Evaluation of a Sticky Trap for Collecting *Aedes* (*Stegomyia*) Adults in a Dengue-endemic Area in Thailand

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Abstract. Development of new operational techniques for collection and monitoring of adult *Stegomyia* mosquitoes is considered a pressing need for surveillance and prevention of arboviruses. Here we report the results from a trial carried out in 2 dengue-endemic villages in Thailand to compare the ability to collect *Aedes* adults of a sticky trap versus a CDC backpack aspirator, which has been used routinely at the study area for entomological/epidemiological surveys. Our comparison was based on a comparable sampling effort required to carry out collections with 2 approaches. Over 19,000 specimens were collected, ~90% of which were *Culex* spp. Sticky traps collected significantly more *Aedes aegypti* and *Aedes albopictus* females than did backpack aspirators when located outdoors. The percentage of positive sticky-trap catches was double for *Ae. aegypti* and almost 20 times higher for *Ae. albopictus*. Operational benefits of the sticky trap are discussed within the context of the results obtained.

INTRODUCTION

*Aedes aegypti* and *Aedes albopictus* (subgenus *Stegomyia*) occur together in many tropical and subtropical parts of the world, and both are involved in transmission of pathogens to humans. *Ae. aegypti* is the most important dengue vector worldwide, is the urban vector of yellow fever virus, and is reported to be mainly a peridomestic species associated with human dwellings, using man-made containers (jars, water pots, discarded tires) as larval habitats. *Ae. albopictus* occupies some of the same larval habitats as *Ae. aegypti* but is considered more exophilic and bites humans and other animals mainly outdoors. Because of this feeding behavior, *Ae. albopictus* is generally considered a less-efficient vector of arboviruses than *Ae. aegypti*, although it can have an important role depending on certain ecological conditions, as in the case of the recent Chikungunya outbreak in La Reunion island, leading to 255,000 suspected cases and more than 200 deaths.

The diurnal activity of adult *Stegomyia* with immature development in containers limits the availability of collection methods for large-scale sampling, which is a major drawback in epidemiological surveillance of arboviruses as well as the evaluation of the impact of control strategies and detection of allochthonous mosquito invasion into nonendemic regions. Development of new operational techniques for collection of adult *Stegomyia* females that can be used to monitor their densities would, therefore, be a valuable contribution to the prevention and control of arboviruses, such as dengue.

Recently, Facchinelli and colleagues described a new sticky trap (ST) for collection of adult *Stegomyia* species, whose females lay eggs in small water containers. They reported that it was effective for collecting *Ae. albopictus* females and monitoring their population dynamics in Rome, Italy. The objective of this study was to evaluate the ability of the ST to collect adult *Ae. aegypti* and *Ae. albopictus* in a dengue-endemic region of Thailand compared with the CDC backpack aspirator (BA). Backpack aspirators are routinely used to collect *Ae. aegypti* and were already being used at the study area to investigate dengue epidemiology and evaluate vector intervention strategies.

MATERIALS AND METHODS

Study area. The trial was carried out in 2 villages in Kamphaeng Phet Province (central Thailand): Nhong Ping (village 1, 16°31’ N, 99°30’ E) and Kon Tee (village 2, 16°22’ N, 99°38’ E). The villages are located along the Ping River about 3 km north and 20 km southeast respectively, from the provincial capital, Kamphaeng Phet. Houses in these villages are either concrete or wood, and approximately half of these are elevated above the ground. A few sheds made of corrugated metal sheets are also present. To protect themselves from mosquito bites, people in this area use coils and bednets, and some houses have screen windows and/or doors. Animals, mainly chickens and dogs, as well as water containers are abundant in the small plot of land surrounding each house. Irrigated rice fields and sugarcane plantations are the main types of agricultural land use in both villages. In the study area, *Ae. aegypti* and *Ae. albopictus* are syntopic, and all 4 dengue virus serotypes (DEN-1, DEN-2, DEN-3, and DEN-4) are transmitted in this area.

Study design. Collections performed by sticky-trap (ST) and by modified CDC backpack aspirator Model 1412 (BA) were carried out from 4 November to 13 December 2005, for a total of 39 sampling days. All households in the study area were georeferenced before the trial and mapped using MapInfo software. A total of 40 houses—16 in village 1 and 24 in village 2—was selected on a grid composed of 150 m x 150 m cells drawn on village maps. Each household was selected within a radius of 25 m from each grid crossing. As a consequence, selected households were located at least 100 m from each other (a distance assumed to be adequate to ensure minimal interaction between the 2 collecting methods used in contiguous households, based on the limited dispersal of *Ae. aegypti*). Over the 40 selected houses, STs were placed in 20...
of them (8 in village 1 and 12 in village 2) and BA was performed in the remaining 20 (8 in village 1 and 12 in village 2). Within each village, collecting methods were alternated in contiguous households.

Six STs were used per household: 3 indoors and 3 outdoors (120 in total). We considered indoor locations to be those spaces surrounded by at least 3 walls and covered by a roof. Indoor STs were located in dark places and room corners, near racks with clothes, and at least 2 m away from water containers. Outdoors, STs were placed within 3 m from the edge of the house roof, close to walls or vegetation, in shaded places, and at least 2 m far from water containers. Because Aedes collections in individual traps were low, for statistical analyses, the 3 STs, based on location (indoors versus outdoors), were considered a single sampling unit.

Sticky traps were serviced twice a week, replacing the adhesive sheets with new ones previously coated with glue with a spatula and filling the trap with fresh water collected from the Ping River. Mosquitoes caught on the adhesive sheets were counted and identified in the field with the help of a magnifying lens.

Backpack aspirator collections were carried out in the remaining 20 households without traps. As with the ST, BA collections were performed twice a week both indoors, in all accessible rooms, and outdoors on objects and vegetation within 3 m from the edge of the roof. The duration of the collection effort varied from 9 to 20 minutes, according to the size and the complexity of each premise. We considered as a single sampling unit to be each collection performed at the same premise and date, either indoors or outdoors. Captured mosquitoes were transported back to the Armed Forces Research Institute of Medical Sciences (AFRIMS) Entomology Laboratory in Kamphaeng Phet, killed by freezing, counted, and identified to species.

Aedes were separated by species and gender. Other Culicidae were identified to genus, and only Culex from ST collections was separated by gender. In only 4 of the 1,320 total STs serviced during the entire investigation, the number of Culex spp. was estimated as > 100, rather than counted.

Our sampling protocol was designed so that the human effort necessary to carry out catches with the 2 methods (i.e., 2 teams of 3 technicians, twice per week) was comparable. Effort necessary to carry out catches with the 2 methods (i.e., minimum and maximum number) of mosquitoes for each catch. All statistical analyses were performed using STATA 9 statistical analysis software (Stata Corp., College Station, TX).

RESULTS

Ninety-eight percent of the overall catches (859/880) carried out in the two villages were performed successfully. Twenty-one catches were excluded from the analysis: 20 were BA collections that were not carried out because houses were not accessible, and 1 was a ST catch with 2 out of 3 traps not operative. Successful catches were carried out as follows: 88 indoors/88 outdoors by ST and 86 indoors/86 outdoors by BA in village 1 and 132 indoors/131 outdoors by ST and 124 indoors/124 outdoors by BA in village 2.

A total of 19,007 adult mosquitoes were collected and identified, of which 10,761 were collected during 348 attempts in village 1 and 8,246 during 511 attempts in village 2, respectively. In both villages, Culex spp. represented the majority (~90%) of mosquitoes collected. The rest of the mosquitoes collected were Aedes spp., Armigeres spp., and Anopheles spp.

Sticky traps and BA collected a total of 11,524 and 7,483 specimens, respectively. Table 1 shows the total number of ST and BA catches and the total number of mosquitoes caught, either indoors and outdoors, in the two villages, subdivided by species and gender for Aedes. Gender and species identification was successful for 98.4% of the Aedes specimens collected by ST and for 100% of those collected by BA.

Table 2 shows the number of catches, the proportion of positive catches, the total number of specimens caught and minimum and maximum number of specimens/positive catch for Ae. aegypti and Ae. albopictus. Data on Ae. albopictus males are not shown; only 3 specimens were collected.

Ae. aegypti. In total, 663 females and 64 males were collected by ST and 313 females and 474 males by BA.

Females. Comparison of the proportion of positive catches obtained by the 2 methods, carried out adjusting for data collected in the 2 villages, showed that outdoors the frequency of positive ST catches was significantly higher than that of BA catches (70.8% versus 9.0%, \( \chi^2 \text{M-H} = 167.9, P < 0.001 \)), whereas indoors, no differences between the 2 methods were observed (52.7% and 51.9%, \( \chi^2 \text{M-H} = 0.01, P = 0.94 \)). This is confirmed by the regression model controlling for clustering of catches repeated in the same household and possible heterogeneity between villages, which shows that the observed difference between the 2 methods was not different indoors (\( \hat{\beta} = 0.14, P = 0.45 \), 95% confidence interval (CI) from −0.499 to 0.796), whereas it was significantly different outdoors (\( \hat{\beta} = −2.92, P < 0.001, 95\% \text{ CI} \) from −3,547 to −2,304).
The proportion of positive catches obtained by ST was significantly higher outdoors than indoors ($\chi^2_{M-H} = 15.24, P < 0.001$), whereas the opposite (more indoors than outdoors) was observed with BA ($\chi^2_{M-H} = 90.59; P < 0.001$).

**Males.** The frequency of positive ST catches was significantly lower than that of BA catches both indoors and outdoors (indoors, 18.2% versus 52.4%, $\chi^2_{M-H} = 55.3, P < 0.001$; outdoors, 3.2% versus 9.0%, $\chi^2_{M-H} = 6.17, P = 0.013$). The proportion of positive catches was significantly higher indoors than outdoors for ST ($\chi^2_{M-H} = 25.22, P < 0.001$) and BA ($\chi^2_{M-H} = 92.3, P < 0.001$).

**Females versus males.** The frequency of positive ST catches was significantly higher for females than for males, both indoors and outdoors (indoors, 52.7% versus 18.2%, $\chi^2_{M-H} = 209.7, P < 0.001$; outdoors, 70.8% versus 3.3%, $\chi^2_{M-H} = 56.6, P < 0.001$). There were no differences in BA catches (indoors, 51.9% versus 52.4%, $\chi^2_{M-H} = 0.01, P = 0.999$; outdoors, 9% versus 9%, $\chi^2_{M-H} = 0.03, P = 0.86$).

*Aedes albopictus.* In total, 149 females and 1 male *Aedes albopictus* were collected by ST and 5 females and 2 males by BA. The frequency of positive ST catches was significantly higher than that of BA catches both indoors (8.6% versus 1.9%) and outdoors (34.7% versus 0.5%) (Fisher's exact test $P < 0.001$). The proportion of female-positive catches obtained by ST was significantly higher outdoors than indoors ($\chi^2 = 54.7, P < 0.001$).

### Table 1

<table>
<thead>
<tr>
<th>Village</th>
<th>Method</th>
<th>No. of catches</th>
<th>$%$</th>
<th>$\chi^2$</th>
<th>$P$</th>
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<tr>
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<td>ST IN</td>
<td>170</td>
<td>38</td>
<td>10</td>
<td>0.813</td>
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<td></td>
<td>ST OUT</td>
<td>196</td>
<td>8</td>
<td>48</td>
<td>0.813</td>
</tr>
<tr>
<td></td>
<td>BA OUT</td>
<td>98</td>
<td>152</td>
<td>2</td>
<td>0.813</td>
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<td>2</td>
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<td>15</td>
<td>0.813</td>
</tr>
<tr>
<td></td>
<td>BA IN</td>
<td>190</td>
<td>1</td>
<td>76</td>
<td>0.813</td>
</tr>
<tr>
<td></td>
<td>BA OUT</td>
<td>192</td>
<td>286</td>
<td>2</td>
<td>0.813</td>
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</tbody>
</table>

### Table 2

<table>
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<tr>
<th>Species</th>
<th>Method</th>
<th>Location</th>
<th>No. of catches</th>
<th>No of positive catches (%)</th>
<th>No. of specimens</th>
<th>Min–Max</th>
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<tbody>
<tr>
<td><em>Aedes aegypti</em></td>
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<td>IN</td>
<td>219</td>
<td>155 (70.8)</td>
<td>386</td>
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<td></td>
<td>OUT</td>
<td></td>
<td>219</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>439</td>
<td>271 (61.7)</td>
<td>663</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BA</td>
<td>IN</td>
<td>210</td>
<td>109 (51.9)</td>
<td>290</td>
<td>1–13</td>
</tr>
<tr>
<td></td>
<td>OUT</td>
<td></td>
<td>210</td>
<td>19 (9.0)</td>
<td>23</td>
<td>1–2</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>420</td>
<td>128 (30.5)</td>
<td>313</td>
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<tr>
<td><em>Aedes aegypti</em></td>
<td>ST</td>
<td>IN</td>
<td>220</td>
<td>38 (17.3)</td>
<td>55</td>
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<tr>
<td></td>
<td>OUT</td>
<td></td>
<td>219</td>
<td>7 (3.2)</td>
<td>9</td>
<td>1–3</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>439</td>
<td>45 (10.3)</td>
<td>64</td>
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<tr>
<td></td>
<td>BA</td>
<td>IN</td>
<td>210</td>
<td>108 (51.4)</td>
<td>438</td>
<td>1–30</td>
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<tr>
<td></td>
<td>OUT</td>
<td></td>
<td>210</td>
<td>19 (9)</td>
<td>36</td>
<td>1–5</td>
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<tr>
<td></td>
<td>Total</td>
<td></td>
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<td>127 (30.2)</td>
<td>474</td>
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<tr>
<td><em>Aedes albopictus</em></td>
<td>ST</td>
<td>IN</td>
<td>220</td>
<td>19 (8.6)</td>
<td>25</td>
<td>1–2</td>
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<tr>
<td></td>
<td>OUT</td>
<td></td>
<td>219</td>
<td>76 (34.7)</td>
<td>124</td>
<td>1–4</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>439</td>
<td>95 (21.6)</td>
<td>149</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BA</td>
<td>IN</td>
<td>210</td>
<td>4 (1.9)</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>OUT</td>
<td></td>
<td>210</td>
<td>1 (0.5)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>420</td>
<td>5 (1.2)</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3

*Culex spp.* Table 3 shows the number of catches, the proportion of positive catches, the total number of specimens caught, and the minimum and maximum number of specimens/catch in the 2 villages. Sticky traps and BA collections resulted in total numbers of 10,535 and 6,635 *Culex* specimens, respectively. Gender determination was carried out only for specimens collected in the ST; females represented 90% of the sample.

Data from the 2 villages were not homogeneous (test of homogeneity: $P = 0.031$). Therefore, we analyzed data separately. In village 1, no differences were observed between ST and BA catches indoors (97.7% versus 98.8%, $\chi^2 = 0.31, P = 0.57$), whereas outdoors the frequency of positive ST catches was significantly higher than that of BA catches (97.7% versus 55.8%, $\chi^2 = 43.15, P < 0.001$). In village 2, the frequency of positive ST catches was significantly higher than BA catches indoors (97.7% versus 86.3%, $\chi^2 = 11.61, P < 0.001$) and outdoors (96.2% versus 38.7%, $\chi^2 = 97.08, P < 0.001$).

Results from the regression model controlling for clustering of collections repeated in the same household and the heterogeneity between villages indicate that there were significant differences between the 2 methods outdoors ($\beta = -1.574, P < 0.001$, 95% CI from $-2.312$ to $-0.837$) but not indoors ($\beta = 0.096, P = 0.793$, 95% CI from $-0.621$ to $0.813$).
DISCUSSION

The aim of the present study was to evaluate whether the ST developed by Facchinelli and colleagues is effective for collecting *Stegomyia* mosquitoes, such as the dengue vectors *Ae. aegypti* and *Ae. albopictus*. The ST was designed specifically for mosquito species with immature development in containers and had been previously tested only in a temperate area (i.e., in Rome, Italy), where it was shown to effectively collect high numbers of female *Ae. albopictus*. Results from the present study demonstrate that, in central Thailand, the ST collected *Culex* spp. (which represented about 90% of the specimens collected), *Ae. aegypti*, *Ae. albopictus*, and *Armigeres* spp. Although only a small sample of *Culex* was identified to species, we suspect that most specimens collected were *Culex quinquefasciatus*, known to be abundant in Thailand.

In our comparison of results from ST catches with those obtained by BA, it is relevant to stress that the 2 approaches collect different fractions of the mosquito populations (i.e., mainly ovipositing females for ST and mainly resting males and females for BA). The ST collects mosquitoes continuously. The BA captures mosquitoes only for the few minutes that the collecting is carried out. Therefore, our comparison was based on overall effort required to carry out sampling activities (from implementation of field work to specimen counting and identification), which was comparable between the 2 approaches.

Taking these differences into consideration, our results show that the ST collects more female *Aedes* than BA, the percentage of positive catches being double in the case of *Ae. aegypti* and almost 20 times higher in the case of *Ae. albopictus*. Results from *Culex* spp. (which represented about 90% of the specimens collected), *Ae. aegypti*, *Ae. albopictus*, and *Armigeres* spp. Although only a small sample of *Culex* was identified to species, we suspect that most specimens collected were *Culex quinquefasciatus*, known to be abundant in Thailand.

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Taking these differences into consideration, our results show that the ST collects more female *Aedes* than BA, the percentage of positive catches being double in the case of *Ae. aegypti* and almost 20 times higher in the case of *Ae. albopictus*. A similar trend, although less marked, was also observed in the case of *Culex* collections. The only result in contrast with this trend concerns *Ae. aegypti* males, which were collected mostly by BA. This result is expected because the ST is targeted at ovipositing females.

When the results are analyzed separately for indoor and outdoor catches, it is clear that the larger number collected by the ST is due mainly to outdoor catches, where ST consistently shows a higher percentage of positive catches and higher numbers of *Aedes* females/catch when compared with BA. The same pattern is also observed for *Culex* and *Armigeres* spp. These results may reflect the fact that the 2 methods collect different fractions of the mosquito populations and may also be linked to intrinsic constrains in the use of BA outdoors. It is likely that mosquitoes are more difficult to locate outdoors and/or may be able to escape aspiration more easily outdoors than when confined indoors.

The comparison between ST catches carried out indoors versus outdoors shows that the percentage of *Aedes*-positive catches, as well as the overall number of females/catch, is higher outdoors; i.e., 57% and 80% positive outdoor ST catches for *Ae. aegypti* and *Ae. albopictus* females, respectively. The higher outdoor collection rate for *Ae. albopictus*, compared with that for *Ae. aegypti*, may reflect a different distribution of females of the 2 species and the greater exophily tendency of *Ae. albopictus*. The higher number of male *Ae. aegypti* collected indoors and the general observation that this species tends to rest indoors suggest that females are leaving the indoor environment to find oviposition sites outside, as assumed by Dibo and others and confirmed by Favaro and others in Brazil. Movement studies and assessment of gonotrophic stages of females collected in the ST, which we have proved to be feasible, are needed to prove that females leave houses when looking for oviposition sites in Thailand.

**CONCLUSIONS**

Our results indicate that the new ST can be successfully used to sample dengue vectors, such as *Ae. albopictus* and *Ae. aegypti*, and possibly other mosquitoes with immature development in containers (e.g., *Cx. quinquefasciatus*, vector of *Wuchereria bancrofti*). Further studies would be needed to evaluate whether the ST can be used as an alternative to other collection methods for monitoring population dynamics of these species. In fact, the ability of the ST to detect the presence of *Ae. aegypti* and *Ae. albopictus* particularly in the outdoor peridomestic environment, suggests that ST could be a conveniently exploited trap for detection and monitoring exophilic vector species. It could be a component of surveillance and monitoring activities in areas where it is not easy/possible to perform indoor collections.

The ST has several operational advantages compared with the BA: i) it operates continuously and is, therefore, less biased by local circumstances during the short period of aspiration; ii) it is not biased by collector expertise; and iii) it does not require a power source. Variation in operator placement of STs can, however, have a significant impact on the results. Compared with other kinds of sampling devices targeted at trapping adult mosquitoes (e.g., the Prince trap, the CDC
Wilton trap, and the BG-Sentinel trap), the ST used herein is cheap (about 15 USD for 1 ST equipped with a set of 4 sticky sheets), easy to manipulate, and does not need a power source. However, it should be stressed that, because the ST collects mainly gravid females, it cannot in principle be used to estimate human-vector contact. It is reasonable to assume, however, that gravid *Ae. aegypti* females must have taken a human blood meal because of their high degree of anthropophily.20 The sticky trap should be further evaluated for surveillance of *Stegomyia* mosquitoes and/or monitoring the impact of mosquito control strategies. When interpreting the results of such investigations, it will be important to consider that efficiency of the ST is likely to be affected by the availability of alternative, natural oviposition sites, a phenomenon that was demonstrated for ovitraps by Focks.20

The ST may also represent an attractive tool for ecological and epidemiological investigations. Specimens collected by the ST could be used i) to study dispersal and longevity—results from the preliminary MRR experiment carried out in Rome by Facchinelli and others8 confirm that specimens dusted with fluorescent powders can be easily detected by observing the sticky sheets under UV light; ii) to study resting and/or ovipositing behaviors with respect to the indoor versus outdoor environments (Valerio and others, unpublished data); iii) to analyze the origin of bloodmeals in specimens captured before completion of bloodmeal digestion (Valerio and others, unpublished data); iv) to assay for the presence of pathogens, e.g., dengue virus21,22; and v) to assay for insecticide resistance alleles.23 We propose that in areas where alternative breeding sites are scarce and/or reduced as a result of integrated source reduction control activities, it will interesting to test the potential of ST as a mass trapping device to reduce vector densities, similar to the study conducted with a lethal ovitrap in Thailand.24

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