Surveillance of adult *Aedes* mosquitoes in Selangor, Malaysia

Sai-Ming Lau¹, Indra Vythingam², Jonathan Inbaraj Doss³, Shamala Devi Sekaran⁴, Tock H. Chua⁵, Wan Yusof Wan Sulaiman³, Karuthan Chinna³, Yvonne Ai-Lian Lim² and Balan Venugopalan¹

1 State Vector Borne Disease Control Unit, Selangor State Health Department, Selangor, Malaysia
2 Department of Parasitology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
3 Julius Centre, Department of Social and Preventive Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
4 Department of Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
5 Department of Pathobiology and Medical Diagnostics, Faculty of Medicine and Health Sciences, University of Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia

**Abstract**

**Objective** To determine the effectiveness of using sticky traps and the NS1 dengue antigen kit for the surveillance of *Aedes* mosquitoes for dengue control.

**Methods** Apartments were selected in a dengue-endemic area, and sticky traps were set to capture adult *Aedes* mosquitoes. NS1 dengue antigen kit was used to detect dengue antigen in mosquitoes, and positive mosquitoes were serotyped using real-time RT-PCR.

**Results** The sticky traps were effective in capturing *Aedes aegypti*, and a minimum of three traps per floor was sufficient. Multiple serotypes were found in individual mosquitoes.

**Conclusion** The sticky trap and the NS1 dengue antigen test kit can be used as surveillance tool in dengue control programmes. This proactive method will be better suited for control programmes than current reactive methods.

**Keywords** *Aedes aegypti*, Dengue, sticky trap, NS1 antigen test kit, surveillance, Malaysia

**Introduction**

Dengue virus, which is transmitted primarily by the *Aedes aegypti* mosquito, is the most important arbovirus affecting humans in tropical and subtropical countries [1], and dengue outbreaks are spreading across more countries [2, 3]. The number of reported dengue cases has increased exponentially since 2007 (WHO Western Pacific). Currently, an estimated 2.5 billion people in 100 countries are at risk [4]. In South-East Asia, dengue emerged as a public health problem during and after World War II [5]; in Malaysia, dengue was first reported in 1902 [6]. Epidemics of dengue have occurred in 1973, 1982 and 1998 [7]. However, the number of reported cases in Malaysia until 9 September 2014 is above the median of 2009–2013. Current total numbers of reported cases and deaths are 70,337 and 133, respectively [8]. In recent years, the epidemics of dengue have become critical in the state of Selangor, Malaysia. Reported cases of dengue have increased fourfold in 2014 compared to the same period in 2013 (from 8258 to 33,564); case mortality increased fivefold from 10 in 2013 to 50 in 2014 [9].

In the absence of a vaccine and drugs against dengue virus, control of the *Aedes* mosquito is the main tool for the surveillance and management of dengue. In Malaysia, *Ae. aegypti* is the principal vector of dengue and *Ae. albopictus* is the secondary vector [10]. Control methods for dengue in Malaysia are environmental sanitation, house-to-house larva surveys, source reduction and health education. Fogging and ULV are conducted when cases are reported or when *Aedes* indices are higher than the sensitive threshold (Ministry of Health Plan of Action for Surveillance and Control of Dengue 2009–2013). These methods may have been effective in the past, but currently, due to mushrooming of houses and unplanned urbanisation and construction, the control of dengue is becoming more challenging.

There is no correlation between the *Aedes* index and the number of dengue cases [11]. Larval mortality can be high, and there is a possibility that larvae may die before emergence as adults [12]. They can also be spatially dissociated from the larval development site. A more appropriate index would be the pupal index because it is the stage that directly precedes the adult mosquito that can transmit dengue [13]. However, to determine the pupal index is also time-consuming and labour-intensive. Determining the adult density may provide a better correlation to the number of cases [11, 14, 15]. The ovitrap has been used as a proxy to gauge the number of adults present from the total number of eggs laid. This has been
used to monitor the spatial and temporal analysis of the
*Aedes* mosquito. However, it is not an efficient tool to be
used for adult density determination because *Ae. aegypti*
exhibits skip oviposition, and thus, the results from
ovitraps may not be accurate [16].

Many methods are available for the collection of adult
*Aedes* mosquitoes. The BG-sentinel can be used to collect
*Aedes* mosquitoes and is very efficient, but the only disad-
vantage is the cost which may not be affordable by underde-
vloped countries where dengue is most endemic [17, 18].
The backpack aspirator can be used to collect resting
mosquitoes inside and outside houses [19], providing an
abundance of adult *Aedes* in the locality in correlation to
host–vector contact [20]. However, this method is also very
dependent on the individual carrying out the process, and it
is also labour-intensive and intrusive. To overcome these
limitations, sticky traps have been deployed in many
countries to test their efficacy [14, 21–24].

The state of Selangor consists of nine districts of which
Petaling District had the most dengue cases, contributing
43.55% of cases and 30.77% of deaths in Selangor [25].
Even with all control measures in place, it remains diffi-
cult to control the rising number of cases, which has been
increasing over the past decades [26, 27]. Operational
research that could complement existing strategies is
urgently needed. Thus, this pilot study was initiated with
the objective to determine the feasibility of using sticky
traps as a surveillance tool for dengue control.

**Methods**

**Study site**

The study took place in Selangor, Malaysia’s most
developed and populated state. Selangor’s geographical
position in the centre of Peninsular Malaysia contrib-
uted to the state’s rapid development as Malaysia’s
transportation and industrial hub that contributes 23% of
the total GDP of Malaysia (Department of Statistics,
Malaysia). Selangor consists of nine districts, of which
we chose Petaling, which had the highest number of
dengue cases and is the most populated district in Se-
langor state (Figure 1). The actual study site was at
Mentari Court Apartments, as the complex had a high
number of dengue cases (28 in 2011, 30 in 2012 and
17 from January to May 2013). Mentari Court Apart-
ments comprises 7 blocks with 17 floors in each block
and a total of 3472 premises. There are also car parks,
24 shop lots, two recreation parks and five refuse
storage areas. Almost 40% of the residents are immi-
grants from Africa, Bangladesh, India, Middle East,
Mongolia and Vietnam. For Trial 1 of the pilot study,
blocks C and D were selected due to the high number
of cases at that point in time. However, for Trial 2 of
the pilot study, all seven blocks were included. The
total population of the blocks was estimated to be
about 12 000.

Figure 1 Map of peninsular Malaysia showing the different states. Insert is the
map of Selangor, showing all districts. The study was carried out in Mentari
court apartments which is situated in Petaling District.
Sticky trap

The sticky trap consisted of two plastic containers which were sprayed black as shown in Figure 2. The bigger container was 11.5 cm in diameter and 10 cm in height; the smaller container was 11.5 cm in diameter and 7 cm in height. The smaller container had netting at the bottom, and the sides of the container were lined with brown disposable paper sprayed with sticky insect catcher®. This consists of synthetic solid rubber (53%), solvent (46.6%) and yellow dye (0.4%) and is produced by SR Megah Chemicals (Taman Klang Perdana, Klang, Malaysia). The larger container was filled with 10% hay infusion water. The smaller container containing the sticky surface was placed inside the larger container. Ovipositing mosquitoes are trapped on the sticky surface, and if a mosquito sits on the netting to lay eggs, the emerging adults will not be able to escape due to the netting.

Field sampling

Trial 1: For the initial study from June 2013 to September 2013, a total of 62 sticky traps were set in blocks C and D, on floors: ground floor (GF), 3rd, 6th, 9th, 12th and 15th. In Block C, the number of sticky traps set on the respective floors starting from ground floor (GF) was 1, 2, 4, 6, 8 and 10, respectively, while in Block D, it was the reverse. Thus, in Block D, the ground floor had the most traps. All traps were labelled accordingly.

All sticky traps were examined twice a week. If any insects were stuck on the surface, the trap was replaced with a new one and taken to the laboratory for insect identification. If no insects had caught on the surface, the sticky paper was changed once a month or as needed when dirty. The hay infusion water was replaced once a week. The sticky traps were set inside the house or outside, under the roof. The sticky trap index was calculated as the percentage of traps positive for *Aedes*. The *Aedes* density was calculated as the total number of *Aedes* divided by the number of positive sticky traps.

From July to September 2013, two ovitraps per floor per block were set to monitor the presence of *Aedes*. All ovitraps contained hay infusion water and were serviced twice a week. The ovitrap index was the percentage of ovitraps positive. The egg density was calculated as the total number of eggs divided by the number of positive traps.

Trial 2

The second trial was carried out for 5 weeks from 1 October 2013 until 6 November 2013. All seven blocks were included in this trial. This trial was carried out to determine the optimum number of traps that would be needed for surveillance. Sticky traps were set on floors: GF, 3rd, 6th, 9th, 12th, 15th and 17th starting with three traps for the first week, five traps on the 2nd week, seven traps on the 3rd week and nine traps on the 4th and 5th week, respectively. Thus, the total number of sticky traps set ranged from 147 to 441. At the same time, one ovitrap was placed on each of the floors mentioned above. All traps were examined and serviced weekly.

Identification and processing of mosquitoes

All sticky papers with insects were examined under stereomicroscope in the laboratory. Mosquitoes were identified to species level. Only *Ae. aegypti* and *Ae. albopictus* were processed for detection of the virus. The abdomens of the mosquitoes were pooled (five in a pool), while the head and thorax of each mosquito were kept individually in Eppendorf tubes at −20 °C until real-time RT-PCR processing.

Detection of dengue viral antigen in abdomen of mosquitoes

To each pool of mosquito abdomens, 50 µl of PBS was added and homogenised lightly using a pestle and handheld homogenizer (Kontes Thompson Scientific). The tube was centrifuged for 3 min at 1006 g. The SD Bioline® NS1 Ag kit (Standards Diagnostic Korea) was used to test for the dengue antigen in the mosquito, following the manufacturer’s protocol. Briefly, the content from each
tube was pipetted using the pipet provided onto the well of the device (test kit). After 10–15 min, the reading was taken. If the sample was positive, two bands will be seen. If negative, only the control band will be seen. For all the pooled abdomens that were positive, the individual head and thorax of the respective mosquito was tested for dengue virus by real-time RT-PCR.

RNA extraction

Individual mosquitoes were ground in pre-chilled Eppendorf tubes with 0.25 ml of a growth medium (Eagle’s minimum essential medium, EMEM). The mosquito suspensions were then centrifuged at 21000 g for 15 min at 4 °C. RNA extraction was carried out with High Pure Viral RNA Isolation Kit (Roche Applied Science) according to the manufacturer’s protocol. The homogenate (200 μl) was mixed with 400 μl of binding buffer and centrifuged at 8000 g for 15 s. The RNA was then washed twice with washing buffer and centrifuged at 8000 g for 1 min. A total of 30 μl of viral RNA were eluted from the sample using elution buffer. The extracted RNA was collected and stored at −80 °C for viral detection through real-time RT-PCR.

One-step TaqMan real-time RT-PCR

The one-step TaqMan real-time RT-PCR was carried out in a CFX96 Thermocycler (Bio-Rad) [28]. Briefly, 5 μl of the sample RNA, 0.5 μM of each primer, four TaqMan probes (0.25 μM each) and 5.0 mM of MgCl2 in final concentration were used in a 25-μl reaction containing the one-step RT-PCR premix (BioNeer). The thermal cycling profile of this assay consisted of an initial RT step at 50 °C for 30 min, and Taq polymerase activation at 95 °C for 15 min, followed by 40 cycles of PCR with the following conditions: denaturation at 95 °C for 30 s, annealing/extension at 60 °C for 1 min.

Statistical analysis

All statistical analyses were performed using R [29] programming language for statistical analysis (version 3.1) and Excel 2010. Data were subjected to analysis of variance (ANOVA), t-test, nonparametric tests (Pearson’s $\chi^2$ test), nonlinear regression (Box–Lucas) and general linearised modelling. The minimum infectious rate (MIR) was calculated by maximum likelihood estimation method [30] based on 45 pools of 5 mosquitoes each.

Results

Trial 1

A total of 249 Ae. aegypti and eight Ae. albopictus were obtained from the two blocks for the 18 weeks of the first trial, while a total of 35 dengue cases were reported for the same period for the two blocks. Figure 3 shows the distribution of the dengue cases, Aedes mosquitoes and the positive mosquitoes throughout the study period. It would appear that the positive mosquito was detected (6–10 June 2013) before a case was reported (8 June 2013). There was no correlation between number of cases and number caught for Ae. aegypti ($r^2 = 0.013$) and Ae. albopictus ($r^2 = 0.012$).

Also, further correlation analysis on the lag time (2, 3 and 4 weeks) of occurrence of cases and number of Aedes caught did not show significant relationship between the two variables. However, the relationship between number of cases and Aedes caught yielded significant relationship using general linearised model (GLM). The relationship can be described with the equation: $y = 1.1517 + 0.0404x$ ($F_{1,20} = 3.95, P < 0.001$).

Analysis of distribution of cases recorded during the study period among the 17 floors (Table S1) in the seven blocks showed that the cases occurred independently of floor and block (Pearson’s $\chi^2 = 112.22$, df = 102, P-value $> 0.05$). The sticky trap index and the ovitrap index are shown in Figure 4. The ovitrap index seemed to follow the same trend as the sticky trap index. However, the ovitrap index was higher than sticky trap which was to be expected because a single mosquito can lay eggs in many containers. ANOVA indicated there was no statistical difference in the index values recorded between the blocks, between the floors and between locations ($P > 0.05$). Besides Ae. aegypti and Ae. albopictus, the sticky trap also collected a large number of Culex quinquefasciatus (results not shown).

The density of Ae. aegypti and the egg density per trap are shown in Figure 5. Both show the same trend. ANOVA indicated there was no difference in egg density per trap between blocks ($F_{6,216} = 1.70, P > 0.05$) nor between weeks ($F_{4,216} = 1.66, P > 0.05$). Similarly, there was no difference in positive sticky traps between blocks ($F_{7,39} = 1.52, P > 0.05$), but a significant difference existed between weeks ($F_{4,39} = 5.82, P < 0.001$). However, there were significant differences in the number of eggs recorded between the floors ($F_{5,336} = 6.66, P < 0.001$), between the locations of the traps ($F_{3,336} = 4.90, P < 0.001$) and between weeks ($F_{12,336} = 3.86, P < 0.001$). Significantly higher number of eggs was recorded on the ground floor ($P < 0.001$). There was a significant correlation between the percent-
The distribution of *Ae. aegypti* among the various floors is shown in Figure 6. In Block C, the highest percentage of *Ae. aegypti* was obtained on the 15th floor, while in Block D, it was on the ground floor. These were the floors that had the highest number of traps. However, on a per trap basis, there was no difference in the number caught between blocks and between floors (*P* > 0.05), although higher number was recorded for ground floor. Interestingly, the one trap on the ground floor of Block C caught 19% of the *Ae. aegypti*.

**Trial 2**

Relationship between *Ae. aegypti* caught and number of traps: There was a significant correlation between sticky traps with *Ae. aegypti* and the proportion of positive ovitraps with *Aedes* eggs (*r*² = 0.43, df = 17, *P* < 0.01). The relationship between *Ae. aegypti* caught (y) and number of traps (x) is best described by a nonlinear model (Box–Lucas 1959). The equation obtained is

\[ y = 19.92 \left(1 - \exp(-0.27x)\right) \]  

(*P* < 0.001) (Figure 7). The equation is asymptotic at around 20 suggesting that 20 traps per block would be sufficient to be deployed for monitoring *Aedes* population.

Detection of dengue virus: A total of eight pools of *Ae. aegypti* were positive for dengue virus using the NS1 antigen detection kit, and thus, the minimum infection rate per 1000 mosquitoes (MIR) was 38.02 (18.00–71.18). Of the 40 mosquitoes (head and thorax) tested individually using real-time RT-PCR, 15 were positive giving an infectious rate of 6.02. Of these, 10 had dual infection of DENV2 and DENV3, two were positive for
DENV3, one was positive for DENV2, and two were positive for DENV1.

**Discussion**

The most important and novel finding of this project was that the virus was detected in the mosquito before a case was reported. Ten mosquitoes carried two serotypes of dengue virus. In this study, all serotypes except DENV-4 were present. According to [27], DENV-4 was the least prevalent of all serotypes and it formed <20% of all serotypes detected in 2000–2012 in Malaysia. Thus, using sticky traps for surveillance would be more cost-effective and help to give warning before large epidemics. During this trial, we did not manage to detect dengue virus in mosquitoes on the day of collection because the traps were sent to the main laboratory for this. However, this is a simple procedure that can be carried out by health staff at the ground level. Studies in Singapore have also...
shown that the antigen detection NS1 kit was useful in detecting the dengue viral antigen in field-collected mosquitoes [23, 31].

Adult mosquitoes can be used for estimating dengue transmission risk [24], and dengue virus has been found in field-collected Aedes mosquitoes in Mexico [32], South-East Asia [33, 34] and India [35]. The peak entomological inoculation seems to precede the human dengue cases by several weeks to a month [32]. However, although these studies showed evidence that the monitoring of adult population would be a useful tool for dengue surveillance, it is not being implemented as a surveillance tool. One reason could be due to the use of RT-PCR for the detection of dengue virus in mosquitoes. This requires expertise and laboratory support and would be very expensive for dengue-endemic countries. However, as the NS1 antigen kit is effective and simple to use, it could be used in future dengue control programmes.

Our trials have also shown that it is unnecessary to set large numbers of traps. Our sticky trap numbers ranged from 147 to 441 and setting as few as three traps in each floor or about 20 traps per block, also supported by the Box–Lucas equation, were sufficient to collect and monitor the adult Ae. aegypti population for control programme purposes. Similar studies in Brazil also showed that setting as many as four traps in their study area was sufficient [36]. However, before the introduction of sticky traps, it will be necessary to determine the number needed for each house type and location.

The ovitrap results also showed that the number of Aedes was falling because the egg density decreased over time. As the Aedes mosquito performs skip oviposition [16], the positivity of the ovitrap may not have fallen as expected compared to the sticky trap. Researchers have tried to correlate the adult density from sticky traps and egg counts from ovitraps [37]. In some studies, a poor correlation was detected when comparing collections from ovitraps and MosquiTRAP [22]. However, similar percentages of positive ovitraps and sticky traps were reported in Australia [15] and in Italy [37]. In our study, there was significant correlation between sticky trap and ovitrap index.

The Aedes larva survey has been the hallmark of the surveillance programme for decades [38], but it has shown that it is currently not sustainable due to the mushrooming of houses and apartments in the urban areas. Health personal have not increased in proportion to the number of housing estates that have developed over the years, thus leading to shortage of manpower to carry out surveys. In most urban areas, people are at work during the day and accessibility to houses for larva surveys is a major problem. However, it would be easier and more effective to use the sticky traps as a surveillance tool. A larger area can be covered by a smaller number of personnel. The true incidence of the disease in Malaysia may be underestimated due to the use of passive reporting system and low levels of reporting from the private sector [39]. Thus, there is the additional benefit of detecting the virus in the mosquito and taking necessary control measures to break the chain of transmission. This would be a more proactive measure for a control programme.

Although many studies have been conducted in different countries [14, 22, 24, 40–42] to show the effectiveness of the sticky trap in collecting the Aedes mosquitoes and its importance in a surveillance programme, this has not been implemented in any control programme in South-East Asia [38] with the exception of Singapore where it is used for dengue cluster management [23]. However, in Brazil, besides using larval surveys, some municipalities are using Intelligent Dengue Monitoring (MI Dengue) which consists of using MosquiTRAP (a sticky trap with a synthetic attractant), palmtops/cell phones and GIS software (Geo-Dengue). The adult indices are used for larviciding and source reduction [21]. Our initial trial has encouraged health workers who worked in this study to observe the benefits of using the sticky trap compared to carrying out house-to-house larval surveys.

New paradigms are wanting for surveillance of dengue because it is the fundamental for setting goals and evaluating success. Reliable surveillance tools for dengue vectors are greatly needed. An inexpensive and effective Ae. aegypti-specific adult trap would be a significant surveillance breakthrough and could also allow for virus testing [33]. Thus, we feel strongly that this simple sticky trap and the NS1 antigen diagnostic kit will serve as a good tool for surveillance of dengue. This needs to be tested in many different ecotypes.

Another important aspect of this trap is that a female Ae. aegypti will have to lay eggs after a blood meal (with or without virus), and thus, if it selects the sticky trap, it will be caught. If it had selected some other container to lay her eggs, there is another opportunity for it to select this sticky trap for its 2nd oviposition and thus will not be able to transmit the virus.

But the major limitation of the sticky trap is that it targets only gravid females seeking ovipositing sites rather than host-seeking ones and its efficacy could be reduced by nearby natural oviposition sites [15]. That is
the reason why hay infusion water was used to make the sticky trap containers more attractive than the surrounding containers. Ideally, attractant would be used instead of preparing hay infusion every week. These sticky traps also have the advantage over other traps that were used for collection for adult mosquitoes, because they do not need to be serviced daily. It would not be practical for a control programme to use a tool that has to be serviced daily. Commercial traps are also very expensive.

It is also important that the sticky adult trap has some mechanism which prevents immatures from developing into adults. Chadee and Ritchie [14] found that due to death stress oviposition, female Aedes aegypti shoot out eggs directly into the water when caught on the sticky surface. Thus, either larvicides can be applied to kill the emerging larvae or a netting can be placed at the bottom of the inner container so as to prevent the escape of the adult mosquitoes.

The number of infected mosquitoes obtained in the study was high. This shows that the survival of these old females was important because they have to survive at least 6–9 days (incubation period) after feeding on an infected blood meal. Indirectly the sticky trap prevented the human–vector contact, which would reduce the infective bites and also eliminate all mosquito progeny. Studies have also indicated that abundance of larvae or pupae was not predictive of abundance of Aedes aegypti females [11]. The relationship between vector abundance and dengue transmission needs to be elucidated [44], to introduce adult mosquito sampling as a routine and current indices like Breteau are not reliable universal dengue transmission thresholds. A recent study in Thailand [45] demonstrated a positive association between infected Aedes aegypti and dengue-infected children in the same and neighbouring houses. The positive mosquito was obtained before the index case was reported.

Conclusion

This preliminary trial has shown that the sticky trap is a cheap and effective way to collect Aedes mosquito. Dengue virus was detected in Aedes aegypti before the epidemic. The NS1 antigen test kit is a simple tool that can be used by public health staff to demonstrate the presence of an infected mosquito and thus preventive action can be taken before an epidemic occurs. A shift to this toolkit would be more efficient for a control programme than the current practice of house-to-house larval surveys.

Acknowledgements

We thank the staff of the Entomology Unit of the Selangor State Health Department for their help in setting and collecting traps. The project was funded by University of Malaya/Ministry of Higher Education. (UM/MOHE) High Impact Research Grant E000010-20001 and UM-MOHEUM.C/625/1/HIR/MOHE/H-20001-00-E000053.

References

S.-M. Lau et al.   Surveillance of adult Aedes mosquitoes


44. Bowman LR, Runge-Ranzinger S, McCall P. Assessing the relationship between vector indices and Dengue transmission:


### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Cases of dengue in seven blocks in Menterai Court from June to November 2013.

---

**Corresponding Author** Indra Vythilingam, Department of Parasitology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia. E-mail: indra.vythilingam@gmail.com