johor State Health Department,
24 Ministry of Health Malaysia, Jalan Persiaran Permai, 81200 Johor Bahru Johor.

25 ⁷ Bayer S.A.S, Environmental Science, Crop Science Division; 16 rue Jean Marie Leclair ;
26 69266 Lyon Cedex 09 ; France

27 ⁸ Innovative Vector Control Consortium, Pembroke Place, L3 5QA, Liverpool, UK

28 ⁹ Hospices Civils de Lyon, Service de Biostatistique et Bioinformatique, F-69003 Lyon, France;
29 Université de Lyon, F-69000 Lyon, France; Université Lyon 1, F-69100 Villeurbanne, France;
30 CNRS, UMR 5558, Laboratoire de Biométrie et Biologie Evolutive, Equipe Biostatistique-
31 Santé, F-69100 Villeurbanne, France.

32 ¹⁰ Service d'Hygiène, Epidémiologie et Prévention, Hospices Civils de Lyon, F-69437 Lyon,
33 France, and Laboratoire des Pathogènes Emergents – Fondation Mérieux, Centre
34 International de Recherche en Infectiologie, Institut National de la Santé et de la Recherche
35 Médicale U1111, Centre National de la Recherche Scientifique, UMR5308, Ecole Normale
36 Supérieure de Lyon, Université Claude Bernard Lyon 1, 21, Avenue Tony Garnier, 69007
37 Lyon, France

38 **Corresponding author**

39 Dr Mitra Saadatian Elahi

40 Service d'Hygiène, Epidémiologie et Prévention, Hospices Civils de Lyon, F-69437 Lyon,
41 France, and Laboratoire des Pathogènes Emergents – Fondation Mérieux, Centre

42 International de Recherche en Infectiologie, Institut National de la Santé et de la Recherche
43 Médicale U1111, Centre National de la Recherche Scientifique, UMR5308, Ecole Normale
44 Supérieure de Lyon, Université Claude Bernard Lyon 1, 21, Avenue Tony Garnier, 69007
45 Lyon, France

46 Mail: mitra.elahi@chu-lyon.fr

47 Phone: +33 6 29 36 49 24

48 **Abstract**

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

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

61 **Principal findings:** Relative to the control site, the TORS and ADD sites showed reduction in
62 the *Aedes* ovitrap index (-6.5%, $p=0.04$ and -8.3%, $p=0.10$ respectively). Analysis by species
63 showed that as compared to the control site, *Ae. aegypti* density was lower in ADD (-8.9%,
64 $p=0.03$) and TORS (-10.4%, $p=0.02$). No such effect was evident in the TORS+ADD site.

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

70 **Key words**

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

72 **Introduction**

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

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

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

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

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

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

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

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

117 ADDs (In2Care®) attract and kill *Aedes* mosquitoes via a combination of a slow killing
118 adulticide, the entomopathogenic fungus *Beauveria bassiana* strain GHA, and the juvenile

119 hormone analogue pyriproxyfen (PPF), a larvicide that can be auto disseminated to
120 surrounding breeding sites (Buckner et al., 2017). ADDs rely on mosquito behaviour to
121 distribute the pesticide to cryptic, hard to find breeding sites and can potentially offer
122 precision-targeted larval control and sustained breeding suppression of vector populations
123 (Farenhorst et al., 2009; Snetselaar et al., 2014). Gravid female mosquitoes enter the trap
124 searching for a place to lay their eggs. When landing on the floater the females contact
125 gauze contaminated with PPF and *B. The latter can take 7-14 days to develop and then kill*
126 *exposed mosquitoes, providing the opportunity to transfer PPF from the ADDs to other*
127 *surrounding larval habitats [Snetselaar et al., 2014].*
128 The results of a field implementation study carried out to evaluate the feasibility and to
129 provide guidance to optimize the methods and procedures for the set-up and conduct of the
130 cRCT are presented here.

131 **Methods**

132 **Setting**

133 The study was carried out from February to June 2018 (3 weeks pre-treatment and 10 weeks
134 intervention) in Johor Bahru, Malaysia. The study included one control site and three
135 intervention sites to be treated with a) targeted outdoor residual spraying only (TORS site),
136 b) deployment of autodissemination devices only (ADD site) and c) combination of outdoor
137 residual spraying and deployment of autodissemination devices (TORS+ADD site). The study
138 sites were located within 10Km radius with each other. TORS+ADD and ADD sites were 3Km
139 apart (Figure 1). The control site comprised three buildings of 17 floors each. TORS site was
140 composed of two buildings of 14 floors each. The number of buildings for ADD and
141 TORS+ADD sites were respectively four (9 floors per building) and three (4 floors per

142 building). This research was approved by the Malaysian Ministry of Health's Medical
143 Research and Ethics Committee (17 Oct 2017).

144 **Insecticide and treatments**

145 Following the collection of pre-treatment data for a period of three weeks, outdoor space
146 spraying was conducted (Ministry of Health Malaysia 2009) in all study sites for a quick and
147 short-term reduction of the *Aedes* population. TORS was applied in TORS and TORS+ADD
148 sites at week five and consisted of spraying semi-indoor and outdoor perimeter concrete
149 walls with K-Othrine Polyzone. The latter contains deltamethrin as its active ingredient (62.5
150 g/l). The insecticide dosage was 25 mg/m² and was applied by using a compression sprayer.
151 ADDs were deployed in two sites (ADD and TORS+ADD).

152 According to manufacturing specification, one ADD is necessary for every 400m². A logical
153 distribution of ADDs generating a similar efficacy would be to treat every floor. But the key
154 element of ADDs being the autodissemination effect, three strategies were evaluated in the
155 intervention sites ADD and ADD+TORS to find a more economical distribution pattern : A)
156 two ADDs on each floor (Strategy A, two buildings in ADD site and one in TORS+ADD site), B)
157 two ADDs every second floor excluding the top floor(Strategy B, one in each site), and C) two
158 ADDs on each of the first 2 floors, and 2 on the top floor (Strategy C, one in each site).
159 Strategy C stems from the concept that most breeding sites are found at ground level, but
160 high-rise buildings often have water reservoirs and potential breeding sites on the roof
161 (Wan-Norafikah et al., 2010; Lau et al., 2013; Zainon et al., 2016).

162 **Monitoring of *Aedes* population**

163 A total of 87 outdoor (near bushes and small plants) and 136 semi-indoor (along the
164 corridor, e.g. near shoe racks and flower pots) ovitraps were placed in the study areas to
165 monitor mosquito density. Semi-indoor was defined as not being completely enclosed by

166 walls (e.g. corridors open on one side) but covered and protected from sunlight and heavy
167 rainfall. Entomological data were collected during the pre-treatment and for 10 weeks
168 following the intervention.

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

176 **Population-based survey and community engagement**

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

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

183 **Statistical analyses**

184 The primary entomological outcome was the weekly ovitrap index (OI), which is the
185 percentage of positive ovitraps (i.e. those with evidence of larvae in the trap). This was
186 calculated as the number of positive ovitraps divided by the total number of recovered
187 ovitraps in each site at the end of each week. We also calculated the number of larvae per
188 ovitrap (the larvae index, or LI) expressed as the total number of *Aedes* sp. larvae in each
189 recovered ovitrap at the end of each week. To quantify the effect of each intervention on OI

190 in comparison to control, a modified ordinary least squares regression model using a robust
191 standard error estimator was implemented (Cheung et al., 2007). The mean LI during the
192 pre-treatment (baseline) period of each site and the ovitrap location (semi-outdoor vs
193 outdoor) were included in the regression model, as well as the intervention applied.
194 The same analysis strategy was applied to quantify the intervention effect on LI using a
195 negative binomial regression model.

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

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

206 **Results**

207 During the surveys, between 80 and 100% of the semi-indoor and outdoor ovitraps were
208 recovered after seven days. Of the total of 65,118 larvae examined, 39,070 (60.0%) were *Ae.*
209 *aegypti*, and 25,982 (39.9%) were *Ae. albopictus*. During the pre-treatment, the highest
210 mean OI (56.5%) were found in TORS+ADD site while the lowest values were observed in the
211 ADD site (mean: 19.0%) (Figure 2). Following the intervention, we observed an increase in
212 the overall OI in all study sites, although there was weekly variation in both control and
213 intervention areas. The overall OI and mean larva index was in general higher in outdoor as

214 compared to semi-indoor areas (Supp.Table S1). Analysis by species showed higher OI and LI
215 for *Ae. aegypti* in semi-indoor areas (Supp.Table S1). As for OI, the mean LI overall was
216 higher in all study sites during the intervention period as compared to the pre-treatment
217 period (Supp. Figure S1).

218 The results of the effect of the intervention on OI are summarized in Table 1. As compared
219 to the control site, the overall outdoor and semi-indoor OI was lower in the intervention
220 sites ADD (-8.3%, p=0.04) and TORS (-6.5%, p=0.10) and slightly higher in TORS+ADD (+1.8,
221 p=0.63). The difference reached statistical significance only in the ADD site. Relative to the
222 control site, the outdoor and semi-indoor OI for *Ae. aegypti* was lower in ADD (-8.9%,
223 p=0.03) and TORS (-10.4%, p=0.02) and slightly higher in TORS+ADD (+4.9%, p=0.29).

224 Regarding *Ae. albopictus*, relative to the control site, outdoor and semi-indoor OI was slightly
225 lower in ADD (-4.2%, p=0.19) and TORS+ADD (-3.4%, p=0.34) and slightly higher in TORS
226 (+4.5%, p=0.18) but none reached statistical significance.

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

231 The relative difference in mean number of larvae per ovitrap in ADD, TORS and TORS+ADD in
232 comparison to the control site was estimated to be -35.4% (95% confidence interval (CI): -
233 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;
234 p=0.81) respectively (Table 2). Similar trends were observed for *Ae. aegypti* but the
235 difference reached statistical significance only in ADD (-37.6%; p=0.002). Regarding *Ae.*
236 *albopictus*, as compared to the control site, the mean number of larvae per ovitrap was
237 lower in all intervention sites but none reached statistical significance. As for OI, the LI

238 showed a greater effect of the intervention during weeks 6-10 compared to weeks 0-5 in
239 TORS ($p<0.0001$) and ADD ($p<0.0001$) (Supp. Table S3).

240 **Distribution of ADDs deployment strategies**

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

245 **Population-based survey and community engagement**

246 Baseline characteristics of the 732 individuals that completed the survey are presented in
247 supplementary material (Supp. Table 5). Income categories were based on the report of
248 Household Income and Basic Amenities 2016-Malaysia as follows: top 20% (T20:
249 >US\$1440/month), middle 40% (M40: US\$720-1440/month), and bottom 40% (B40:
250 <US\$720/month) (Department of Statistics Malaysia; 2017). The highest percentage of
251 individuals with primary school education and low income was observed in the TORS+ADT
252 site. This site had also the highest rate of unemployed individuals. Television and radio were
253 identified as the preferred source of information about dengue (71.5%), followed by internet
254 (31%) and relatives (28.2%). COMBI volunteers were available in all study sites but did not
255 participate to the study in the TORS+ADT site. Lower education level in this site might
256 explain the observed lack of participation of COMBI volunteers.

257 **Discussion**

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

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

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

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

275 As reported in other investigations (Lee et al., 2015; Hamid et al., 2019) and in agreement
276 with our results, TORS or ADDs effectively reduced the mosquito population. It can
277 therefore be expected that co-application of these techniques together with public
278 cooperation would further enhance the vector control efficacy. The lack of an observed
279 additive effect of the combined TORS+ADD on the mosquito population may be related to
280 socio-economic, waste management measures and architectural differences of the
281 TORS+ADD site compared to the other intervention sites. Frequent presence of objects such
282 as pet cages, fish aquarium, furniture and edible plants in the semi-indoor areas in this site
283 led to TORS coverage of 50% as compared to 100% in the TORS site. An average coverage of
284 70% of walls is requested for an effective action of TORS. More discarded, often plastic,

285 waste was also observed in the TORS+ADD site that could slow down the autodissemination
286 effect of the ADDs. As plastic waste forms breeding sites, fewer females choose the ADD as
287 primary breeding site. In addition, for those females that did choose the ADD first, the more
288 breeding sites are available on leaving the ADD, the smaller is the initial effect. Some larger
289 breeding sites need more than one mosquito visit to reach the appropriate threshold for
290 killing over 80% of the pupae. Finally, 26% of ADDs were subject to vandalism in this site as
291 compared to only 3% in the site with ADDs alone. The lower education level of the
292 population and the lack of COMBI activities in TORS+ADT site could have contributed to
293 higher vandalism, and presence of bird cages and aquaria at the time of TORS spraying, thus
294 leading to the lack of effect. Moreover, the architecture of the buildings in TORS+ADD site
295 make the semi-indoor walls more subject to rainfall and hence, plausibly, quicker wash-off of
296 K-Othrine Polyzone during the heavy rainfall that occurred after the introduction of the
297 intervention.

298

299 The observed greater effect of the intervention on the mosquito population overtime in the
300 ADD site fit well with the slow killing effect of this device. ADD is designed to attract
301 mosquitoes and then contaminate the adults which then carry pyriproxyfen to other sites
302 before dying from the exposure to the *Beauveria* within approximately 10 days. The PPF
303 targets the next generations; it prevents the pupae from transforming to the adult stage and
304 tarsal contact with pyriproxyfen has been shown to suppress egg production and
305 hatchability in adult females (Ohba et al., 2013). Thus, we do not expect to see much effect
306 of PPF within the first 2 weeks. Over time accumulation of PPF occurs in surrounding
307 breeding sites increasing the effectiveness of the ADDs. Depending on the size of the
308 breeding site, a single contaminated mosquito might not be enough to kill the larvae in this

309 breeding sites. Multiple visits might be necessary to reach this threshold, which again will
310 delay the effect. A trend towards a lower proportion of positive ovitraps in the TORS+ADD
311 area was observed although it is was not statistically significant. We do not have a specific
312 explanation for the observed greater effect of TORS during weeks 6-10. An efficacy lag of
313 one month on 24h mortality rates of *Anopheles gambiae* on wood panels treated with K-
314 Othrine Polyzone was also reported by Dunford and collaborators (Dunford et al., 2018).
315 The attempt to evaluate three ADD deployment strategies, including potentially suboptimal
316 one, may have led to the effect of ADD being underestimated. However, the main objective
317 of this study was to obtain information on the optimization of the intervention procedures
318 for the cRCT, rather than obtaining a precise estimate of the intervention effect. Despite the
319 reduced power resulting from multiple ADD deployment strategies across limited numbers
320 of buildings, the results did give some insight as to optimal deployment. We found that
321 strategy C (ADDs on the first 2 floors and on the top floor) seems to be a valid alternative to
322 reduce the number of ADD needed while keeping the quality of the expected results.
323 Strategy A with ADDs in every floor did not perform better than strategy C. Factors such as
324 different overall population levels between buildings within a site or different distributions
325 over the floors have been reported in the past (Lau et al., 2013) and could explain the
326 observed results. The better result of strategy C compared to strategy B, even though more
327 ADDs were deployed under strategy B, could be due to better/smarter distribution as
328 strategy B did not include the second and the top floor. These floors have been reported as
329 sometimes having a higher infestation than other floors (Wan-Norafikah et al., 2010; Zainon
330 et al., 2016). If we were to draw a conclusion from these results, it would be that, in
331 buildings up to 9 floors, reducing the ADD coverage from every floor to the first two and top
332 floors seems to be possible without necessarily lowering the impact.

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

337 **Conclusions and lessons learned**

338 The combination of multiple insecticides with different modes of action in one package is
339 innovative, although we could not demonstrate the additive effect of TORS+ADD.

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

351

352 Offering a better understanding of a proactive IVM approach on *Aedes*-related diseases by
353 conducting large scale randomized controlled trial is key to further reduce their incidence
354 and improve global health. Successful implementation of such large-scale studies requires
355 the existence of appropriate infrastructure (expertise in vector control management, strong
356 social mobilization capacities, existence of surveillance systems) and high dengue

357 endemicity. Furthermore, the Ministry of Health has an epidemiological and entomological
358 surveillance system specifically for the *Aedes*-borne diseases: dengue, Zika and chikungunya.
359 This system also records post-outbreak vector control activities and dengue virus serotypes.
360 These are the main reasons for carrying out the planned trial in Malaysia. We believe that
361 the planned cRCT will allow us to further expand upon and validate the entomological
362 evidence generated here, to evaluate the impact of the proposed IVM approach on dengue
363 incidence and to help shift the conception of policies to handle *Aedes*-borne diseases from
364 treatment to prevention, thus saving public funding.

365 **Acknowledgments**

366 The authors express their gratitude to the Director General of Health and Director, Institute
367 for Medical Research, Kuala Lumpur for support. We are also thankful to the Malaysian
368 Ministry of Health for providing the laboratory place and equipment.

369 Thanks are also due to David Malone (Integrated Vector Control Consortium) and all staff of
370 Johor Bahru health office and Institute for Medical Research (IMR, Kuala-Lumpur) for help
371 rendered in the field.

372 **Declaration of interest**

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

375 NA receives salary support from the MRC UK and DFID - MRC Grant Reference
376 MR/K012126/1: This award is jointly funded by the UK Medical Research Council (MRC) and
377 the UK Department for International Development (DFID) under the MRC/DFID Concordat
378 agreement and is also part of the EDCTP2 programme supported by the European Union.

379 **Funding**

380 The study was funded by budget allocation from Bayer who supplied also the product for
381 residual spraying. Autodissemination devices were provided by In2Care.

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495

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

	Pre-treatment		Intervention		Difference in OI	
Study area	N	OI (%)	N	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
<i>Ae. aegypti</i>						
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
<i>Ae. albopictus</i>						
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

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

501 *Adjusted for baseline and for ovitrap location

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

505 **Table 2:** Outdoor and semi-indoor mean larval index (overall and per species) in study sites
 506 during pre-treatment and intervention period and estimate of the mean larvae index relative
 507 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
<i>Ae. aegypti</i>						
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
<i>Ae. albopictus</i>						
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

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

510 *Adjusted for baseline and for ovitrap location

511

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

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

6
7 Nurulhusna Ab Hamid¹, Neal Alexander², Remco Suer³, Nazni Wasi Ahmed¹, Rose Nani
8 Mudin⁴, Topek Omar⁴, Rahmat Dapari⁴, Shahrom Nor Azian Che Mat Din⁵, Roslinda Abdul
9 Rahman⁵, Ropiah Jaraee⁶, Frederic Baur⁷, Frederic Schmitt⁷, Nick Hamon⁸, Jason H.
10 Richardson⁸, Carole Langlois-Jacques⁹, Muriel Rabilloud⁹, Mitra Saadatian-Elahi¹⁰

11

12 ¹Medical Entomology Unit, Institute for Medical Research, WHO Collaborating Centre,
13 Institute for Medical Research, Ministry of Health Malaysia, Jalan Pahang, 50588 Kuala
14 Lumpur

15 ²MRC Tropical Epidemiology Group, Department of Infectious Disease Epidemiology, London
16 School of Hygiene and Tropical Medicine, Keppel St, London, WC1E 7HT

17 ³In2Care B.V., Marijkeweg 22, 6871SE Wageningen, the Netherlands

18 ⁴ Vector Borne Disease Sector, Disease Control Division, Ministry of Health Malaysia, Level 4,
19 Block E10, Complex E, Federal Government Administrative Center, 62590 Putrajaya,
20 Malaysia

21 ⁵Public Health Division, Johor, Johor State Health Department, Ministry of Health Malaysia,
22 Jalan Persiaran Permai, 81200 Johor Bahru Johor.

23 ⁶ Entomology and Pest Unit Public Health Division, Johor, Johor State Health Department,
24 Ministry of Health Malaysia, Jalan Persiaran Permai, 81200 Johor Bahru Johor.

25 ⁷ Bayer S.A.S, Environmental Science, Crop Science Division; 16 rue Jean Marie Leclair ;
26 69266 Lyon Cedex 09 ; France

27 ⁸ Innovative Vector Control Consortium, Pembroke Place, L3 5QA, Liverpool, UK

28 ⁹ Hospices Civils de Lyon, Service de Biostatistique et Bioinformatique, F-69003 Lyon, France;
29 Université de Lyon, F-69000 Lyon, France; Université Lyon 1, F-69100 Villeurbanne, France;
30 CNRS, UMR 5558, Laboratoire de Biométrie et Biologie Evolutive, Equipe Biostatistique-
31 Santé, F-69100 Villeurbanne, France.

32 ¹⁰ Service d'Hygiène, Epidémiologie et Prévention, Hospices Civils de Lyon, F-69437 Lyon,
33 France, and Laboratoire des Pathogènes Emergents – Fondation Mérieux, Centre
34 International de Recherche en Infectiologie, Institut National de la Santé et de la Recherche
35 Médicale U1111, Centre National de la Recherche Scientifique, UMR5308, Ecole Normale
36 Supérieure de Lyon, Université Claude Bernard Lyon 1, 21, Avenue Tony Garnier, 69007
37 Lyon, France

38 **Corresponding author**

39 Dr Mitra Saadatian Elahi

40 Service d'Hygiène, Epidémiologie et Prévention, Hospices Civils de Lyon, F-69437 Lyon,
41 France, and Laboratoire des Pathogènes Emergents – Fondation Mérieux, Centre

42 International de Recherche en Infectiologie, Institut National de la Santé et de la Recherche
43 Médicale U1111, Centre National de la Recherche Scientifique, UMR5308, Ecole Normale
44 Supérieure de Lyon, Université Claude Bernard Lyon 1, 21, Avenue Tony Garnier, 69007
45 Lyon, France

46 Mail: mitra.elahi@chu-lyon.fr

47 Phone: +33 6 29 36 49 24

48 Abstract

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

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

65 **Principal findings:** Relative to the control site, the TORS and ADD sites showed reduction in
66 the *Aedes* ovitrap index (-6.5%, $p=0.04$ and -8.3%, $p=0.10$ respectively). Analysis by species
67 showed that as compared to the control site, *Ae. aegypti* density was lower in ADD (-8.9%,
68 $p=0.03$) and TORS (-10.4%, $p=0.02$). No such effect was evident in the TORS+ADD site.

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

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

76 **Key words**

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

78 **Introduction**

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

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

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

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

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

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

111 We plan to set-up a cluster randomized controlled trial (cRCT) to evaluate the effectiveness
112 of a proactive IVM strategy on the incidence of dengue in Malaysia. The IVM strategy will
113 combine targeted outdoor residual spraying (TORS) by K-Othrine Polyzone, deployment of
114 auto-dissemination devices (ADDs) and extensive public engagement activities.
115 The active ingredient of the TORS, K-Othrine Polyzone, has been prequalified by the WHO for
116 use in vector control activities [WHO, 2018]. K-Othrine Polyzone indoor residual spraying
117 (IRS) application has been proven to reduce adult and immature *Aedes* populations
118 (Paredes-Esquivel et al., 2016). K-Othrine Polyzone kills host-seeking and resting adult
119 mosquitoes landing on the treated substrate, thereby lowering the number of the adult
120 mosquitoes in the area [Dunford et al., 2018]. Its use in TORS can potentially reduce the
121 frequency of current insecticide applications for *Aedes* control due to its longer residual
122 effect (Hamid et al., 2019).

123 ADDs (In2Care®) attract and kill *Aedes* mosquitoes via a combination of a slow killing
124 adulticide, the entomopathogenic fungus *Beauveria bassiana* strain GHA, and the juvenile

125 hormone analogue pyriproxyfen (PPF), a larvicide that can be auto disseminated to
126 surrounding breeding sites (Buckner et al., 2017). ADDs rely on mosquito behaviour to
127 distribute the pesticide to cryptic, hard to find breeding sites and can potentially offer
128 precision-targeted larval control and sustained breeding suppression of vector populations
129 (Farenhorst et al., 2009; Snetselaar et al., 2014). Gravid female mosquitoes enter the trap
130 searching for a place to lay their eggs. When landing on the floater the females contact
131 gauze contaminated with PPF and *B. The latter can take 7-14 days to develop and then kill*
132 exposed mosquitoes, providing the opportunity to transfer PPF from the ADDs to other
133 surrounding larval habitats [Snetselaar et al., 2014].
134 The results of a field implementation study carried out to evaluate the feasibility and to
135 provide guidance to optimize the methods and procedures for the set-up and conduct of the
136 cRCT are presented here. ~~The present study reports the results of a pilot study that was~~
137 ~~carried out to optimize the methods and procedures to be used for the main trial.~~

138 **Methods**

139 **Setting**

140 The ~~pilot~~ study was carried out from February to ~~May-June~~ 2018 (3 weeks pre-treatment and
141 10 weeks intervention) in Johor Bahru, Malaysia. The study included one control site and
142 three intervention sites to be treated with a) targeted outdoor residual spraying only (TORS
143 site), b) deployment of autodissemination devices only (ADD site) and c) combination of
144 outdoor residual spraying and deployment of autodissemination devices (TORS+ADD site).
145 The study sites were located within 10Km radius with each other. TORS+ADD and ADD sites
146 were 3Km apart (Figure 1). The control site comprised three buildings of 17 floors each.
147 TORS site was composed of two buildings of 14 floors each. The number of buildings for ADD
148 and TORS+ADD sites were respectively four (9 floors per building) and three (4 floors per

149 building). This research was approved by the Malaysian Ministry of Health's Medical
150 Research and Ethics Committee (17 Oct 2017).

151 **Insecticide and treatments**

152 Following the collection of pre-treatment data for a period of three weeks, outdoor space
153 spraying was conducted (Ministry of Health Malaysia 2009) in all study sites for a quick and
154 short-term reduction of the *Aedes* population. TORS was applied in TORS and TORS+ADD
155 sites at week five and consisted of spraying semi-indoor and outdoor perimeter concrete
156 walls with K-Othrine Polyzone. The latter contains deltamethrin as its active ingredient (62.5
157 g/l). The insecticide dosage was 25 mg/m² and was applied by using a compression sprayer.
158 ADDs were deployed in two sites (ADD and TORS+ADD).

159 According to manufacturing specification, one ADD is necessary for every 400m². A logical
160 distribution of ADDs generating a similar efficacy would be to treat every floor. But the key
161 element of ADDs being the autodissemination effect, three strategies were evaluated in the
162 intervention sites ADD and ADD+TORS to find a more economical distribution pattern : A)
163 two ADDs on each floor (Strategy A, two buildings in ADD site and one in TORS+ADD site), B)
164 two ADDs every second floor excluding the top floor(Strategy B, one in each site), and C) two
165 ADDs on each of the first 2 floors, and 2 on the top floor (Strategy C, one in each site).

166 Strategy C stems from the concept that most breeding sites are found at ground level, but
167 high-rise buildings often have water reservoirs and potential breeding sites on the roof
168 (Wan-Norafikah et al., 2010; Lau et al., 2013; Zainon et al., 2016).

169 **Monitoring of *Aedes* population**

170 A total of 87 outdoor (near bushes and small plants) and 136 semi-indoor (along the
171 corridor, e.g. near shoe racks and flower pots) ovitraps were placed in the study areas to
172 monitor mosquito density. Semi-indoor was defined as not being completely enclosed by

173 walls (e.g. corridors open on one side) but covered and protected from sunlight and heavy
174 rainfall. Entomological data were collected during the pre-treatment and for 10 weeks
175 following the intervention.

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

183 **Population-based survey and community engagement**

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

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

190 **Statistical analyses**

191 The primary entomological outcome was the weekly ovitrap index (OI), ~~i.e. the number~~
192 which is the percentage of positive ovitraps (i.e. those with evidence of larvae in the trap).
193 This was calculated as the number of positive ovitraps divided by the total number of
194 recovered ovitraps in each site at the end of each week~~expressed as a percentage~~. We also
195 ~~analysed the weekly larval~~ calculated the number of larvae ~~number~~ per ovitrap (the larvae
196 index, or LI) expressed as the total number of *Aedes* sp. larvae ~~per~~ in each recovered ovitrap

197 at the end of each week. To quantify the effect of each intervention on OI in comparison to
198 control, a modified ordinary least squares regression model using a robust standard error
199 estimator was implemented (Cheung et al., 2007). The mean LI during the pre-treatment
200 (baseline) period of each site and the ovitrap location (semi-outdoor vs outdoor) were
201 included in the regression model, as well as the intervention applied. The analysis was
202 adjusted on the LI at pre-treatment and the ovitrap location (semi-indoor vs outdoor).
203 The same analysis strategy was applied to quantify the intervention effect on LI using a
204 negative binomial regression model.

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

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

216 **Results**

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

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

228 The results of the effect of the intervention on OI are summarized in Table 1. As compared
229 to the control site, the overall outdoor and semi-indoor OI was lower in the intervention
230 sites ADD (-8.3%, $p=0.04$) and TORS (-6.5%, $p=0.10$) and slightly higher in TORS+ADD (+1.8,
231 $p=0.63$). The difference reached statistical significance only in the ADD site. Relative to the
232 control site, the outdoor and semi-indoor OI for *Ae. aegypti* was lower in ADD (-8.9%,
233 $p=0.03$) and TORS (-10.4%, $p=0.02$) and slightly higher in TORS+ADD (+4.9%, $p=0.29$).

234 Regarding *Ae. albopictus*, relative to the control site, outdoor and semi-indoor OI was slightly
235 lower in ADD (-4.2%, $p=0.19$) and TORS+ADD (-3.4%, $p=0.34$) and slightly higher in TORS
236 (+4.5%, $p=0.18$) but none reached statistical significance.

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

241 The relative difference in mean number of larvae per ovitrap in ADD, TORS and TORS+ADD in
242 comparison to the control site was estimated to be -35.4% (95% confidence interval (CI): -
243 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;
244 $p=0.81$) respectively (Table 2). Similar trends were observed for *Ae. aegypti* but the

245 difference reached statistical significance only in ADD (-37.6%; p=0.002). Regarding *Ae.*
246 *albopictus*, as compared to the control site, the mean number of larvae per ovitrap was
247 lower in all intervention sites but none reached statistical significance. As for OI, the LI
248 showed a greater effect of the intervention during weeks 6-10 compared to weeks 0-5 in
249 TORS (p<0.0001) and ADD (p<0.0001) (Supp. Table S3).

250 **Distribution of ADDs deployment strategies**

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

255 Population-based survey and community engagement

256 Baseline characteristics of the 732 individuals that completed the survey are presented in
257 supplementary material (Supp. Table 5). Income categories were based on the report of
258 Household Income and Basic Amenities 2016-Malaysia as follows: top 20% (T20:
259 >US\$1440/month), middle 40% (M40: US\$720-1440/month), and bottom 40% (B40:
260 <US\$720/month) (Department of Statistics Malaysia; 2017). The highest percentage of
261 individuals with primary school education and low income was observed in the TORS+ADT
262 site. This site had also the highest rate of unemployed individuals. Television and radio were
263 identified as the preferred source of information about dengue (71.5%), followed by internet
264 (31%) and relatives (28.2%). COMBI volunteers were available in all study sites but did not
265 participate to the study in the TORS+ADT site. Lower education level in this site might
266 explain the observed lack of participation of COMBI volunteers.

267

268

269 Discussion

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

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

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

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

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

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

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

305 As reported in other investigations (Lee et al., 2015; Hamid et al., 2019) and in agreement
306 with our results, TORS or ADDs effectively reduced the mosquito population. It can
307 therefore be expected that co-application of these techniques together with public
308 cooperation would further enhance the vector control efficacy. The lack of an observed
309 additive effect of the combined TORS+ADD on the mosquito population may be related to
310 socio-economic, waste management measures and architectural differences of the
311 TORS+ADD site compared to the other intervention sites. ~~the reduced TORS coverage due to~~
312 requent-Frequent presence of objects such as pet cages, fish aquarium, furniture and edible
313 plants in the semi-indoor areas in this site led to TORS coverage of 50% as compared to
314 100% in the TORS site. An average coverage of 70% of walls is requested for an effective
315 action of TORS. More discarded, often plastic, waste was also observed in the TORS+ADD

316 site that could slow down the autodissemination effect of the ADDs. -As plastic waste forms
317 breeding sites, fewer females choose the ADD as primary breeding site. -In addition, for
318 those females that did choose the ADD first, the more breeding sites are available on leaving
319 the ADD, the smaller is the initial effect. Some larger breeding sites need more than one
320 mosquito visit to reach the appropriate threshold for killing over 80% of the pupae. -Finally,
321 26% of ADDs were subject to vandalism in this site as compared to only 3% in the site with
322 ADDs alone. The lower education level of the population and the lack of COMBI activities in
323 TORS+ADT site could have contributed to higher vandalism, and presence of bird cages and
324 aquaria at the time of TORS spraying, thus leading to the lack of effect. Moreover, the
325 architecture of the buildings in TORS+ADD site make the semi-indoor walls more subject to
326 rainfall and hence, plausibly, quicker wash-off of K-Othrine Polyzone during the heavy
327 rainfall that occurred after the introduction of the intervention.
328 ~~Extensive involvement of the community is a key parameter of the IVM approach and has~~
329 ~~been shown to enhance the effectiveness of vector control programs [Andersson et al.,~~
330 ~~2015]. Community engagement was conducted by meeting with local officials, head of~~
331 ~~COMBI committees and local COMBI volunteers prior to the start of the study to explain its~~
332 ~~purpose and secure their cooperation and good will. COMBI teams were available in all study~~
333 ~~sites but were actively involved in the study only in TORS and ADD sites. The lack of COMBI~~
334 ~~activities in the TORS+ADD site could have contributed to higher vandalism, and presence of~~
335 ~~bird cages and aquaria at the time of TORS spraying, thus leading to the lack of effect.~~
336 The observed greater effect of the intervention on the mosquito population overtime in the
337 ADD site fit well with the slow killing effect of this device. ADD is designed to attract
338 mosquitoes and then contaminate the adults which then carry pyriproxyfen to other sites
339 before dying from the exposure to the *Beauveria* within approximately 10 days. The PPF

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

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

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

375 **Conclusions and lessons learned**

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

380 Higher education level in TORS and ADT sites suggests better health literacy and could
381 explain tangible results in these sites. Health education of the public will be the first step in
382 community engagement for the planned cRCT epidemiological trial. Active public
383 engagement will start before the intervention and will be maintained throughout the study
384 period. Banners, posters, and announcement brochures will be distributed to explain the
385 objectives of the study. Random allocation of eligible sites for the planned cRCT will be
386 stratified on socio-economic status. The use of indoor ovitraps was not initially planned due
387 to reluctance of the study population. However, regular contact between the study

388 population and the field workers during the collection of baseline data created public trust
389 and some flat owners accepted the ovitraps to be deployed in their homes (results not
390 shown). For the cRCT, it is planned to place indoor ovitraps in volunteers' flats.
391 ~~Moreover, using a combination of several active ingredients (chemical and biological with~~
392 ~~different modes of action) in the same program is expected to have significant benefits for~~
393 ~~insecticide resistance management. Many national programs are using combinations of~~
394 ~~methods but evidence on best practices and the most cost-effective integrated approaches~~
395 ~~is needed.~~

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

409 **Acknowledgments**

410 The authors express their gratitude to the Director General of Health and Director, Institute
411 for Medical Research, Kuala Lumpur for support. We are also thankful to the Malaysian
412 Ministry of Health for providing the laboratory place and equipment.

413 Thanks are also due to David Malone (Integrated Vector Control Consortium) and all staff of
414 Johor Bahru health office and Institute for Medical Research (IMR, Kuala-Lumpur) for help
415 rendered in the field.

416 **Declaration of interest**

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

419 NA receives salary support from the MRC UK and DFID - MRC Grant Reference
420 MR/K012126/1: This award is jointly funded by the UK Medical Research Council (MRC) and
421 the UK Department for International Development (DFID) under the MRC/DFID Concordat
422 agreement and is also part of the EDCTP2 programme supported by the European Union.

423 **Funding**

424 The study was funded by budget allocation from Bayer who supplied also the product for
425 residual spraying. Autodissemination devices were provided by In2Care.

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539

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

Study area	Pre-treatment		Intervention		Difference in OI	
	N	OI (%)	N	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
<i>Ae. aegypti</i>						
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
<i>Ae. albopictus</i>						
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

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

545 *Adjusted for baseline and for ovitrap location

546 The number of oviposition sites was the same during the pre-treatment and intervention
547 periods, but the positivity of the ovitraps was measured every week for 10 weeks during the
548 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
 550 during pre-treatment and intervention period and estimate of the mean larvae index relative
 551 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
<i>Ae. aegypti</i>						
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
<i>Ae. albopictus</i>						
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
 553 interval

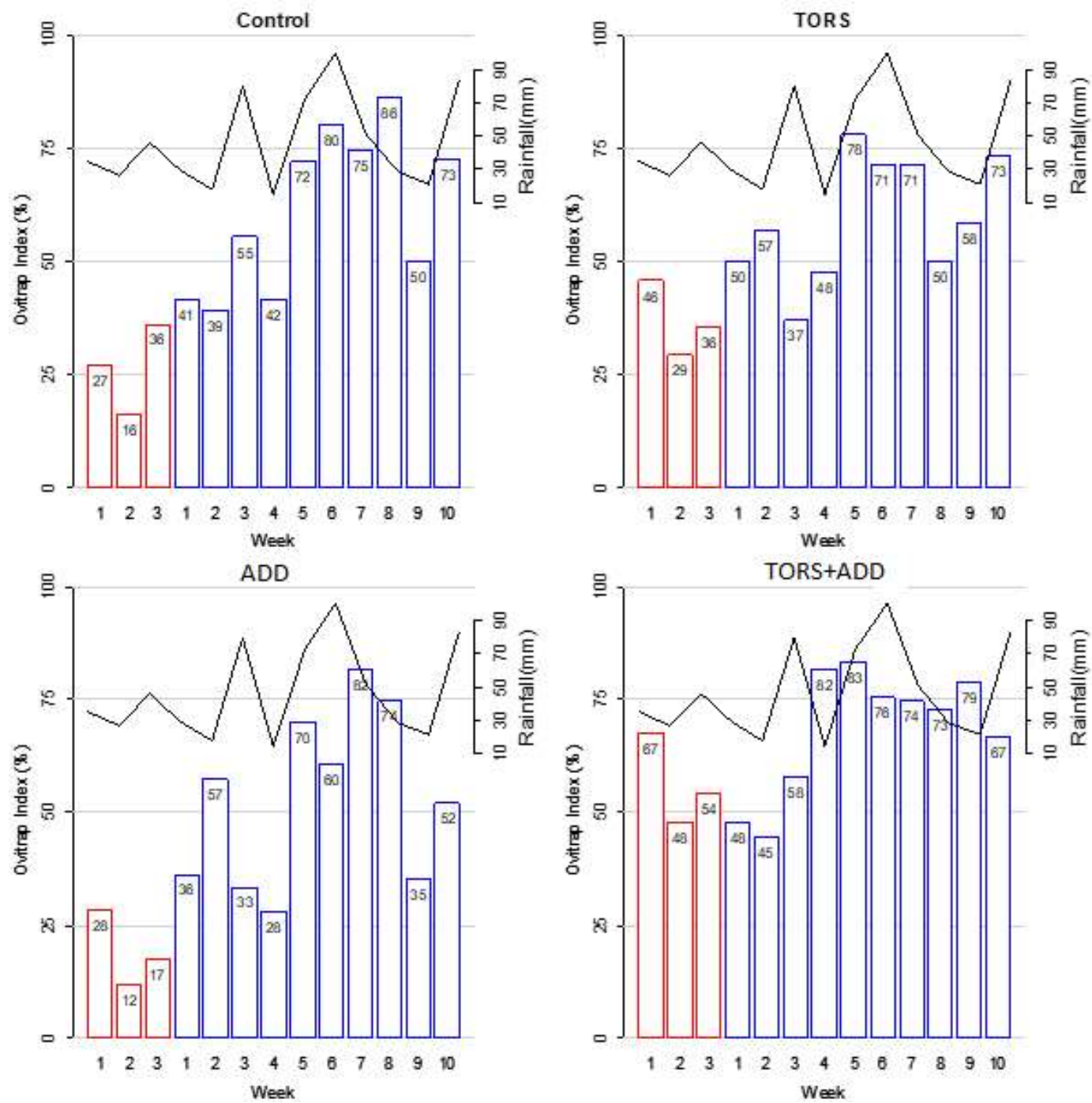
554 *Adjusted for baseline and for ovitrap location

555

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	<i>Ae. aegypti</i>	<i>Ae. albopictus</i>	Overall	<i>Ae. aegypti</i>	<i>Ae. albopictus</i>
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: Larvae index and rainfall (mm) per week during the baseline (red) and the intervention period (blue) in study sites

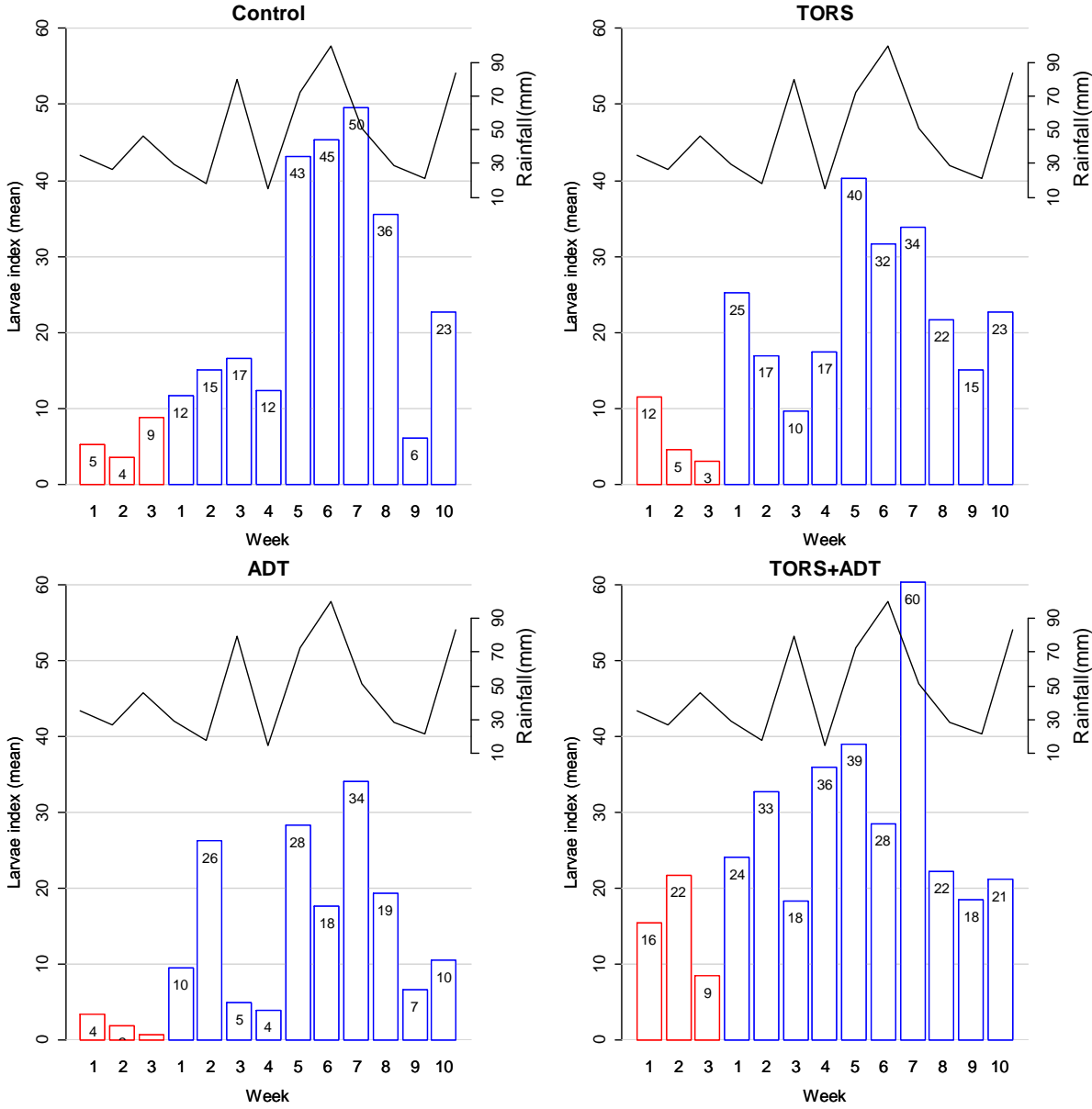


Table S2: Estimate of outdoor and semi-indoor (overall and by species) ovitrap index (OI) differences in comparison to the control site according to the intervention period

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
<i>Ae. aegypti</i>				
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
<i>Ae. albopictus</i>				
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
<i>Ae. aegypti</i>				
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
<i>Ae. albopictus</i>				
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.