Ceylon Journal of Science 53 (3) 2024: 439-445

RESEARCH ARTICLE

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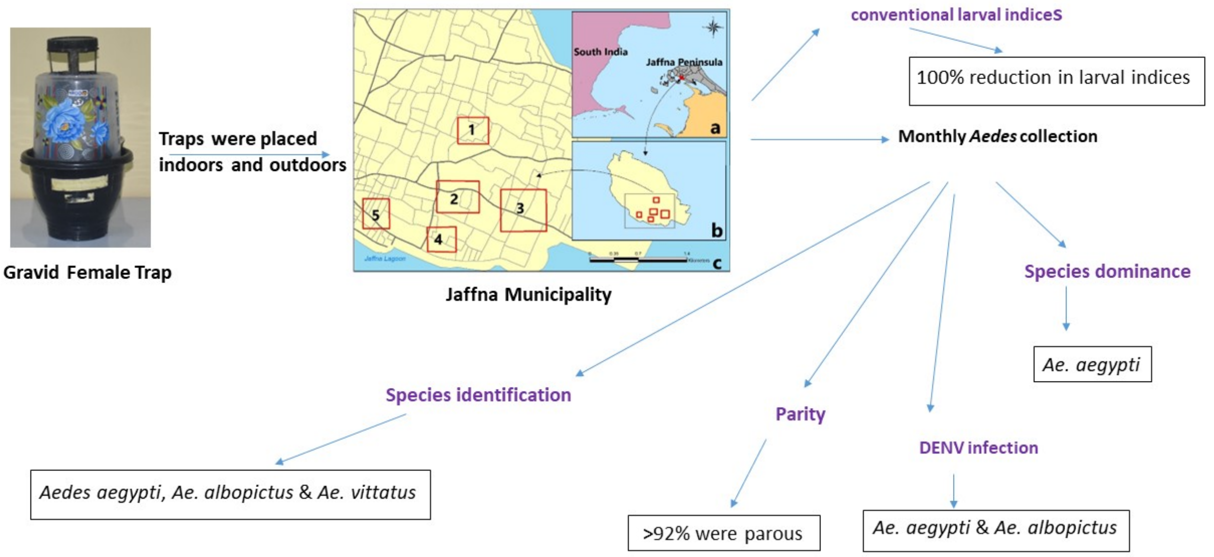
Graphical Abstract



Highlights

- A simple inexpensive gravid female traps were locally fabricated.
- The traps were used to survey dengue vectors in Jaffna municipality area.
- By the end of the survey a significant reduction was observed in conventional larval indices and adult indices.
- *Aedes aegypti* was the dominant species.
- *Aedes aegypti* and *Ae. albopictus* were found infected with DENV.
- The trap is useful to be used in routine vector survey/control purposes

Ceylon Journal of Science 53 (3) 2024: 439-445
DOI: https://doi.org/10.4038/cjs.v53i3.8435

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A Pilot Field Study on Dengue Vectors with Locally Manufactured Inexpensive Gravid Female Traps

Y.D. Wanasinghe[1], H. Ganesalingam[1], M.P. Bienvenu[1], T. Eswaramohan[1], T.T.P. Jayadas[2], G.N. Malavige[2], C. Jeewandara[2], R. Selvarajah[3], A. Thileepan[4], R. Ramasamy[1] and S. N. Surendran[1]*

[1]*Department of Zoology, University of Jaffna, Sri Lanka*
[2]*Department of Immunology and Molecular Medicine, University of Sri Jayawardasnepura, Sri Lanka*
[3]*Department of Geography, University of Jaffna, Sri Lanka*
[4]*Coordinator, Provincial Public Health, Northern Province, Jaffna, Sri Lanka*

Received: 10.06.2024; *Accepted: 05.08.2024*

Abstract: High prevalence of *Aedes aegypti* and *Ae. albopictus* vectors and dengue incidence as well as the presence of the four dengue virus serotypes underscore the need for effective and simple tools to monitor dengue vectors in Sri Lanka. Field studies were therefore undertaken with inexpensive gravid female trap (GFT) fabricated from local materials in Jaffna city in northern Sri Lanka. The study was initiated in April 2022, and GFT-based mosquito collection was conducted from June 2022 to April 2023. Five blocks of 30 to 40 houses, were selected in areas of high dengue prevalence in Jaffna city. Thirty houses were selected within each block and provided with five traps to be placed indoor and outdoor in each house. Conventional larval indices were determined at the beginning, middle and end of the study. Nine rounds of GFT collections were performed to identify and quantify trapped mosquitoes, their parity, and infection with virus serotypes. A total of 2177 and 3069 *Aedes* were collected respectively in indoor and outdoor GFTs, during nine rounds of collections and identified at the species level where possible. A total of 679 and 433 *Ae. aegypti* and *Ae. albopictus* were collected in indoor traps and 377 and 1288 in outdoor traps respectively. Adult and larval indices decreased respectively by 50% and 100% by the end of the study. Parous mosquitoes comprised 93% (of 120) and 94% (of 288) of the collected *Ae. aegypti* and *Ae. albopictus* respectively. In 110 pools of female *Ae. aegypti* and *Ae. albopictus* screened for virus serotype by PCR, five pools of *Ae. aegypti* and six pools of *Ae. albopictus* from both indoor and outdoor collections were positive for DENV-2. GFTs fabricated with locally available and inexpensive materials described here can be effective for monitoring the prevalence of *Aedes* vector species, parity and dengue virus infection status. The larval indices suggest that the use of GFTs can contribute to reducing dengue transmission.

***Keywords*:** *Aedes aegypti*; *Aedes albopictus*; Dengue control; Dengue surveillance; Dengue virus serotypes; Gravid female traps; Jaffna, Sri Lanka

INTRODUCTION

Dengue is prevalent in Sri Lanka with all four dengue virus serotypes (DENV1-4) in circulation (Jayadas et al., 2021). This is also the case in Jaffna city in the northern Jaffna peninsula (Figure 1) (Jayadas et al., 2021; Surendran et al., 2021). *Aedes aegypti* and *Ae. albopictus*, the primary and secondary vectors of dengue respectively, are prevalent in Jaffna city and throughout Sri Lanka (Jayadas et al., 2021; Surendran et al., 2021). Vector surveillance provides important information for the effective implementation of dengue vector control programs by public health authorities. A pertinent recent example from Sri Lanka was the relationship observed between people movement restrictions for controlling the COVID-19 pandemic, dengue incidence and collections of *Ae. aegypti* and *Ae. albopictus* larvae from ovitraps. The incidence of dengue fell by 89% in the Jaffna district compared to that predicted from pre-pandemic data, and this was accompanied by an 89% reduction in the collection of *Ae. aegypti* and *Ae. albopictus* larvae from ovitraps in the Jaffna city during the height of people movement restrictions from March 2020 to April 2021 in Sri Lanka (Surendran et al., 2022 a & b). The findings suggested that dengue control measures involving *Aedes* vector larval source reduction need to be more stringently applied than at present in public places where people congregate in Sri Lanka and other countries with similar dengue epidemiology (Surendran et al., 2022a; 2022b).

Ovitraps (Barrera et al., 2014; Zeichner & Perrich, 1999) and adult mosquito traps have been shown to be useful for dengue vector surveillance and dengue control (Barrera et al., 2014; Degner et al., 2014; Barrera et al., 2017). Trapping gravid females not only aids in surveillance but also reduces DENV transmission because some of the trapped females would have ingested blood containing DENV. Modified ovitraps composed of small black plastic water-filled containers that contain an insecticide impregnated strip acts as a lethal toxicant for adult female mosquitoes laying eggs and the resulting larvae (Zeichner et al., 1999; Eiras et al., 2014; Paz-Soldan et al., 2016).

As an alternative to insecticide impregnated strips, sticky cards are used to capture females entering the trap (Heringer et al., 2016; Lee et al., 2013) and are reported to be an effective and inexpensive method of capturing and monitoring *Aedes* vectors and estimating DENV

**Corresponding Author's Email: noble@univ.jfn.ac.lk*
https://orcid.org/0000-0002-7861-2037

transmission (Lau et al., 2017; Liew et al., 2021). We describe here the pilot field evaluation of a simple, locally fabricated and inexpensive (costing USD 1.5) gravid female trap (GFT) (Thiruchenthooran et al., 2019) as a dengue surveillance and intervention tool in Jaffna city.

MATERIALS AND METHODS

Study Site and Study Period

Jaffna city with an area of 20.2 km^2 is the most densely populated (~ 85,000 population) urban centre in the Jaffna peninsula (Jaffna District Secretariat, 2023). The dengue incidence in the city was 1002 and 1375 in 2022 and 2023 respectively (source: Office of the Provincial Public Health, Northern Province). This study was performed in the Jaffna city from April 2022 to April 2023. Five blocks of houses in areas of high dengue incidence were identified within Jaffna city limits for the study. Each block consisted of 30 - 40 houses with a minimum distance between the blocks of 300 m (Figure 1).

Figure 1: Jaffna city and peninsula in northern Sri Lanka in relation to India showing the five selected study sites; **a.** northern Jaffna peninsula in relation to South India, **b**. Jaffna city and **c**. study sites named as **1**: Bosco, **2**: Eachamoddai, **3**: Koyyathottam, **4**: Chundukkuli and **5**: Gurunagar

Ethics and Research Governance

Informed consent for larval surveys and adult collections using traps within private premises was obtained from heads of households. Ethical clearance was provided by the Animal Research Ethics Committee (AERC/2019/03) of the University of Jaffna. Written approval from the Commissioner of Jaffna Municipal Council was obtained to conduct this study.

Fabrication of Gravid Female Traps

Gravid Female Traps (GFTs) were fabricated using locally available plastic containers as shown in Figure 2. An individual trap (A) has 3 major components: (B) black plastic bucket base (bottom diameter 11 cm, top diameter 20.5 cm, height 14.5 cm); (C) translucent plastic chamber (bottom diameter 16.5 cm, top diameter 14.8 cm and height 19 cm) coated with Vaseline® (Blue Seal Petroleum Jelly) containing a deltamethrin strip [Hitline chalk containing 0.5% deltamethrin; Godrei Households Product Lanka (Pvt) Ltd.] pasted inside to trap and kill entering mosquitoes. A black nylon mesh (mesh size 1mm) (D) was placed between the bottom base (B) and the translucent middle part (C) to prevent gravid females laying eggs in the water infused with hay in the bottom base (B). A black plastic container (E) with a lid on top to prevent entry of rain water and other particulates such as leaves, and side openings (diameter 9 cm and height 11.3 cm) was attached to the middle compartment (C) to permit entry of mosquitoes throughout the day. The bottom base (B) contained 500 ml of tap water, 1.5 g sugar, 0.05 g baker's yeast powder and 0.5 g of dried-hay straw. Traps were re-coated with Vaseline® and the water infused with hay and yeast was replaced after each round of collection. The total material cost for fabricating a GFT was USD 1.5. The efficacy of the trap was determined, by counting the number of *Aedes* mosquitoes per month per trap, was previously evaluated against control traps with water in the base (similar to a conventional ovitrap) found to be significantly better ($p<0.05$) at trapping adult *Aedes* mosquitoes (Thiruchenthooran et al., 2019). The GFT trapped 14 *Aedes* mosquitoes per trap per month and the control trapped 2 *Aedes* mosquitoes per trap per month (Thiruchenthooran et al., 2019).

Procedure for Trapping Gravid Female Mosquitoes

Because user acceptance was essential for the effectiveness of this study, a community outreach program was undertaken from April 2022 to May 2022, with door-to-door visits to all households in the selected areas to introduce the traps and explain the strategy and likely reduction of vector populations by diverting gravid mosquitoes towards the GFTs. The GFTs were placed at 30 houses per block, and if a block had more than 30 houses, thirty houses were randomly selected. From each block, 2 indoor GFTs (mainly in living areas) and 3 outdoor GFTs (near shady areas close to gardens and water sources like wells and

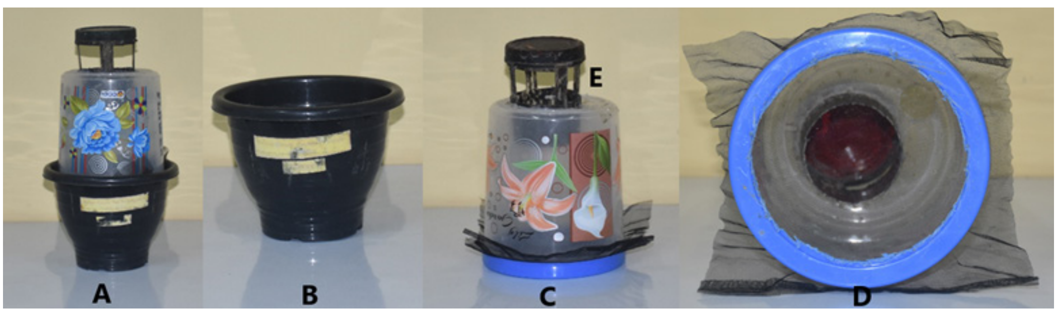

Figure 2: Components of a gravid female trap (GFT)

water storage tanks) were placed in each household. The traps were kept in the study blocks throughout the study period, and mosquito collection was carried out from June 2022 to April 2023. Although monthly collections were planned, due to logistical problems and limited human resources, only nine *Aedes* collections were completed during this period.

Aedes Larval Survey

In order to assess the role of GFTs in reducing *Aedes* population, before the initiation of the GFT study, pre-survey, mid-survey and post-survey larval sampling was conducted, in April, 2022, October, 2022 and April 2023 respectively. Larval survey was carried out as described previously (Surendran et al., 2019; 2021). Essentially preimaginal stages of *Aedes* were collected from all possible outdoor habitats of houses selected for GFT placement, using a standard procedure consisting of ten dips with 350-ml dippers for water containers with wide openings (e.g. for water storage tanks), pipetting with 5-ml plastic pipettes for smaller containers (e.g. plastic cups and paper cups) and ten dips with string-connected conical drop nets (diameter: 15 cm, depth: 10 cm) for wells. Collected preimaginal stages were brought to the laboratory where live larvae were maintained under contained insectary conditions. Adult mosquitoes emerging from collected larvae were identified to the species level with a standard key (Mahadevan & Cheong, 1974).

Aedes larval indices as outlined by the World Health Organization (2009) were measured; House (premise) Index (HI= Number of houses found positive for the presence of *Aedes* larvae/ Number of houses surveyed x 100), Container Index (CI= Number of containers found positive for *Aedes* larvae/Number of wet containers survey x 100) and Breteau Index (BI) =Number of containers found positive for *Aedes* larvae/Number of houses surveyed x 100). In addition to the larval indices, species dominance index (D) was determined during each larval sampling period using the following formula (May, 1975); D=Yn/Ym, where, Yn = the number of larvae of a particular *Aedes* species collected during the larval sampling period and Ym= total number of all *Aedes* larvae collected.

Entomological Data

The primary outcome determined was the numbers of *Ae. aegypti* and *Ae. albopictus* females captured in the GFTs. Their parity and dengue infection status were additionally determined. Other mosquito species, that were collected in the GFTs were not studied. Collected *Aedes* adults were identified at the species level where possible with a standard key (Mahadevan & Cheong, 1974). A proportion of mosquitoes collected in GFTs were decomposed, and could only be identified as belonging to the genus *Aedes* but not at the species level. Parity of *Ae. aegypti* and *Ae. albopictus* was determined by screening the ovaries of females under a stereomicroscope (Olympus, Japan). The presence of coiled tracheole skeins indicate a nulliparous mosquito and uncoiled skeins indicate a parous mosquito (Detinova, 1962). This method has been found to be accurate and relatively simple (Hugo et al., 2008). The remaining carcass of female *Ae. aegypti* and *Ae. albopictus* were stored at -20 ^{o}C to screen for the presence of DENV serotypes by RT-PCR. The adult index for *Ae. aegypti* and *Ae. albopictus* was calculated by dividing the total number of female adults identified for each species by the total number of intact functional GFTs (Liew et al., 2021; Ong, 2016). The *Aedes* index was determined using the total number of adult females identified as *Aedes*.

Detection of Dengue Serotypes

DENV serotype was detected separately in *Ae. aegypti* and *Ae. albopictus* collected from indoor and outdoor GFTs. Viral RNA from 5 to 15 mosquito pools representing each species was extracted with the QIAamp® Viral RNA Mini Kit (Qiagen, Hilden, Germany) and transcribed to cDNA using the high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. Assays were performed on Applied Biosystems—7500 Real Time PCR system as previously described (Jayadas et al., 2021, Surendran et al., 2021; Santiago et al., 2013).

RESULTS

Aedes Larval Indices

A total of twenty-five preimaginal development sites in outdoor premises were identified with *Aedes* larvae during the pre- and mid-larval surveys. As reported previously (Surendran et al., 2021), *Aedes* larvae were found in discarded plastic and metal containers, glass and ceramic containers, flower pots, paper cups, and cement water storage tanks. In general, during the larval survey carried out at three different stages namely pre-, mid-, and post-survey there has been a reduction in all larval indices such as HI, CI and BI. In particular, considering the most important larval index, CI, there was a 28% reduction from pre-survey to mid-survey and 100% from pre-survey to end of post-survey. All larval indices decreased during the survey period as shown in Table 1. The species dominance index (D) showed that *Ae. aegypti* is the dominant species among the three *Aedes* species collected during the larval sampling (Table 1).

Aedes Adult Indices and Parity

A total of 750 GFTs were placed in 150 houses (5 traps per house) comprising 300 indoor traps and 450 outdoor traps at inception of the study. This changed due to household preference and support as well as physical damage to the GFTs. At the conclusion of the study, 212 indoor and 297 outdoor traps were retained in 148 (out of 150) houses.

Adult entomological indices for *Aedes* (total number of mosquitoes identified as *Aedes*), *Ae. aegypti*, and *Ae. albopictus* were determined by considering the nine rounds of collections over eleven months, during the initial, mid, and final rounds of collections to arrive at a meaningful interpretation and the results are given in Table 2. There was a 50%, 67% and 50% reduction respectively in the total *Aedes*, *Ae. aegypti* and *Ae. albpictus* trapped in the GFTs from the initial to final survey (Table 2). During the study period, 15, 31 and 102 houses were positive for *Aedes* in indoor traps, outdoor traps and both indoor and outdoor traps respectively. Out of 750 traps 233, of which

Table 1: Larval indices and species dominance index for *Aedes* species calculated during the pre- mid and post-survey periods

Period	Survey stage	No. of houses inspected	No. of positive houses	No. of wet containers inspected	No. of positive wet containers	HI	CI	BI	% reduction in			D		
									HI	CI	BI	*AA*	*AL*	*AV*
April, 2022	Pre-survey	150	20	269	20	13.3	7.4	13.3				0.5	0.4	0.1
October, 2022	Mid-survey	150	3	56	3	2.0	5.3	2.0	84	28	84	0.6	0.4	0
April, 2023	Post-survey	150	0	3	0	0	0	0	100	100	100	0	0	0

HI (House Index): Percentage of houses with *Aedes* larvae; CI (Container Index): The % of water holding containers found with *Aedes* larvae; BI (Breteau Index); The number of *Aedes* positive containers per 150 houses; D: Species Dominance Index; AA: *Aedes aegypti*; AL: *Aedes albopictus*; AV: *Aedes vittatus*

23% only indoor, 33% only outdoor and 44% both indoor and outdoor, were positive for *Aedes* species. Other mosquito species including *Culex* sp., *Anopheles* sp. and *Mansonia* sp. were also trapped in a ratio of 4:1 (*Aedes* genus: other mosquito genus) but were not analyzed further.

A total of 5246 *Aedes* mosquitoes were collected from both indoor and outdoor GFTs representing the collection 2117 and 3069 individuals in indoor and outdoor traps respectively during the study period. Only 13 *Aedes* mosquitoes were collected alive from the traps. Both *Ae. aegypti* and *Ae. albopictus* were collected together in 15 indoor and 48 outdoor GFTs.

Of the total collection of *Aedes* mosquitoes from indoor traps there were 1005, 679 and 433 as unidentified *Aedes*, *Ae. aegypti* and *Ae. albopictus* respectively. Of the *Aedes* mosquitoes from outdoor traps there were 1404, 377 and 1288 as unidentified *Aedes*, *Ae. aegypti* and *Ae albopictus* respectively. A total of 1056 *Ae. aegypti* (679 from indoor traps and 377 from outdoor traps) and 1721 *Ae. albopictus* (433 from indoor traps and 1288 from outdoor traps) were identified. Chi-square analysis revealed significant (Chi-square = 417.57; $p<0.0001$) difference for *Ae. aegypti* and *Ae. albopictus* to be trapped in indoor and outdoor GFTs with more *Ae. albopictus* being trapped outdoors. A portion of the collected samples were screened for parity based on the physical state of the samples at collection. A total of 120 *Ae. aegypti* and 288 *Ae. albopictus* were dissected and of these 93 % and 94% were found to be parous respectively.

DENV Serotyping

A total of 1021 female *Aedes* comprising 45 pools of *Ae. aegypti* and 65 pools of *Ae. albopictus* were screened for DENV serotypes (Table 3). Mosquitoes collected from both indoor and outdoor traps were found with DENV serotype-2. This was also the first detection of DENV-infected *Ae. albopictus* in field samples from the Jaffna peninsula.

Dengue Incidence in the Study Sites

Throughout the study period, 11 dengue cases were recorded from the study blocks whereas 21 cases were recorded in the preceding year January – December, 2021 (Source: Nallur Medical Health Office). However, data on serotypes were not available to associate with the outcome based on screening *Aedes* mosquitoes.

DISCUSSION

The locally fabricated GFTs proved to be a simple and efficient tool to survey dengue vectors and possibly also directly reduce *Aedes* vector populations. The reduction of vector population can be inferred from the progressive decrease in larval indices during the period of the study. The larval survey revealed that *Aedes aegypti* was the dominant species detected followed by *Ae. albopictus* and *Ae. vittatus*. *Aedes vittatus*, a potential vector of dengue and a global invasive species (Mejfa-Jurado et al., 2024), was also previously detected in Jaffna city (Surendran et al., 2021). The progressive lowering of *Aedes* adult indices as during the course of the study shows diminishing adult densities, and this is compatible with the larval indices data and a reduction in dengue cases in the studied houses during the same period. Adult indices are a measure for vector control activities as pre-emptive vector control measures can be implemented when adult mosquito indices exceed a certain threshold (Azil et al., 2015).

The primary vector *Ae. aegypti* is highly anthropophilic (Scott and Morrison, 2010) and remains infected for life (Harrington et al., 2014) to cause dengue epidemics in highly populated areas (Harrington et al., 2014). The presence of both *Ae. aegypti* and *Ae. albopictus*, which is considered to be a more rural or peri-urban species, in highly populated areas of Jaffna city with the preponderance of *Ae. aegypti* indoors and *Ae. albopictus* outdoors is consistent with other findings from Jaffna and mainland Sri Lanka (Herath et al., 2024, Surendran et al., 2022b, Surendran et al., 2021). This differential behavioral pattern of both vector species and the role of *Ae. albopictus* in the transmission of DENV is important for vector surveillance and control of dengue transmission in Jaffna.

Only DENV-2 was detected in 11 pools comprising *Ae. aegypti* and *Ae. albopictus* that were collected from both indoor and outdoor GFTs. DENV-2 has been the predominant

Table 2. Adult indices determined during initial, mid and final rounds of mosquito collection and in the Gravid Female Traps (GFT)

Period	**Adult indices**					
	Aedes sp.	**% reduction**	***Ae. aegytpi***	**% reduction**	***Ae. albopictus***	**% reduction**
June, 2022	0.4		0.3		0.2	
November, 2022	0.3	25	0.2	33	0.2	0
April, 2023	0.2	50	0.1	67	0.1	50

Table 3: Outcome of DENV serotype screening of trapped *Aedes aegypti* and *Aedes albopictus*

Category	***Aedes aegypti***		***Aedes albopictus***		**Total**
	Indoor traps	**Outdoor traps**	**Indoor traps**	**Outdoor traps**	
Number of adults screened	209	114	186	512	1021
Number of pools tested	27	18	21	44	110
Number of DENV-2 positive pools	4	1	3	3	11

serotype circulating in Jaffna in recent years (Jayadas et al., 2021, Surendran et al., 2021). *Aedes albopictus* is well established to be a dengue vector (Janaki et al., 2022, World Health Organization, 2024, Centers for Disease Control and Prevention, 2024), but this is the first report of DENV in field collected *Ae. albopictus* in Jaffna. The trapping of DENV positive *Aedes* in both indoor and outdoor GFT traps confirm that infection can occur inside and outside houses.

The effectiveness of dengue vector control measures is dependent on community participation and support (Liew et al., 2021). Households that participated in this study were made aware of dengue control measures through the explanations provided by the investigators at the inception and then throughout the study. This could have led to the households taking measures to reduce larval development in the premises, which in turn might have reduced larval and adult indices during the course of the study. Our findings underscore the importance of programmes encouraging public involvement for more effective dengue control.

To improve the efficacy of the GFTs as a surveillance and intervention tool, trapped samples may need to be collected and analyzed more frequently than the long intervals used in this pilot study. This may permit more of the collected mosquitoes to be used for morphology-based identification and molecular screening. In order to detect DENV serotypes RT-qPCR was used which is highly sensitive but requires samples that retain integrity of viral RNA It is possible that the dengue nonstructural antigen 1 (NS1) rapid test can be a useful adjunct for detecting DENV in mosquitoes trapped in GFTs (Liew et al., 2021, Surendran et al, 2018; Selvarajoo et al., 2022).

The GFT described here with an estimated manufacturing cost of US$ 1.5, is likely to be a simple and effective tool for dengue vector surveillance. The traps are easy to deploy with community engagement in indoors and outdoors. The traps may have reduced human- vector contact and vector population by trapping gravid females. More than 90% of the trapped females, that we screened for parity, were parous –indicating that they were ready to lay eggs. The GFT kills gravid female *Aedes* mosquitoes before they have an opportunity to lay their eggs, thus lowering the biting rate and reducing fertility by preventing *Aedes* population expansion (Barrera et al., 2014).

The trapping and investigation of adult *Aedes* mosquitoes for DENV are essential to understand dengue transmission (Liew et al., 2021). Eliminating gravid females reduces DENV transmission as the gravid females might have had an infective previous blood meal. Apart from an effective surveillance tool to collect, identify and screen parous female for the presence of DENV, the GFTs may also be effective as potential intervention tools to reduce dengue transmission as suggested by the preliminary dengue incidence findings reported in this small pilot study. The limitation of the study was that it was conducted for one year with monthly collections. Larger and more extensive field studies by relevant public health authorities are needed to further evaluate the GFT described here in different countries with varying dengue transmission patterns to more completely assess their value for dengue surveillance and control. With these limitations, the GFT-based surveillance described in the study lie in its simplicity (cheap and reusable) and efficacy. The GFT can be considered as a pragmatic tool for dengue vector surveillance/control.

ACKNOWLEDGEMENT

Logistical assistance of the public health officers affiliated to the Medical Health Office at Nallur, Jaffna is gratefully acknowledged. The study received financial assistance from National Science Foundation of Sri Lanka (RG/2019/HS/02) and partial support from the NIH, USA (grant number: 5U01AI151788-02).

DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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