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ORIGINAL ARTICLE

Study on the Behavior of Dengue Viruses during Outbreaks with Reference to Entomological and Laboratory Surveillance in the Cuddalore, Nagapattinam, and Tirunelveli Districts of Tamil Nadu, India

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Abstract
Objectives: This study was carried out in order to understand the behavior of dengue viruses through the entomological and laboratory surveillance of outbreaks. The aim of the study was to provide additional research to support current knowledge of epidemiological, clinical, and laboratory diagnosis of dengue virus and ultimately to use this information to forecast dengue as well as to justify intervention measures.
Methods: Data on the presence of *Aedes* larvae in human dwellings during the entomological surveillance in Cuddalore, Nagapattinam, and Tirunelveli dengue outbreaks were taken to compute indices, namely the House Index (HI), Container index (CI), and the Breteau Index (BI). Standard procedures were followed for nonstructural Protein 1 (NS1) and immunoglobulin M enzyme linked immunosorbent assay for the confirmation of dengue. Serovar confirmation was made in the Kottayam field station of the Vector Control Research Center, Puducherry.
Results: Larval indices HI $<$ 2–3% and BI $<$ 20 contributed to halting the outbreak. Incubation of the dengue viruses in humans was detected at 15 days, NS1 was identified as a tool for the early diagnosis of dengue cases and its presence indicated the need to implement all available interventions. It was also discovered that it is helpful to search for hidden habitats of *Aedes* when dengue cases have not been reduced even after the sustainable management of the larval indices, HI $<$ 5% and BI $<$ 20. Based on the observed incidences of stopping dengue outbreaks, it was learnt that neighborhood areas of the outbreak villages, around 400 m, should have permissible larval indices $<$ 5% HI and BI $<$ 20. Heterogeneous serovars that led to dengue hemorrhagic fever and Dengue Shock

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Syndrome (DSS) were identified using reverse transcription polymerase chain reaction and reconfirmed in the field as DEN-1 and DEN-3 viruses and were circulating in Tirunelveli during the outbreak.
Conclusion: The behaviors of dengue viruses experienced in experimental, clinical, epidemiological, entomological, and laboratory surveillance did not deviate from observations in the field during dengue outbreaks in the Cuddalore, Nagapattinam, and Tirunelveli districts of Tamil Nadu, India.

1. Introduction

Since dengue is not currently a vaccine preventable communicable disease, vector control remains the only way to prevent dengue transmission [1−3].Vector control programs are essentially based on source reduction, eliminating *Aedes aegypti* larval habitats from the domestic environment with increasing community involvement and inter sectoral action in recent decades [4,5]. Various efforts have been made to detect this disease early to determine transmission risk, define thresholds for dengue epidemic alerts, or set targets for vector control programs [6−8]. Among larval indices, the House Index (HI, percentage of houses positive with *Aedes* larvae) and the Breteau Index (BI, number of positive containers per hundred houses) have been the most widely used indices [9,10]. Since HI $<$ 1% or BI $<$ 5 were proposed to prevent yellow fever transmission, these values have also been applied to dengue transmission but without much evidence whereas the Pan American Health Organization described three levels of risk for dengue transmission: low (HI $\leq$ 1%), medium (HI = 0.1−5%), and high (HI $>$ 5%) [11,12]. Pertaining to the determination of the critical threshold levels of HI and BI, it is known that these values were obtained in a specific location and are not applicable to all locations, hence these values need to be verified [13]. The vector density, below which dengue transmission does not occur, continues to be a topic of much debate and conflicting empirical evidence. For example, dengue outbreaks occurred in Singapore when the national overall HI was $<$ 1% [14]. By contrast, researchers from Fortaleza, Brazil found that dengue outbreaks never occurred, when HI was $<$ 1% [15]. However, different geographic levels are used to calculate the indices in various studies and the appropriated level for entomological indices is in itself an issue of debate [16]. Lizet Sanchez et al [17] have attempted to incorporate larval indices for identifying high risk areas for dengue virus transmission. From their study, it was found that the influence of measurements at different geographical levels can establish a threshold for epidemic outbreaks and provide a discussion point for determining their utility for community based *Aedes* control programs.

Since no protective vaccine or specific treatments are available for dengue fever, accurate diagnosis is critical for the early initiation of specific preventative health measures to curtail epidemic spread and reduce economic losses as well as the timely monitoring of patients to prevent fatalities. Commonly used diagnostic methods for confirming dengue infection involve virus isolation, detection of virus antigens or RNA, and the presence of dengue virus-specific antibodies [18]. Among diagnostic tools available for dengue diagnosis are nonstructural Protein 1 (NS1) and immunoglobulin (Ig)M and IgG enzyme-linked immunosorbent assay (ELISA). NS1 uses the nonstructural protein of the virus and dengue virus specific antibodies respectively. When these tools undertake the detection of dengue infection, it is known that the IgM antibody can be detected by Day 5 of illness, and 93−99% of cases have detectable IgM by Days 6−10 of illness, which may then remain detectable for $>$ 90 days [19]. The NS1 based assays provide a simplified method of diagnosis during the acute stage of dengue infection compared to viral isolation or nucleic acid detection (the detection of viral antigens in the blood stream) [18], its precision in diagnosis of dengue has been enhanced and it may be used for detection in patients in the febrile phase. Other than NS1, acute infection with dengue virus can be confirmed when the virus is isolated from serum or the specific dengue virus genome is identified by reverse transcription polymerase reaction (RT-PCR) from serum or plasma. This diagnostic tool has been widely used to identify more than one serovar of dengue virus circulating in the community and it has been shown to directly elucidate the degree of the transmission of dengue hemorrhagic fever (DHF) from dengue fever (DF) [20,21].

Even though efforts have been made to stop dengue outbreaks in different parts of the world and particularly in tropical countries such as India and South East Asian countries, with reference to the significance of entomological surveillance, current literature study findings agreeing on the behavior of dengue viruses during outbreak situations is scarce. Hence, the present study was undertaken to reaffirm the facts derived from existing experimental and entomological laboratory surveillances. The current research on the control and prevention of dengue during outbreaks in the field, with reference to entomological and laboratory surveillance alone is unique to this study.

2. Materials and methods

Data on the presence of *Aedes* larvae in human dwellings in villages and urban areas of, Tirunelveli (Latitude 8°42′ N; Longitude 77°42′ E), Nagapattinam (Latitude 10°46′ N; Longitude 79°50′ E), and Cuddalore (Latitude 11°75′ N; Longitude 79°75′ E) were collected when dengue outbreaks occurred in May 2012, September 2012, and August 2012 to January 2013, in these respective districts. These data were used to compute various indices, namely: the HI, Container index (CI, percentage of wet containers positive for larvae), and the BI. To obtain these data, active searches for *Aedes* larvae were made by the domestic breeding checkers (DBCs) temporarily placed by the government of Tamil Nadu with political commitment, by going house to house. There were 10 DBCs in each district block and thus, in the current study, 120 DBCs, 130 DBCs, and 110 DBCs were employed in the above districts respectively. Prior to this assignment, full hands on training was given to all DBCs so as to be able to recognize *Aedes* larvae. Training included identification of wriggling movements in stored water, which is a bottom dwelling property of these larvae and could be seen only with a torch light. A dosage of 2 mL of larvicide temephos, an organophosphorus compound at a 50% emulsifier concentration in 1 L of water was used in the field as 1 mL per 1 L of stored water in wet containers (containers that were not able to drain in a dwelling place etc.). The same intervention was undertaken in neighboring areas of the outbreak villages and in parallel municipal areas to avoid epidemic spread from the affected places.

Standard procedures were followed for NS1 and IgM ELISA for the confirmation of dengue. NS1 kits supplied by Pan Bio (Inverness Medical Innovations, Australia, Pty Ltd., Brisbane, Queensland, Australia) and IgM ELISA kits supplied by the National Institute of Virology, Government of India, were used in this study at the sentinel hospitals with laboratory facilities in Tirunelveli, Nagapattinam, the laboratory of the Zonal Entomological Team (ZET), Cuddalore, and in the Public Health Laboratory, Cuddalore.

Serovars of dengue: DEN-1, DEN-2, DEN-3, and DEN-4, confirmations were made with RT-PCR following the standard procedure of the Vector Control Research Center field station, Kottayam, India, and the premier institute of the Indian Council of Medical Research in New Delhi, India. Statistical analysis for this study was carried out using SPSS version 20 (SPSS Inc., Chicago, IL, USA). A p value < 0.05 was taken to be significant.

3. Results

In 2012 there were 1500 dengue cases reported in the Cuddalore district. Among them, 1179 dengue cases were confirmed by NS1 ELISA; 211 cases were confirmed by IgM ELISA; 100 cases were confirmed by the NS1 rapid diagnostic test (RDT); and 10 dengue cases were confirmed by IgM RDT (Tables 1 and 2; Figures 1 and 2). These dengue cases were distributed amongst 13 blocks and five areas in urban municipalities. In 2013 the number of cases of dengue was considerably reduced to 827 cases which were confirmed from all these diagnostic tools (Figure 1). To reduce cases in all affected villages and to halt some outbreaks in the district, various interventions had been used with the help of entomological and laboratory surveillance. In order to implement interventions early in a village, fever information had been accumulated every day from different resources, namely the passive surveillance (institutional surveillance) in primary health centers (PHCs), sentinel surveillance hospitals, government hospitals, and medical college hospitals, information from weekly morbidity reports of the Integrated Disease Surveillance Project, malaria fortnightly report (MF9) in PHCs, rumors and news flashes in the media (television, newspapers etc.). Based on this information interventions were implemented as a priority when even one fever case was confirmed as dengue in a village. Simultaneously, any villages that had reported cases in previous years and villages neighboring those experiencing fever outbreaks were also included in the intervention.

For the present study, selective villages and urban areas of the Cuddalore district had been taken to analyze the above strategy and data collected during the course of outbreaks were depicted in Table 3. Scrutinizing the HI before and after its course from outbreak to the halting of the disease, it was observed through entomological surveillance that the mean HI from 30 outbreaks was 15.3% before the outbreak whereas it was reduced to 2.2% once the intervention was implemented. The associative CI was reduced from 2.5% to 1.7% and BI was reduced from 16.4 to 2.4 (Figure 3).

On examination of the time required to halt the disease from the day of intervention, it was found to be 13−15 days. In some places this time extended more than 15 days due to hidden habitats of *Aedes*. In urban Cuddalore in one area, Devanampattinam, dengue was not stopped even after HI was reduced to $< 5\%$ for more than a month. This provoked further searches for *Aedes* and it was found that refrigerators were the source of breeding, thus a justifiable reason for extending more than one incubation period (4−14 days) was found (Table 4 and Figure 4). Since most outbreaks had ended within 15 days, it was ascertained that the incubation of dengue virus is 4−14 days. This study found that extension over 15 days was due to an insufficiently strong intervention and demonstrates the need for further strengthening of the intervention in these cases.

In the Nagapattinam district, dengue outbreaks occurred during the months of August to October 2012.

Table 1. The number of dengue cases reported in the Cuddalore district by month in 2012 and 2013.

	Month	2012	2013
1	Jan	3	273
2	Feb	3	100
3	Mar	6	44
4	Apr	1	32
5	May	0	19
6	Jun	4	7
7	Jul	8	19
8	Aug	17	14
9	Sep	19	26
10	Oct	142	117
11	Nov	563	99
12	Dec	734	77
	Total	**1500**	**827**

Table 2. The number of positive cases and the various diagnostic tools used to predict them.

Diagnostic tools	No. of cases
NS1 ELISA	1179
IgM ELISA	211
RDT NS1	100
RDT NS1	10
Total	1500

ELISA = enzyme linked immunosorbent assay; Ig = immunoglobulin; NS1 = nonstructural Protein 1; RDT = rapid diagnostic test.

There were 446 dengue cases reported in seven villages (Vadugacherry, Sirkali, Keelaiyur, Vadavoor, Keechankuppam, Akkaraipettai, Thideerkuppam, and Kallar) and in eight areas of Nagapattinam urban (8, 11, 12, 13, 14, 21, 35, and 36). This was a total of 298 and 258 dengue positives from the rural and urban areas respectively (Table 5). The various diagnostic tools used to predict dengue cases in the Nagapattinam district are listed in Table 6.

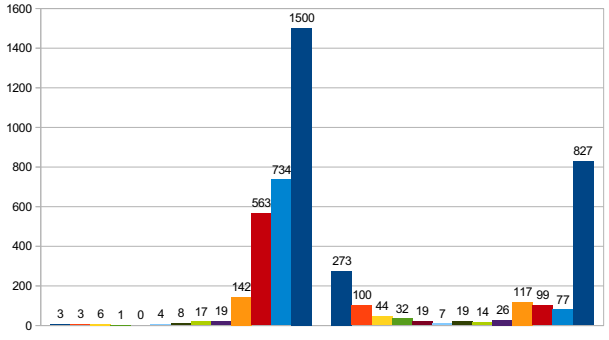

Figure 1. Dengue cases reported in the Cuddalore district by month for the years 2012 and 2013.

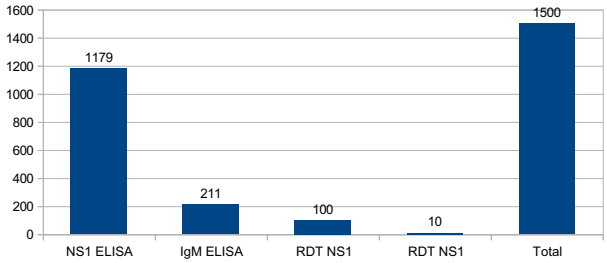

Figure 2. Diagnostic tools used to confirm dengue cases in the Cuddalore district in 2013. ELISA = enzyme linked immunosorbent assay; Ig = immunoglobulin; NS1 = nonstructural Protein 1; RDT = rapid diagnostic test.

On examination of the data from indices obtained in urban Nagapattinam from the beginning of the outbreak to the end, it was observed that the HI in Sevabharathi was 24% on the 1st day of intervention and it had declined to 1.7% when the outbreak was halted. In Old Nambiyar Nagar the HI was 5.7% initially and declined to 2.6% at the halting of the outbreak. In New Nambiyar Nagar the HI declined to 2.2% from 16.2%, likewise, in Tatanagar, the HI was 4.0% initially and reduced to 2.1% when dengue cases had been stopped (Table 7 and Figures 5–8). Since all these declining trends of HI occurred on 15th day post intervention, it has been ascertained that the maximum incubation of dengue is 15 days as previously reported by Gubler [22]. The outbreak that began in Tatanagar with a HI of 4.0%, which was less than the permissible level advised by the World Health Organization (i.e., < 5%), was explained by the identification of refrigerators that acted as hidden habitats and provided breeding grounds for *Aedes* and meant that the HI value determined at the beginning was inaccurate as it was based on observations made in wet containers in and around human dwellings alone.

Prediction of dengue positivity in both the rural and urban areas of the Nagapattinam district with the diagnostic tools NS1 and IgM ELISA were related to the presence of *Aedes* larvae. Furthermore, it is known that the NS1 diagnostic tool has been proven to detect the presence of dengue in the community as early as the 2nd day after the onset of fever. The early confirmation of dengue leads to early investigation into the location of *Aedes* habitats in the affected areas. The testing for dengue by IgM ELISA is limited as an affected individual can only be tested after 7 days from the onset of fever; consequently the implementation of an intervention is delayed. Thus the NS1 tool has received attention from public health program managers as it has been shown to be useful for the initiation of early interventions both in entomological and case management dengue control activities (Figure 9).

The degree of involvement amongst DBCs was assessed in a place where the *Aedes* survey was carried

Table 3. *Aedes* larval density monitoring in primary health centers (PHCs) and the Cuddalore Municipality during the dengue outbreak in the Cuddalore district in 2013.

	Name of the block	Name of the PHC	Name of the village	Period of the fever outbreak	No. of dengue positives	Initial House Index (HI)	Container Index (CI)	Breteau Index (BI) if applicable as 100 houses searched	HI	CI	BI	Time taken to halt the outbreak in d
1	Annagiramam	Melpattampakkam	Keezharungunam	01 Aug 2013 to 31 Dec 2013	*5*	*8*	1.6	*10*	*2.1*	*0.49*	*2.82*	*13*
2	Cuddalore	Naduveerapattu	Nariankuppam	01 Aug 2013 to 31 Dec 2013	*1*	*6*	1.2	*8*	*1.8*	*0.5*	*1.8*	*12*
3	Cuddalore	Karaikadu	Manakuppam	01 Aug 2013 to 31 Dec 2013	*1*	*15.6*	3.12	*17.6*	*1.6*	*0.7*	*1.6*	*11*
4	Cuddalore	Karaikadu	Sangolikuppam	01 Aug 2013 to 31 Dec 2013	*2*	*8.2*	1.64	*10.2*	*1.7*	*0.7*	*1.7*	*14*
5	Cuddalore	Madalapattu	Periyakattupalayam	01 Aug 2013 to 31 Dec 2013	3	20	4	22	2.4	1.2	2.4	15
6	Cuddalore	Madalapattu	Tazhanguda	01 Aug 2013 to 31 Dec 2013	2	13.2	2.64	15.2	1.9	0.8	1.9	14
7	Cuddalore	Vellakarai	Vellakarai	01 Aug 2013 to 31 Dec 2013	8	14.8	2.8	16.8	2.9	1.3	3.9	10
8	Panruti	Kadampuliyur	Malingampattu	01 Aug 2013 to 31 Dec 2013	5	40	5.88	42	1.8	0.4	1.8	15
9	Panruti	Kadampuliyur	South Melmampattu	01 Aug 2013 to 31 Dec 2013	4	28.2	3.81	30.2	3.2	1.1	3.2	13
10	Vadalur	Venpettai	Vadakutthu	01 Aug 2013 to 31 Dec 2013	2	16	3.03	18	3.3	1.5	3.3	16
11	Parangipettai	Gavarapattu	Kovilampoondi	01 Aug 2013 to 31 Dec 2013	5	8.8	1.62	10.8	2.5	1.5	2.5	12
12	Parangipettai	Killai	Ponnanthittu	01 Aug 2013 to 31 Dec 2013	4	6.6	1.25	8.6	2.3	0.7	2.3	14
13	Parangipettai	Gavarapattu	Gavarapattu	01 Aug 2013 to 31 Dec 2013	3	10	1.8	12	3.2	1.13	3.2	13
14	Parangipettai	Ayyepuram	Ayyepuram	01 Aug 2013 to 31 Dec 2013	2	7.2	1.4	9.2	2.7	0.8	2.7	11
15	Kammapuram	Arasakuzhi	Arasakuzhi	01 Aug 2013 to 31 Dec 2013	3	22	3.7	24	3.8	1.1	6.4	14
16	Kammapuram	Kammapuram	Kammapuram	01 Aug 2013 to 31 Dec 2013	1	22	0.44	4.2	2	0.19	2	13
17	Kammapuram	Arasakuzhi	Gangaikondan	01 Aug 2013 to 31 Dec 2013	2	13.2	0.54	15.2	1.5	0.28	1.5	12
18	Kammapuram	Palakollai	Palakollai	01 Aug 2013 to 31 Dec 2013	3	40.1	7.7	42.1	1.2	0.8	2.3	14
19	Mangalampettai	Mangalampettai	Mangalampettai	01 Aug 2013 to 31 Dec 2013	1	21	0.68	4.1	1.7	0.32	1.7	12
20	Nallur	Veppur	Veppur	01 Aug 2013 to 31 Dec 2013	2	13.7	3.03	15.7	1.5	0.8	1.5	11
21	Nallur	Nallur	Nallur	01 Aug 2013 to 31 Dec 2013	2	15	3.31	17	1.7	0.8	1.7	13
22	Nallur	Sirumangalam	Elangaiyanur	01 Aug 2013 to 31 Dec 2013	2	8	1.94	10	1.8	0.8	1.8	11
23	Mangalur	Thozhudur	Thozhudur	01 Aug 2013 to 31 Dec 2013	8	14.4	2.45	16.4	4.2	0.8	4.2	14
24	Mangalur	Sirupakkam	Sirupakkam	01 Aug 2013 to 31 Dec 2013	1	7.2	1.51	9.2	1	0.5	1	12
25	Mangalur	E.keeranur	Vasistapuram	01 Aug 2013 to 31 Dec 2013	5	12.6	2.99	14.6	1	0.4	1	10
26	Cuddalore mty	Devanampattinam	Devanampattinam	01 Aug 2013 to 31 Dec 2013	6	13.9	2.56	15.9	2.6	0.7	2.6	18
27	Cuddalore mty	Pudupalayam	Pudupalayam	01 Aug 2013 to 31 Dec 2013	2	7.6	1.4	9.6	1.4	0.7	1.4	12
28	Cuddalore mty	Tsunami nagar	Tsunami nagar	01 Aug 2013 to 31 Dec 2013	3	22	1.9	24	2.6	0.7	2.6	15
29	Cuddalore mty	Annanagar	Annanagar	01 Aug 2013 to 31 Dec 2013	2	8.5	2.1	10.5	2.1	0.9	1.7	14
30	Nellikuppam	Vanpakkam	Vanpakkam	01 Aug 2013 to 31 Dec 2013	3	8.5	1.63	10.5	2.2	1.2	2.2	16
			Total			**15.27**	**2.45**	**16.37**	**2.2**	**1.77**	**2.4**	**13.13**

Italics denoted the results of intervention as indices reached to its own threshold level to stop dengue.

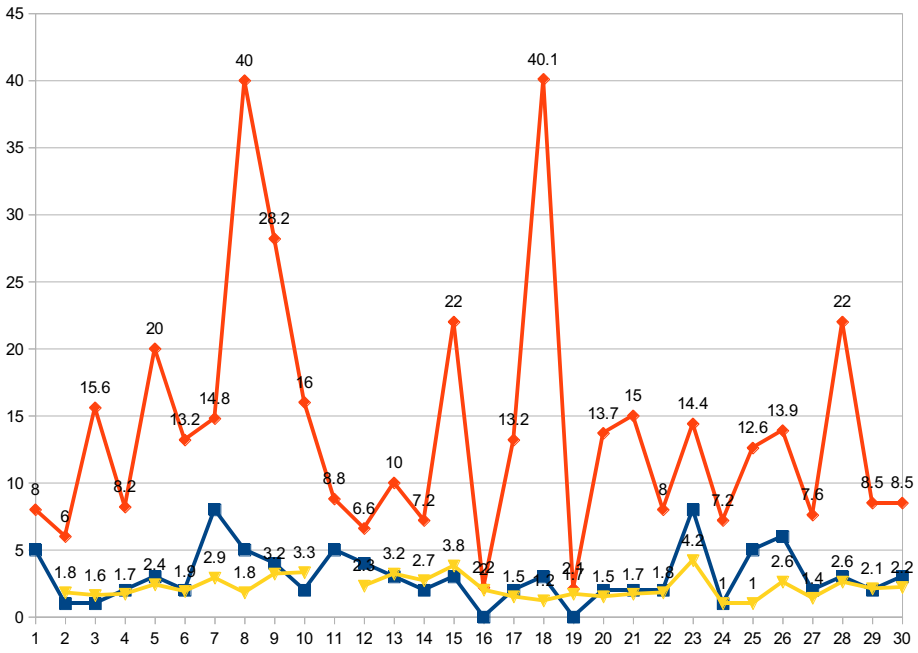

Figure 3. The House Index status in some outbreak villages in the Cuddalore district before and after an outbreak.

out under different categories of supervision (Health Inspectors, Block level supervisors and district level supervisors) Senior Entomologist based on reports of indices HI, CI and BI arrived. Where it is not significant (<0.1), efforts were made to strengthen to bring down permissible level of indices HI < 1% and BI < 20 (Tables 8 and 9).

There was a dengue outbreak in the Tirunelveli district in May 2012, data from the PHC in Mukkudal was taken for the present study. In the 1st week of May 2012, there were six IgM ELISA confirmed dengue cases reported from the villages of Mukkudal, Vaddakku Ariyanayagipuram, Kumarasamy Puram, and Arikesavanallur and four deaths were reported in the Maruthamputhur PHC area. All these villages were under entomological surveillance from the day of intervention to 30 days after. Study findings in the form of HI, CI, and BI are reported in Tables 10 and 11 and Figure 10.

On examination of the daily indices of *Aedes,* it was shown that the HI was 13.2%, CI 11.2%, and BI 13.2 on the day of intervention, subsequently these indices decreased day by day until the 15th day of intervention when these indices were 3%, 0.9%, and 3.8, respectively. Some cases among the six cases identified in the Mukkudal area were reported after the day of intervention. It has been shown that individuals that were infected before the intervention was implemented expressed symptoms within the incubation period of 4−14 days on different dates; hence the impact of the intervention on the dengue outbreak could best be measured a minimum of 15 days from the day of intervention implementation.

The current intervention was extended beyond the first incubation period in these villages, and findings are reported in Table 11. On the 16th day, the HI was 2.8%, CI was 0.9%, and BI was 4.4, these values decreased to 0.7%, 0.2%, and 0.7 respectively on the 30th day and no new positive cases were reported in these villages. From these observations, it has been concluded that the maximum human incubation period of dengue is 15 days. It was also shown that cases confirmed by the IgM ELISA and NS1 tools are related to the presence of the *Aedes* larvae. During the dengue outbreaks, cases were reported in most of the blocks in the district, some adjacent blocks such as Nanguneri which did not report any positive cases were analyzed in parallel with reference to the *Aedes* indices: HI, CI, and BI (Table 12). From this observation, it was shown that all these indices were within permissible levels (HI 1.63%, CI 1.06%, and BI 2.2). These values demonstrate the importance of entomological surveillance and its value in the sustainable management of maintaining a dengue free area. Thus the availability of HI, CI, and BI values play an important role in dengue prevention and control.

Similar observations were made in the Cheranmahadevi block where the HI was 1.6%; CI 0.5%, and BI 1.6 on the day of intervention. These indices decreased day by day, the values after 23 days were HI 0.5%; CI 0.2%, and BI 0.4. No positive cases of dengue were reported in Cheranmahadevi (Table 13).

Apart from these findings, it has also been established that the co-existence of DEN-1 and DEN-3 viruses circulating in the community led to positivity among susceptible groups of all ages, fatalities in both male and female infants of up to 4 years were reported (Table 14).

Table 4. The habitats of *Aedes species* in some rural and urban areas of the Cuddalore district that exhibited dengue positives and permissible *Aedes* indices.

	Name of the block	Name of the villages that took > 15 d to stop dengue	No. of cases reported from NS1 tool	Occult habitats of dengue & its index	NS1	IgM	Total
1	Annagiramam				25	8	33
2	Cuddalore	Kondur	10	Refrigerator	180	19	199
		Koothapakkam	7	Refrigerator			
		Pathirikuppam	8	Refrigerator			
		Thiruvandipuram	7	Refrigerator			
		Knpettai	9	Refrigerator			
		Tazhanguda	9	Refrigerator			
		Ramapuram	12	Refrigerator			
3	Panruti	Silambinathanpettai	23	Refrigerator	103	15	118
				Refrigerator			
4	Vadalur	Kurinjipadi	14	Refrigerator	112	23	135
5	Parangipettai				38	6	44
		Killai	4	Refrigerator			
		Parangipettai	6	Refrigerator			
6	Kammapuram	Seplanatham	6	Refrigerator	35	9	44
		Nadiyapattu	5	Poclain tyres			
7	Kumaratchi	Annamalainagar	5	Refrigerator	17	9	23
8	Nallur	Veppur	5	Refrigerator			
		Pennadam	4	Refrigerator			
9	Virudhachalam				28	7	35
		Thottikuppam	3	Refrigerator			
10	Mangalur				15	4	19
		Alathur	6	Refrigerator			
11	Cuddalore urban				209	31	
		Devanampattinam	27	Refrigerator			
		Yandipalayam	16	Refrigerator			
		Semmandalam	10	Refrigerator			
		Manjakuppam	19	Refrigerator			
		Kammiyampettai	9	Refrigerator			
		Thirupathiripuliyur	17	Refrigerator			
		Pudupalayam	10	Refrigerator			
12	Nellikuppam urban				54	19	73
		Nellikuppam	7	Refrigerator			
		Thirukandeeswaram	13	Refrigerator			
		Vazhapattu	7	Refrigerator			
		Jeevanagar	5	Refrigerator			
13	Chidambaram urban	Chidambaram	26	Refrigerator	26	6	32
14	Panruti urban	Panruti	19	Refrigerator	26	0	26
		Thiruvadhigai	4	Refrigerator			
		Andipalayam	3	Refrigerator			
15	Virudhachalam urban	Virudhachalam	33	Refrigerator	33	2	35
16	Neyveli township	Neyveli	8	Tree holes	8	0	8

Ig = immunoglobulin; NS1 = nonstructural Protein 1.

From this observation, a case fatality rate of 10% was determined during the dengue outbreak in the Mukkudal block.

4. Discussion

The various findings determined in previous studies on the behavior and association of dengue viruses, *Aedes aegypti* and *Aedes albopictus,* both in man and vectors, have been reconfirmed by the current study observations during the course of the dengue epidemic in several parts of three districts, namely Cuddalore, Nagapattinam, and Tirunelveli of Tamil Nadu, India. As these observations were made to forecast the disease as well as to halt the outbreak, the present study has received much attention.

The incubation period of the dengue virus in humans was determined to be a maximum of 15 days and a minimum of 4 days in susceptible individuals among all

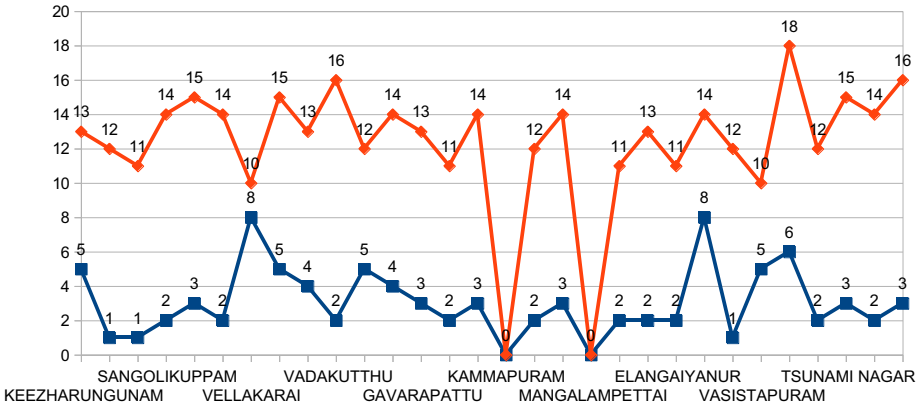

Figure 4. The time taken in days to halt the dengue outbreaks in the Cuddalore district.

Table 5. Basic data describing the dengue situation in the Nagapattinam district in 2013.

	Month	No. of dengue cases in urban areas	No. of dengue cases in Keechankuppam	Other villages	Total
1	August	10	0	0	10
2	September	190	41	81	312
3	October	98	26	110	234
	Total	298	67	191	556

age groups of both sexes. A similar observation was made by Mungrue [18] who noted that following the bite of an infected mosquito there is an incubation period of 3–14 days but it is usually 5–7 days when symptoms begin to occur.

Of the heterogeneous serovars of dengue in circulation in the community, it was known that 47 patients who travelled to India and Singapore in August 2004 displayed a dual coinfection with two serotypes (Type 1 and 2) of dengue virus (DENV). The first documented case of concurrent infections with more than one serotype of DENV was reported in Puerto Rico in 1982 [23]. Since then, several cases have been reported in New Caledonia, Thailand, Somalia, Mexico, and China [24–27]. The reason for dual infections is explained by the unique feeding behavior of *Aedes aegypti* mosquitoes. As the female mosquito often feeds on several individuals during a single gonotrophic cycle, there is a chance that it will be dually infected and then in turn transmit multiple viruses to a single individual [27,28]. In the present study, similar observations were made when deaths occurred among children (from 4 months to 4 years) due to DEN 1 and DEN 3 which were identified during the outbreak in the Tirunelveli district.

Furthermore it was evident that when Cassens [29] described the epidemiological concepts with their components as the agents of disease, host factors, and environmental factors, the host factors in which (1) young children are more likely to have subclinical infection, (2) adults are immune to certain diseases because of prior exposure, and (3) children and adults are exposed to different agents of disease, were emphasized. These observations are relevant to the current study as it has been shown that children were more susceptible and fatalities occurred in the heterogeneous strains during the course of the dengue outbreak.

Table 6. The number of dengue cases reported in rural and urban areas of the Nagapattinam district and the various diagnostic tools used to confirm them.

	Diagnostic tools						
Location	NS1 RDT	NS1 ELISA	IgM RDT	IgM ELISA	IgG RDT	IgG ELISA	Total
Rural	235	6	9	1	8	0	258
Urban	262	5	27	1	3	0	298
	Total						556

ELISA = enzyme linked immunosorbent assay; Ig = immunoglobulin; NS1 = nonstructural Protein 1; RDT = rapid diagnostic test.

Table 7. The declining trends of the House Index (HI) and Container Index (CI) from the intervention days to the halt of the dengue outbreaks in the urban areas of Sevabharathi, Old and New Nambiyar Nagar, and Tatanagar of the Nagapattinam district of Tamil Nadu, India.

Sevabharathi			Old Nambiyar Nagar		New Nambiyar Nagar		Tatanagar	
Time from the intervention (d)	HI	CI	HI	CI	HI	CI	HI	CI
1	24	3.8	5.7	0.9	16.1	4.9	4	1
2	10.2	1.6	4.4	0.9	19.4	2.2	10	2.7
3	14.1	3.5	13.5	3.1	27.3	3.8	8.8	1.1
4	14.6	2.4	2.1	0.4	19.2	2	5.5	2.4
5	13.3	2.2	4.1	1.3	22.2	3.5	4	1
6	16	4.3	4.2	0.9	4.1	1.3	5.2	2.8
7	14.8	1.5	17.2	2.7	4.2	0.9	5.9	2.6
8	15.4	3.7	20.9	2.7	17.2	2.7	4.8	2.4
9	38.5	5.1	5.4	1.1	20.9	2.7	4.1	2
10	9.5	2.1	5.9	1.6	5.4	1.1	3.8	1.6
11	12.5	2	2.1	0.4	4.8	1.6	3.4	1.4
12	7.4	1.3	1.2	0.6	4.2	1.6	4.1	1
13	3.7	1.2	4.3	0.9	3.9	1	3.2	0.9
14	2.9	1.3	3	0.7	2.9	2	2.7	1
15	1.7	0.8	2.6	0.6	2.2	1.6	2.1	0.9
Average	**13.2**	**2.5**	**6.4**	**1.3**	**11.6**	**2.2**	**4.8**	**1.7**

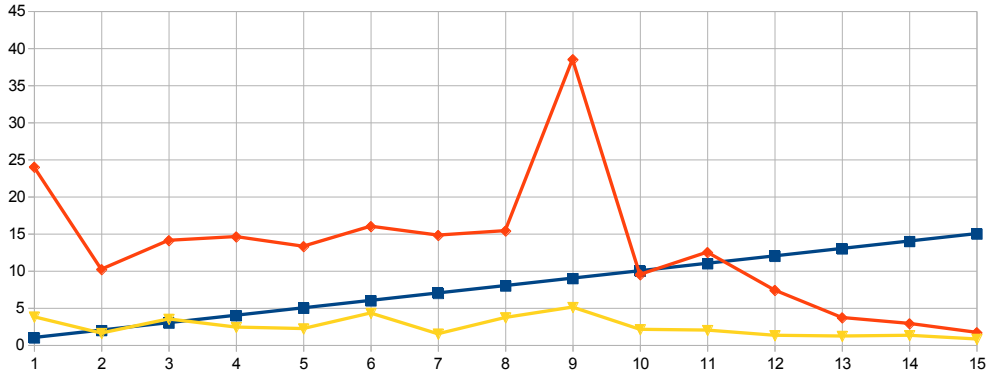

Figure 5. The declining trend of larval indices from the day of intervention to the halting of the dengue outbreak in Sevabharathi of the Nagapattinam district.

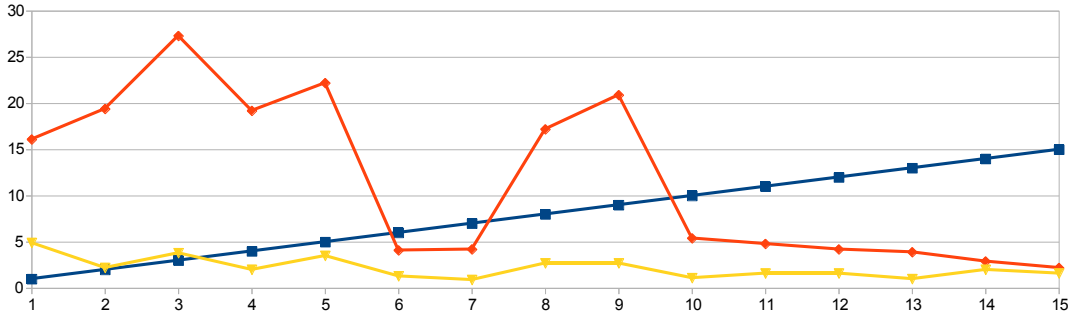

Figure 6. The declining trend of larval indices from the day of intervention to the halting of the dengue outbreak in New Nambiyar Nagar of the Nagapattinam district.

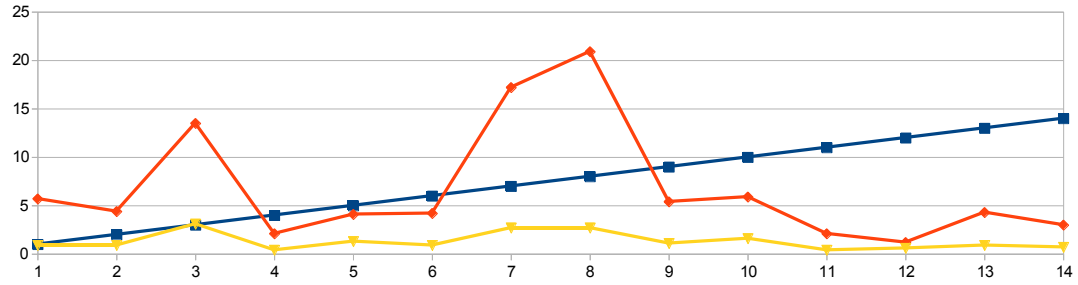

Figure 7. The declining trend of larval indices from the day of intervention to the halting of the dengue outbreak in Old Nambiyar Nagar of the Nagapattinam district.

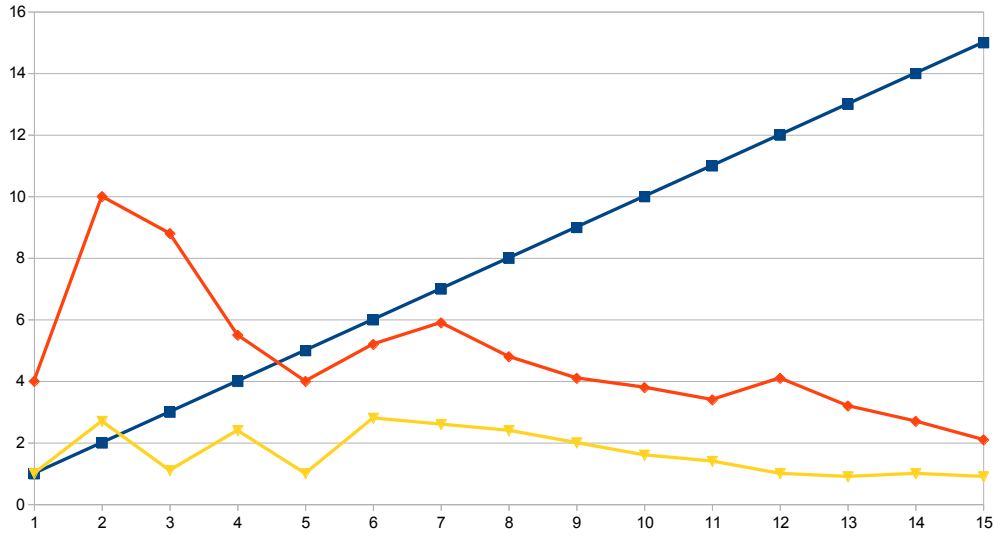

Figure 8. The declining trend of larval indices from the day of intervention to the halting of the dengue outbreak in Tatanagar of the Nagapattinam district.

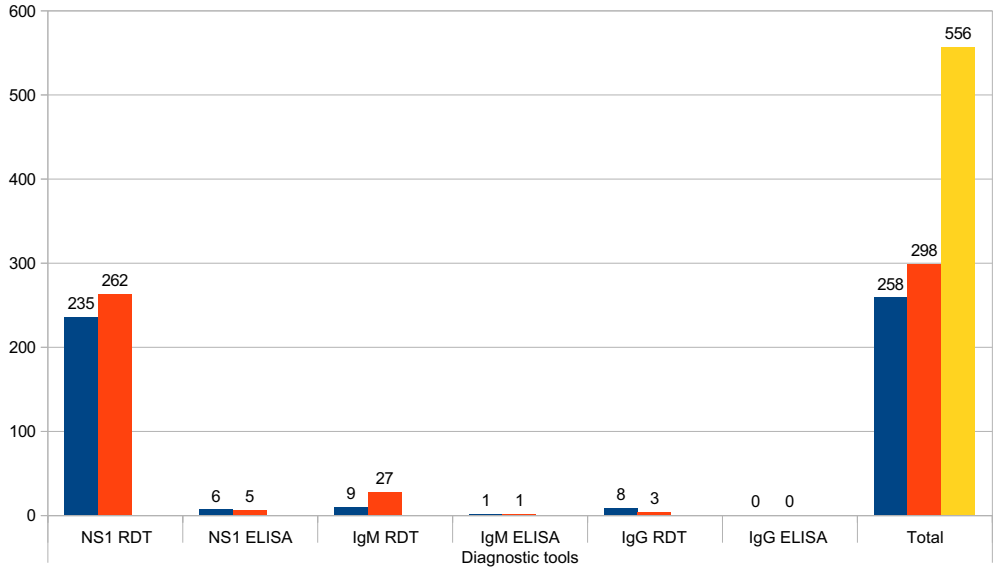

Figure 9. The diagnostic tools used to predict dengue cases in rural and urban areas of the Nagapattinam district. ELISA = enzyme linked immunosorbent assay; Ig = immunoglobulin; NS1 = nonstructural Protein 1; RDT = rapid diagnostic test.

Table 8. The course of larval (AL) indices from the beginning and the end of an outbreak in the urban area of the Nagapattinam district by different supervisory tiers during cross checking of domestic breeding checker (DBC) activities of AL work.

			Larval indices from SE cross checks			Larval indices from junior entomologist cross checks			Larval indices from health inspectors cross checks		
	Name of the urban areas	D	HI	CI	BI[a]	HI	CI	BI[a]	HI	CI	BI[a]
1	Sevabharthi	1	62.1	17	0	24	3.8	0	6.3	1.3	0
		2	28.1	10.4	0	10.2	1.6	0	4.1	1.1	0
		3	9.4	3.1	0	14.1	3.5	0	6.8	4.1	0
		4	8	3.2	0	9.5	2.1	0	8.2	1.5	0
		5	5.9	1.3	0	3.7	1.2	0	2.5	0.4	0
		6	5.4	0.9	0	5.2	1	0	3.1	2.3	0
		7	4	1	0	4.4	1	0	2.2	0.8	0
2	New Nambiyar Nagar	1	13.3	2.7	0	16.1	4.9	0	4.1	1.3	0
		2	8.8	1.3	0	19.4	2.2	0	4.2	0.9	0
		3	23.5	3.7	0	27.3	3.8	0	17.2	2.7	0
		4	15.6	3.4	0	19.2	2	0	20.9	2.7	0
		5	10	1	0	22.2	3.5	0	7.2	1.1	0
		6	7.8	0.9	0	8.2	1	0	4.2	0.7	0
		7	4	1	0	5	1.2	0	2.3	0.8	0
3	Tatanagar	1	10	2.7	0	9.1	2.2	0	1.8	0.4	0
		2	8.8	1.1	0	8	1.2	0	4.2	0.6	0
		3	5.5	2.4	0	4.8	1	0	2.3	0.7	0
		4	4	1	0	3.8	0.9	0	1.9	0.4	0
		5	4.1	1	0	5	1.1	0	2.6	1	0
		6	3.2	0.9	0	3.1	1	0	1.5	0.8	0
		7	2.8	1	0	3.1	1	0	0.5	0.1	0
4	Old Nambiyar Nagar	1	5	1.2	0	5.7	0.9	0	5.9	1.6	0
		2	4.3	1.2	0	4.4	0.9	0	2.1	0.4	0
		3	5	1	0	13.5	3.3	0	1.2	0.6	0
		4	4.3	1.9	0	2.1	0.4	0	4.3	0.9	0
		5	4	0.9	0	5	1	0	3	0.7	0
		6	3.3	1.3	0	3.2	1.3	0	2.6	0.6	0
		7	4	0.9	0	4.2	1	0	2.1	0.8	0

[a]*To compute Breteau Index (BI), minimum hundred houses should be visited to ensure the presence of Aedes larvae in containers whereas BI has not been arrived when searches for Aedes larvae happened to fall <100 houses. BI = Breteau Index; CI = Container Index; HI = House Index; SE = senior entomologist.*

Various studies have been found in literature on the relationships of *Stegomyia* or *Aedes* indices namely HI and BI to the transmission of dengue and these indices remain central to the monitoring of the dengue vector population [30]. Little is known about the relationship between the differing proportions of various sampled larval instars and the accuracy of these data as proxy measures of adult mosquito abundance [31]. Despite these doubts, many dengue control authorities worldwide routinely collect vector population data based on these indices although the mathematical relationship between any indices and dengue transmission is far from clear. Thresholds indicating dengue outbreak risks for HI and BI (HI = 1%, BI = 5) have been used for many years [32,33], even though these values were developed for yellow fever many decades earlier. Simple thresholds may be valid in some situations [8], but a universal critical threshold applicable across many contexts, has never been determined for dengue. These findings support the observations noted in this study as there was a concrete relationship between the recommended HI and BI levels necessary to control dengue outbreaks when their threshold levels fell to between 3% and 5% and < 5, respectively, based on the topography and type of habitat. At the same time, it was found that the impact of dengue in areas where the threshold levels of these indices were sustained for more than an incubation period suggested the existence of hidden habitats such as refrigerators and other unexpected rain dependent materials which accumulate in and around human dwellings. Further, it is also known that some rural villages had not experienced dengue in subsequent years and new villages raised might be based on the proportions of susceptible and immune individuals and in turn the establishment of herd immunity in the group. Epidemics or outbreaks of disease occur when the proportion of susceptible individuals is high, and disappear as the proportion of individuals with immunity increases. This observation was correlated to the present study which noted that a few villages presented dengue cases year

Table 9. Data appraised by the health inspectors of primary health centers (PHCs) and the senior entomologists (SEs).

	Sevabharathi		Old Nambiyar Nagar		New Nambiyar Nagar		Tatanagar	
Days from the intervention	HI of SE	HI of PHC	HI of SE	HI of Hinsp	HI of SE	HI of Hinsp	HI of SE	HI of Hinsp
1	62.1	6.3	2.8	0.5	13.3	4.1	4	2.3
2	28.1	4.1	5	5.9	8.8	4.2	10	1.8
3	9.4	6.8	4.3	2.1	23.5	17.2	8.8	4.2
4	8	8.2	5	1.2	15.6	20.9	5.5	2.3
5	5.9	2.5	4.3	4.3	10	7.2	4	1.9
6	5.4	3.1	4	3	7.8	4.2	4.1	2.6
7	4	2.2	3.3	2.6	6.6	3.8	3.2	1.8

HI = House Index; Hinsp = health inspector.

Table 10. The declining trend of *Aedes* indices within one incubation (15 days) during the dengue outbreak in the Mukkudal primary health center of the Tirunelveli district of Tamil Nadu, India.

Date	Houses checked	Houses positive with *Aedes* larvae	Total containers checked	Containers positive	House Index (HI)%	Container Index (CI)%	Breteau Index (BI)	Positive dengue cases confirmed by IgM ELISA
12 May 2012	715	95	845	95	13.2	11.2	13.2	6
13 May 2012	1008	197	5412	197	19.5	3.6	19.5	0
14 May 2012	3159	250	14,542	275	8	1.8	8.7	0
15 May 2012	3519	232	16,252	429	6.5	2.6	12.2	0
16 May 2012	3464	261	17,607	362	7.5	2	10.4	0
17 May 2012	4067	263	18,864	305	6.5	1.6	7.5	0
18 May 2012	2561	112	11,773	155	4.3	1.3	6	0
19 May 2012	3727	250	14,542	275	8	1.8	8.7	0
20 May 2012	592	40	4883	47	6.7	0.96	7.9	0
21 May 2012	3778	144	18,838	165	3.8	0.9	4.4	0
22 May 2012	3277	101	16,782	109	3.1	0.6	3.3	0
23 May 2012	2315	66	13,913	85	2.9	0.6	3.7	0
24 May 2012	2962	113	16,394	140	3.8	0.9	4.7	0
25 May 2012	2691	72	13,259	98	2.7	0.7	3.6	0
26 May 2012	2794	84	11,042	108	3	0.9	3.8	0
				Mean	**6.6**	**2.10**	**7.84**	

ELISA = enzyme linked immunosorbent assay; Ig = immunoglobulin.

after year in the same block. In this situation, maintaining the threshold level of the *Aedes* indices helped prevent an outbreak.

The above observations agree with the study findings of Tun-Lin et al [8] which showed that the blockwise analysis of *Aedes* indices to fix threshold levels in areas helped stop dengue transmission. In this context the lowest level of threshold (HI<1%) and BI 0.9) yielded from the average of the both the lowest and highest indices among blocks of the same town. The difference between the real lowest threshold and highest indices (up to 17.9% HI) was 400 meters and 40 days as the spatial and temporal boundaries of maximum dengue transmission in a dengue focus [8,34]. Along with this, a study by Lizet Sanchez [17] showed that BI $>$ 1 and maximum BI $\geq$ 4 seemed to be a suitable action threshold and target respectively in community based dengue prevention. However, these results are derived from the analysis of epidemic data, and the thresholds identified may not constitute suitable targets in another epidemic or in a location where different ecologic conditions prevail [34]. Similar observation was also made in this research paper which found that some places with low thresholds of *Aedes* indices yielded cases which were due to different ecological factors and unusual habitats.

Cassens [29] observed that social traits take part in disease transmission as the host goes through life. Marital status, lifestyle, diet, place of residence, and travel are some of the factors that determine disease outcomes and have been shown to be direct factors affecting dengue outbreaks in an area. These factors are further strengthened by many scientists who have reported that DENV infection is a potential risk for travelers to tropical areas where dengue is endemic or epidemic. Furthermore, it is known that the growth of

Table 11. The declining trend of *Aedes* indices in the second incubation (15−30 days) during the dengue outbreak in the Mukkudal primary health center of the Tirunelveli district of Tamil Nadu, India.

Date	Houses checked	Houses positive with *Aedes* larvae	Total containers checked	Containers positive	House Index(HI)%	Container Index (CI)%	Breteau Index(BI)	Positive dengue cases confirmed by IgM ELISA
27 May 2012	1111	31	5719	49	2.8	0.9	4.4	0
28 May 2012	2929	47	11,068	49	1.6	0.4	1.9	0
29 May 2012	3177	34	14,438	39	1.1	0.3	1.2	0
30 May 2012	2961	32	12,759	35	1.1	0.3	1.2	0
31 May 2012	3018	30	12,941	30	0.9	0.2	0.9	0
01 Jun 2012	2686	52	11,825	65	1.9	0.5	2.4	0
02 Jun 2012	2404	54	9632	55	2.2	0.6	2.3	0
03 Jun 2012	2199	39	9568	40	1.8	0.4	1.8	0
04 Jun 2012	2866	22	9733	22	0.8	0.2	0.8	0
05 Jun 2012	2836	37	9723	41	1.3	0.4	1.4	0
06 Jun 2012	2513	19	8887	19	0.8	0.2	0.8	0
07 Jun 2012	2922	26	12,922	26	0.9	0.2	0.9	0
08 Jun 2012	2804	23	11,499	23	0.8	0.2	0.8	0
09 Jun 2012	2286	15	8022	15	0.7	0.2	0.7	0
				Mean	1.34	0.36	1.54	

ELISA = enzyme linked immunosorbent assay; Ig = immunoglobulin.

imported cases is increasingly recognized as a serious public health problem in nonendemic countries [30−33]. A study carried out in Cuba by Lizet Sanchez [17] observed that during the inspection cycle, before the outbreak, the overall municipal BI and HI were 0.92 and 0.87% respectively. The mean values of the indices calculated at the health area level were also ∼1 for areas with or without dengue cases during the subsequent epidemic. However, the mean BI and HI were > 1 in neighborhoods reporting cases and substantially < 1 for neighborhoods without cases.

Morrison et al [35] established the degrees of variation of larval prevalence in the Playa Municipality, Cuba, before, during, and after the dengue epidemic. This provided a unique opportunity to analyze entomologic information at different geographic levels. Entomologic data were collected through routine systems; however, this saw some limitations. Firstly, larval prevalence was possibly slightly underestimated in

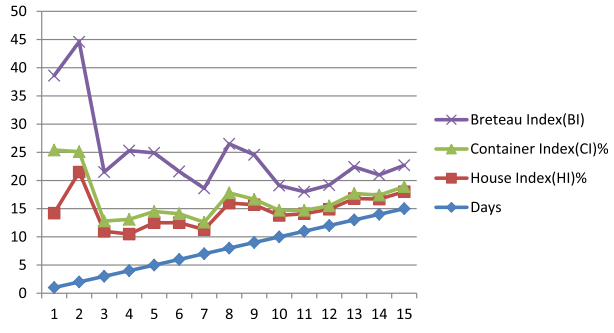

Figure 10. The declining trend of House Index, Container Index, and Breteau Index during the dengue outbreak in the Mukkudal primary health center of the Tirunelveli district of Tamil Nadu, India.

blocks which were inspected by different vector control technicians, procedures used may not have been completely standardized, and few data (randomly) went missing. Secondly, when dengue cases were reported, the control program intensified and more *Aedes* foci may have been detected. Thirdly, sampling *Aedes aegypti* can be time sensitive [32].

Furthermore, it is known that the peak incidents of confirmed infection followed the peak larval density by ∼1 month. In Salvador, Brazil, sentinel surveillance in 30 areas detected a significant 1.4× higher serovar incidence when the HI was > 3%. Recently, Scott and Morrison [16] showed that traditional larval indices in Peru are correlated with the prevalence of human dengue infections. The variety of thresholds proposed in these and other studies could be partially explained by different methods and geographic levels of analysis used, but other factors influence the relationship between *Aedes* density and transmission risk, such as herd immunity, population density, mosquito-human interaction, virus strain, and climate, which affects mosquito biology and most virus interaction [11,16].

Diagnosis of DENV infection using a commercial test kit alone is not reliable in terms of sensitivity and specificity, and a definitive diagnosis should be made in conjunction with other laboratory findings [27,34]. There is a lacuna on the early implementation of preventive measures and dengue control without complications in an area. Probing its determination with reference to diagnostic tools available to confirm dengue in the laboratory, it is known that NS1 is immensely helpful in the initiation of early entomological surveillance in an outbreak and in bringing down *Aedes* indices to their permissible level within a fortnight i.e., one incubation period, barring the cases already infected during the intervention. On the

Table 12. The *Aedes* indices within permissible levels in Nangunery where no dengue positives were reported in May 2012 or June 2012.

Date	Houses checked	Houses positive with *Aedes* larvae	Total containers checked	Containers positive	House Index (HI)%	Container Index (CI)%	Breteau Index (BI)	Positive dengue cases confirmed by IgM ELISA
25 May 2012	5905	101	11,321	244	1.7	2.2	4.1	0
26 May 2012	3553	78	7644	114	2.1	1.5	3.2	0
27 May 2012	3338	48	6215	56	1.6	0.9	1.7	0
28 May 2012	4929	89	10,162	101	1.8	1.0	2.0	0
29 May 2012	4523	85	9487	105	1.8	1.1	2.3	0
30 May 2012	3945	71	8326	87	1.7	1.0	2.2	0
31 May 2012	4024	78	9280	97	1.9	1.0	2.4	0
01 Jun 2012	3744	70	8285	106	2.1	1.3	2.8	0
02 Jun 2012	3766	86	8366	127	2.2	1.5	3.4	0
03 Jun 2012	3797	80	9374	114	2.1	1.2	3.0	0
04 Jun 2012	4935	71	10,931	85	1.4	0.8	1.7	0
05 Jun 2012	5587	65	10,645	85	1.2	0.8	1.5	0
06 Jun 2012	4024	56	8837	86	1.4	1.0	2.1	0
07 Jun 2012	4242	86	10,343	118	2.0	1.1	2.8	0
08 Jun 2012	4384	66	10,180	91	1.5	0.9	2.1	0
09 Jun 2012	4156	50	9379	73	1.2	0.8	1.8	0
10 Jun 2012	4290	58	8437	76	1.4	0.9	1.8	0
11 Jun 2012	4989	64	11,531	88	1.3	0.8	1.8	0
12 Jun 2012	4587	65	9843	65	1.4	0.7	1.4	0
13 Jun 2012	3994	46	8841	66	1.2	0.7	1.7	0
14 Jun 2012	4279	62	9710	52	1.4	0.5	1.2	0
15 Jun 2012	4282	60	4469	73	1.4	1.6	1.7	0
Mean					1.63	1.06	2.22	

ELISA = enzyme linked immunosorbent assay; Ig = immunoglobulin.

Table 13. The *Aedes* indices within permissible levels in the Cheranmahadevi district where dengue positives had not been reported in May 2012 or June 2012.

Date	Houses checked	Houses positive with Aedes larvae	Total containers checked	Containers positive	House Index (HI)%	Container Index (CI)%	Breteau Index (BI)	Positive dengue cases confirmed by IgM ELISA
25 May 2012	5984	93	20,593	93	1.6	0.5	1.6	0
26 May 2012	5409	41	20,900	41	0.8	0.2	0.8	0
27 May 2012	60	2	136	2	3.3	1.5	0	0
28 May 2012	5589	27	26,498	27	0.5	0.1	0.5	0
29 May 2012	6240	108	27,215	108	1.7	0.4	1.7	0
30 May 2012	5411	21	22,569	21	0.4	0.1	0.4	0
31 May 2012	5911	15	23,579	17	0.3	0.1	0.3	0
01 Jun 2012	5139	12	20,381	15	0.2	0.1	0.3	0
02 Jun 2012	4626	21	19,758	25	0.5	0.1	0.5	0
03 Jun 2012	3094	10	11,145	12	0.3	0.1	0.4	0
04 Jun 2012	5314	13	23,097	13	0.2	0.1	0.2	0
05 Jun 2012	5312	9	22,613	10	0.2	0.0	0.2	0
06 Jun 2012	5524	3	20,880	3	0.1	0.0	0.1	0
07 Jun 2012	5569	11	17,548	13	0.2	0.1	0.2	0
08 Jun 2012	5073	12	19,772	12	0.2	0.1	0.2	0
09 Jun 2012	4310	9	15,811	10	0.2	0.1	0.2	0
10 Jun 2012	2947	2	10,433	2	0.1	0.0	0.1	0
11 Jun 2012	5577	8	20,929	8	0.1	0.0	0.1	0
12 Jun 2012	5546	8	22,586	8	0.1	0.0	0.1	0
13 Jun 2012	5381	9	21,861	9	0.2	0.0	0.2	0
14 Jun 2012	5233	10	21,395	11	0.2	0.1	0.2	0
15 Jun 2012	4897	8	19,089	8	0.2	0.0	0.2	0
16 Jun 2012	5215	7	2066	7	0.1	0.3	0.1	0
				Mean	0.50	0.17	0.37	

ELISA = enzyme linked immunosorbent assay; Ig = immunoglobulin.

Table 14. The number of dengue IgM ELISA confirmed cases and deaths by age and sex in the Mukkudal block of the Tirunelveli district of Tamil Nadu, India.

			Deaths	
Age groups (y)	Male	Female	Male	Female
0−1	1	3	1	1
1−4	2	2	2	0
5−8	3	2	0	0
9−14	10	7	0	0
> 15	3	13	0	0

ELISA = enzyme linked immunosorbent assay; Ig = immunoglobulin.

contrary, interventions have been delayed about 1 week when the suspected serum sample is confirmed by IgM ELISA after 6 days from the onset of fever [19]. Even though, NS1 specificity and sensitivity differs between manufacturers, cross reaction, etc., confirmation of dengue with this tool relates to the search for *Aedes* at an immature stage in the place where the case resides and it is known that the serum is eligible for testing the day after the onset of fever. Hence, the NS1 tool contributes to early intervention implementation even before the new complication of dengue cases emerge in an outbreak area. Supporting this view, some studies have shown the significance of the eligibility of serum samples to confirm dengue [18,19]. It is anticipated that clinical diagnosis methods will be strengthened as NS1 plays an important role in the early implementation of entomological interventions.

When discussing the titer of IgM and IgG antibodies during the primary and secondary infection of dengue, it was found that the IgM to dengue virus appears late during the febrile phase of illness, often preceded by IgG. In primary infection, only low levels of IgG to dengue are detected in the febrile or early convalescent phase of infection, whereas levels of IgM are high and greatly exceed IgG levels for 2−4 weeks. In secondary infections, high levels of IgG are detectable even in the acute phase and rise dramatically over the next 2 weeks whereas IgM levels are absent or low, raise little in comparison with IgG and decline quickly. Hence, the detection of IgM is not specific for acute dengue, as IgM may persist for 2−3 months following infection, IgM against other flaviviruses can cross react in some tests [27,34], and IgG does not distinguish current from past infection, an important issue in endemic areas were secondary infection is common.

To detect early infection of the dengue virus, serum or plasma can be tested using the RT-PCR tool. Based on its cost effectiveness, NS1 based testing should be considered for detection in acute febrile illness of dengue as it allows the implementation of an early intervention in an area even before complications have become apparent [34]. Its use is supported by the current study. Similar to previous study findings, this study also noted the degrees of involvement among DBCs. The first and second tiers of supervision were not statically significant ($p > 0.1$), whereas the supervision of higher officials is qualitatively significant ($p < 0.05$). The results of findings on the impact of cases after numerous intervention cycles were made during an outbreak and showed that it is difficult to halt an outbreak within a single span of dengue incubation in humans unless there is a commitment to obtain uniform indices in detecting positive habitats of *Stegomyia* in an outbreak area irrespective of DBC and the tier of supervision.

In summary, the following study findings have been ascertained: (1) the incubation of DENV in human is 4−14 days; (2) the presence of the heterogeneous serotype DENV leads to increased case fatality rates in an epidemic; and (3) cyclic larval checkups are important as they can detect permissible threshold levels of *Aedes* indices and in turn help to stop dengue transmission within 15 days from the day of intervention when there is a significant level of commitments in all field staff. Above all, the NS1 ELISA is the tool which can be used for the early implementation of entomological interventions to reduce dengue complications in the community. Other than these findings, a new area of research has to be necessitated to study the cross reaction of dengue virus in places where other flavi viruses are co-existing. Since the Cuddalore district was previously endemic for JE, vaccination with SA 14−14−2 live attenuated vaccine has been administered to children aged from 9.5 months to 1.5 years since 2008 after its inclusion in the routine national immunization program. Hence efforts need to be made to resolve the cross reaction between dengue and other flaviviruses during outbreaks, it is confirmed that this may give a new dimension to dengue diagnosis.

Conflicts of interest

The authors declare no conflicts of interest.

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