Study on the Behaviour of Dengue viruses during outbreaks with reference to Entomological and Laboratory surveillance in the Cuddalore, Nagapattinam and Thirunelveli Districts of Tamil Nadu, India.

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Aedes aegypti and Aedes albopictus

ENTOMOLOGICAL SURVEILLANCE

"Entomological surveillance is used to determine changes in the geographical distribution and density of the vector, evaluate control programmes, obtain relative measurements of the vector population overtimes and to facilitate appropriate and timely decisions regarding intervention"

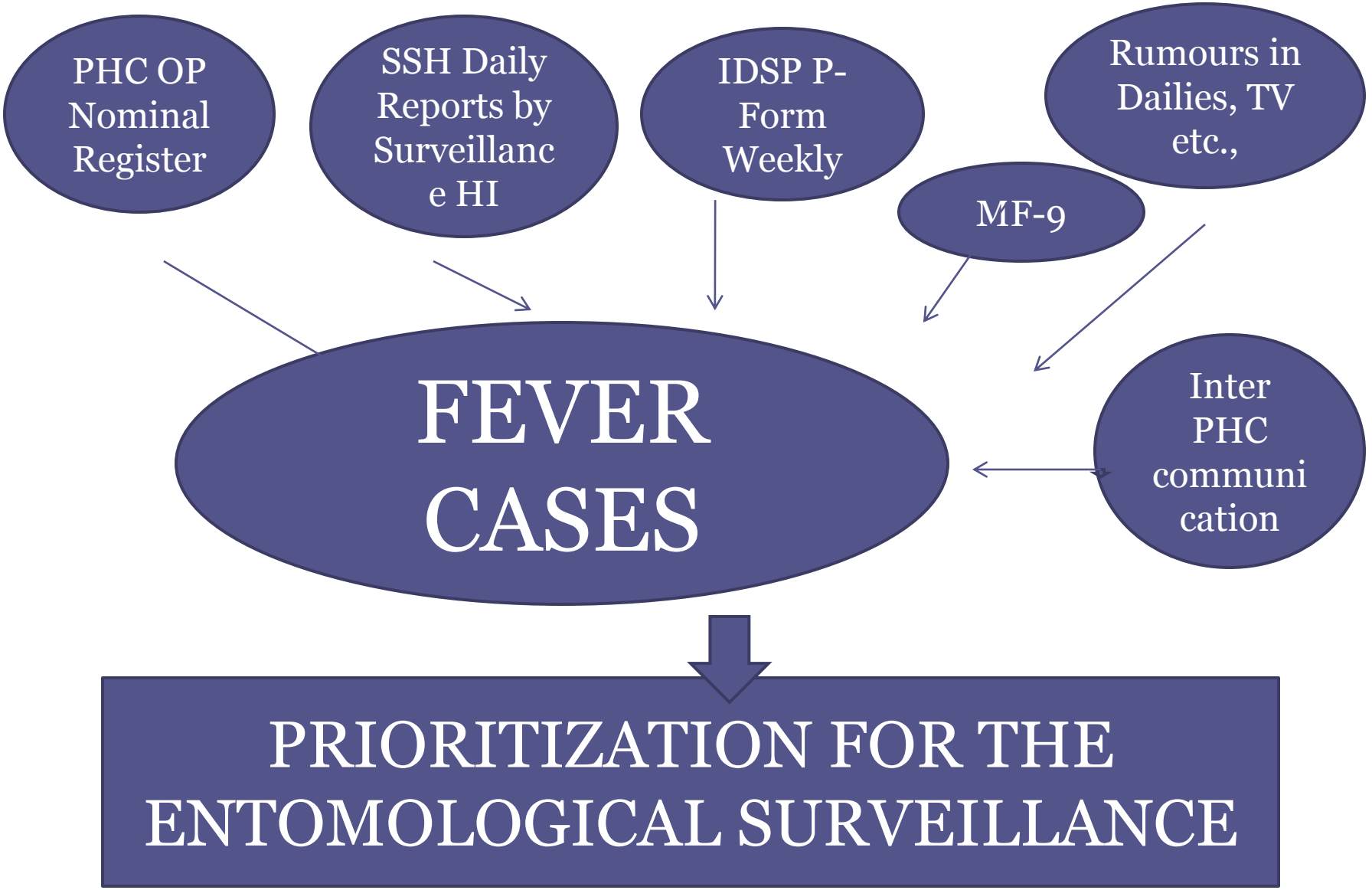
WHO

LABORATORY SURVEILLANCE

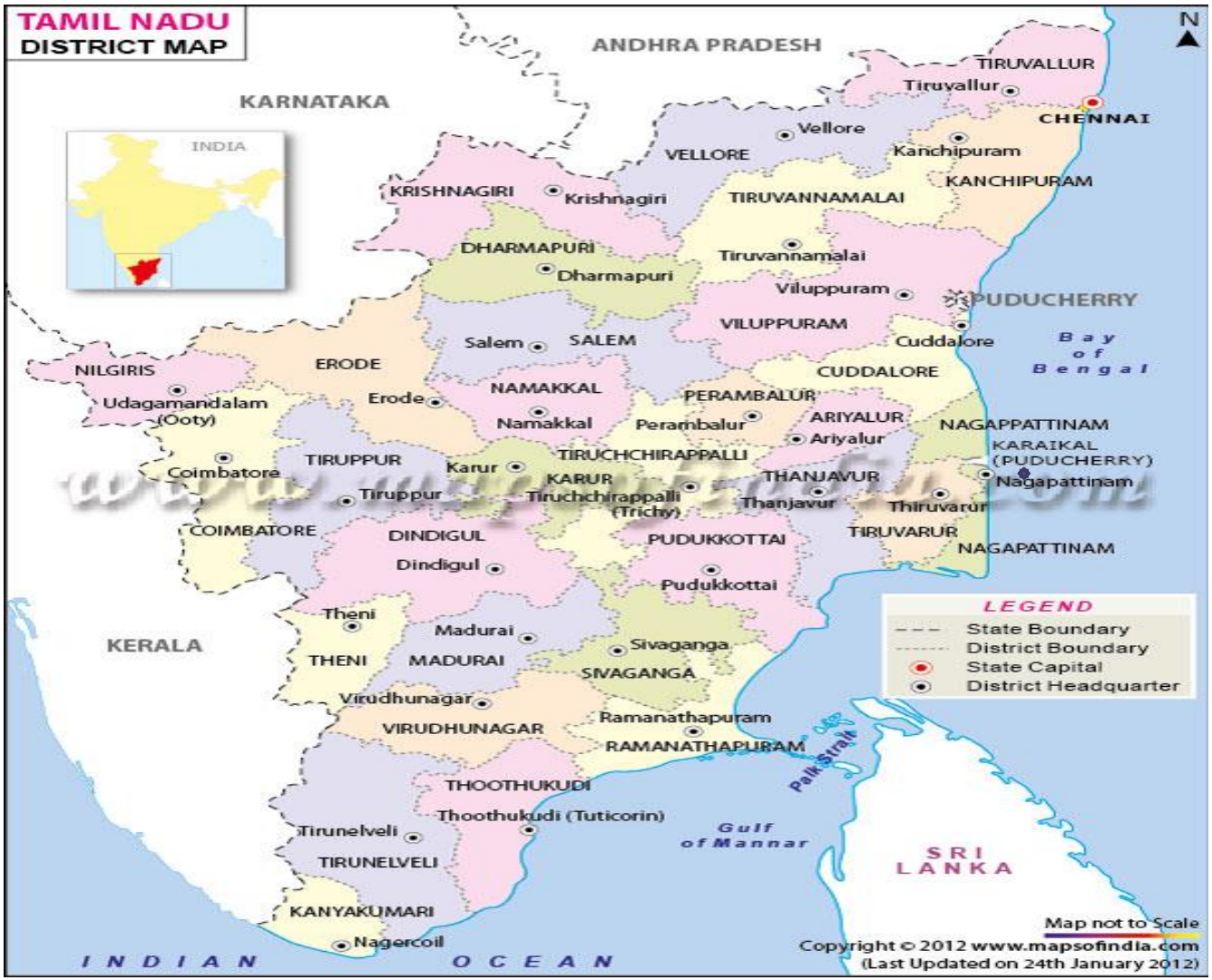
Laboratory surveillance must be stepped up in anticipation or in the event of an outbreak. Serological and other Laboratory based surveys are sometimes Conducted as research projects to collect baseline prevalence rates or to identify high risks Factors, age groups or population sub-groups.

The identification of new agents and changes in the behavior of micro-organisms especially in relation to susceptibility to Anti-microbial are also important components of laboratory Surveillance.

Flow Chart of fever convergence mechanism



STUDY AREAS


TAMIL NADU
DISTRICT MAP
N
INDIA
ANDHRA PRADESH
KARNATAKA
KERALA
TIRUVALLUR
Tiruvallur
CHENNAI
Vellore
VELLORE
Kanchipuram
KANCHIPURAM
KRISHNAGIRI
Krishnagiri
TIRUVANNAMALAI
DHARMAPURI
Dharmapuri
Tiruvannamalai
Viluppuram
PUDUCHERRY
VILUPPURAM
Cuddalore
CUDDALORE
Bay of Bengal
Salem
SALEM
ERODE
NILGIRIS
Udagamandalam (Ooty)
Erode
NAMAKKAL
Namakkal
PERAMBALUR
Perambalur
ARIYALUR
Ariyalur
NAGAPPATTINAM
KARAIKAL (PUDUCHERRY)
Nagapattinam
TIRUPPUR
Coimbatore
Karur
KARUR
TIRUCHCHIRAPPALLI
Tiruchirappalli (Trichy)
THANJAVUR
Thanjavur
Thiruvarur
Tiruppur
COIMBATORE
DINDIGUL
Dindigul
PUDUKKOTTAI
Pudukkottai
TIRUVARUR
NAGAPATTINAM
Theni
THENI
Madurai
MADURAI
Sivaganga
SIVAGANGA
Virudhunagar
VIRUDHUNAGAR
Ramanathapuram
RAMANATHAPURAM
Palk Strait
THOOTHUKUDI
Thoothukudi (Tuticorin)
Gulf of Mannar
SRI LANKA
Tirunelveli
TIRUNELVELI
KANYAKUMARI
Nagercoil
INDIAN OCEAN
LEGEND
State Boundary
District Boundary
State Capital
District Headquarter
Map not to Scale
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(Last Updated on 24th January 2012)

Objectives of the Study

This study was carried out in order to understand the behaviour of dengue viruses through the entomological and laboratory surveillance of outbreaks with the following objectives are

1.To provide additional research

2.To support current knowledge of epidemiological, clinical, entomological and on laboratory diagnosis of dengue virus

3.To apply these information to forecasting dengue as well as

4.To justify intervention measures.

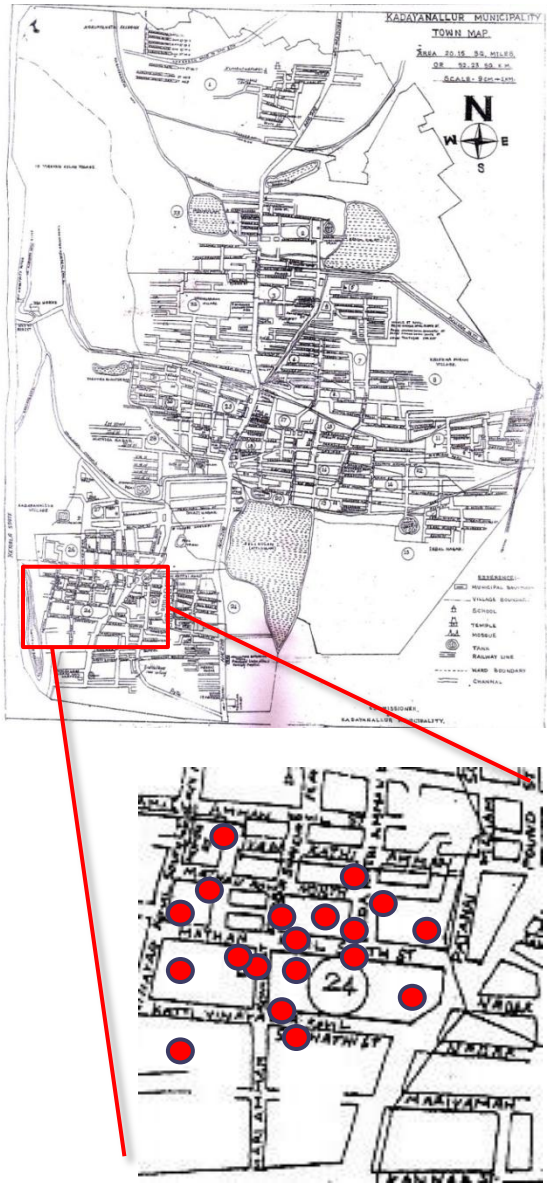
Materials and Methods

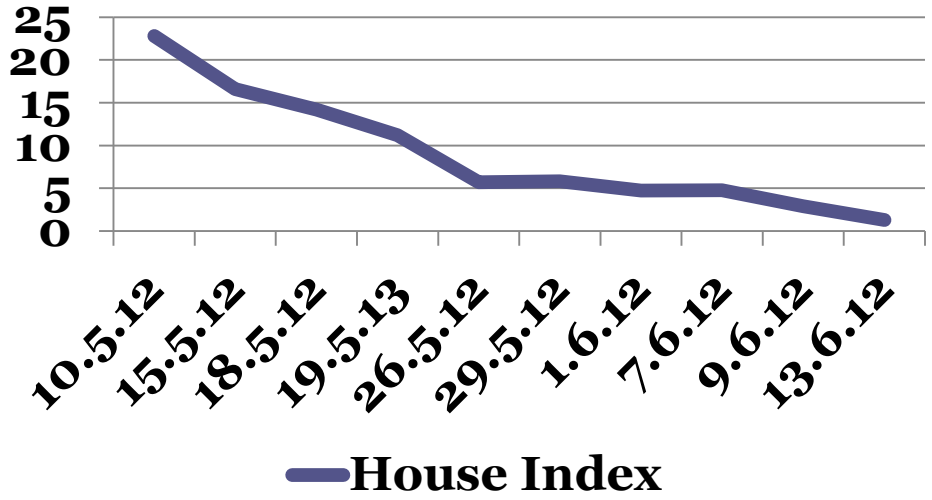
- To arrive House Index (HI), Container Index (CI) and Breteau Index (BI), data on the presence of *Aedes* Spp larvae in habitats in and around house holds in the study area had been taken. DBC, JE, SE and officials involved in it

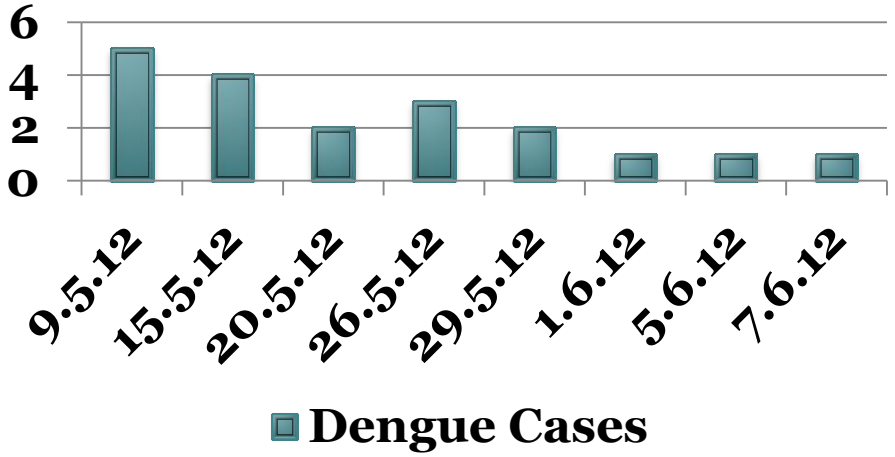
- Standard procedures were followed for non-structural Protein 1 (NS1) and immunoglobulin M enzyme linked immuno sorbant assay for the confirmation of dengue positive cases.

- Serovar confirmation was made by the RT-PCR in the Kottayam field station of the Vector Control Research Center, Puducherry.

Aedes Survey conduced at the Outbreak Area – Kadayanallur Municipality (W.no.24),Thirunelveli Dist







Results

- Larval indices HI < 2-3% and BI < 20 has fixed as threshold to halting the outbreak.
- Incubation of the dengue viruses in humans was detected as 4- 14 days.
- NS1 is a tool for the early diagnosis of dengue cases as it has initiated to implement all available interventions.
- It is also discovered that it is helpful to search for hidden habitats of *Aedes* when dengue cases have not been declined even the larval indices brought down to HI < 5% and BI < 20 within one incubation.
- It was learnt that neighbourhood areas of the outbreak, around 400 m, should be in permissible larval indices < 5% HI and BI < 20.
- Heterogeneous serovars that led to dengue hemorrhagic fever and Dengue Shock Syndrome (DSS) were identified.

Supportive Data (Cuddalore)

Diagnostic tools	No. of cases
NS1 ELISA	1179
Ig-M ELISA	211
RDT NS1	100
RDT NS1	10
Total	1500

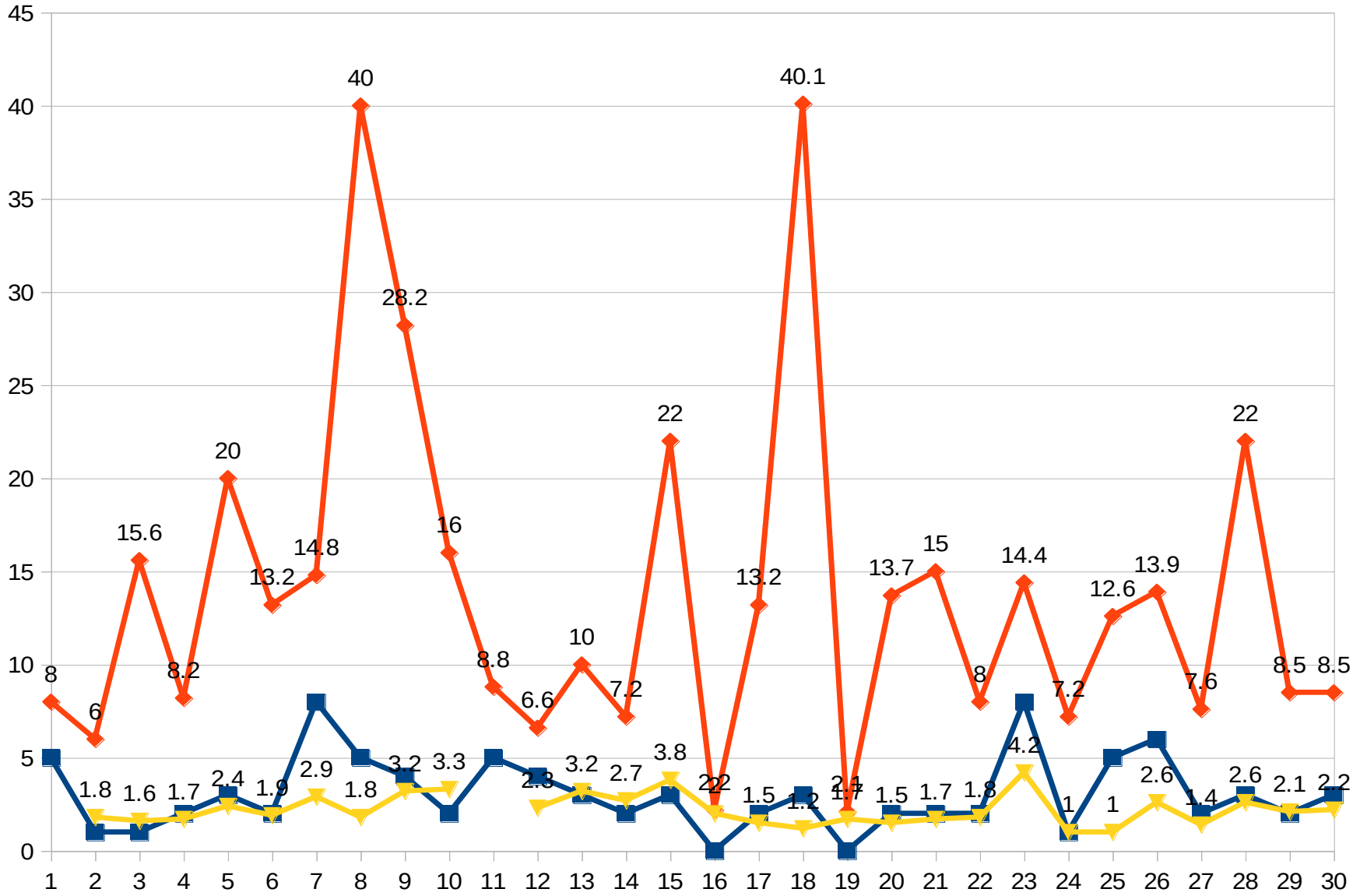
Aedes larval Indices before and after outbreaks and their threshold in eak in the Cuddalore District in 2013.

Sl.No.	Name of the block	No. of dengue positives	Initial House Index (HI)	Container Index (CI)	Breteau Index (BI) if applicable as 100 houses searched	HI	CI	BI
1.	Annagramam	5	8	1.6	10	2.1	0.49	2.82
2.	Cuddalore	1	6	1.2	8	1.8	0.5	1.8
3.	Cuddalore	1	15.6	3.12	17.6	1.6	0.7	1.6
4.	Cuddalore	2	8.2	1.64	10.2	1.7	0.7	1.7
5.	Cuddalore	3	20	4	22	2.4	1.2	2.4
6.	Cuddalore	2	13.2	2.64	15.2	1.9	0.8	1.9
7.	Cuddalore	8	14.8	2.8	16.8	2.9	1.3	3.9
8.	Panruti	5	40	5.88	42	1.8	0.4	1.8
9.	Panruti	4	28.2	3.81	30.2	3.2	1.1	3.2
10.	Vadalur	2	16	3.03	18	3.3	1.5	3.3

Sl.No.	Name of the block	No.of dengue positives	Initial House Index (HI)	Container Index (CI)	Breteau Index (BI) if applicable as 100 houses searched	HI	CI	BI
11.	Parangipettai	5	8.8	1.62	10.8	2.5	0.7	2.3
12	Parangipettai	4	6.6	1.25	8.6	2.3	0.7	2.3
13	Parangipettai	3	10	1.8	12	3.2	1.13	3.2
14.	Parangipetti	2	7.2	1.4	9.2	2.7	0.8	2.7
15.	Kammapuram	3	22	3.7	24	3.8	1.1	6.4
16.	Kammapuram	1	22	0.44	4.2	2	0.19	2
17.	Kammapuram	2	13.2	0.54	15.2	1.5	0.8	1.5

Sl.No.	Name of the block	No.of dengue positives	Initial House Index (HI)	Container Index (CI)	Breteau Index (BI) if applicable as 100 houses searched	HI	CI	BI
21	Nallur	2	15	3.31	17	1.7	0.8	1.7
22.	Nallur	2	8	1.94	10	1.8	0.8	1.8
23.	Mangalur	8	14.4	2.45	16.4	4.2	0.8	4.2
24.	Mangalur	1	7.2	1.51	9.2	1	0.5	1
25	Mangalur	5	12.6	2.99	14.6	1	0.4	1
26.	Cuddalore Mpty	6	13.9	2.56	15.9	2.6	0.7	2.6
27.	Cuddalore Mpty	2	7.6	1.4	9.6	1.4	0.7	1.4
28.	Cuddalore Mpty	3	22	1.9	24	2.6	0.7	2.6
29.	Cuddalore Mpty	2	8.5	2.1	10.5	2.1	0.9	1.7
30.	Nellikuppam	3	8.5	1.63	10.5	2.2	1.2	2.2
	Total		15.27	2.45	16.37	2.2	1.77	2.4

Graphic representation of *Aedes Larval indices before and after the outbreak in Cuddalore*

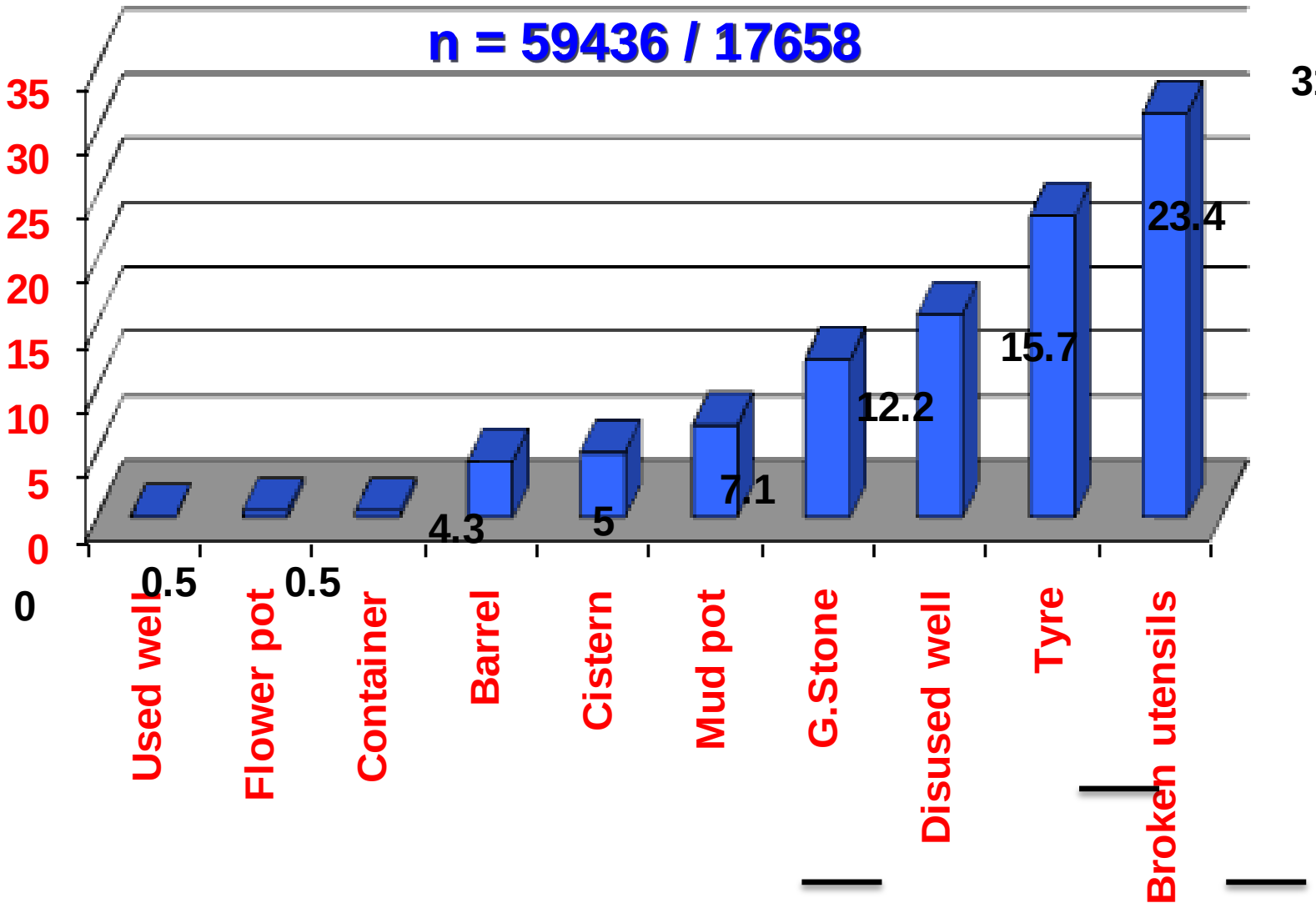


The declining trend of *Aedes* indices in the second incubation (15-30 days) during the dengue outbreak in the Mukkudal primary health center of the Tirunelveli district of Tamil Nadu, India.

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 Ig-M ELISA
27/05/12	1111	31	5719	49	2.8	0.9	4.4	0
28/05/12	2929	47	11068	49	1.6	0.4	1.9	0
29/05/12	3177	34	14438	39	1.1	0.3	1.2	0
30/05/12	2961	32	12759	35	1.1	0.3	1.2	0
31/05/12	3018	30	12941	30	0.9	0.2	0.9	0
01/06/12	2686	52	11825	65	1.9	0.5	2.4	0
02/06/12	2404	54	9632	55	2.2	0.6	2.3	0
03/06/12	2199	39	9568	40	1.8	0.4	1.8	0
04/06/12	2866	22	9733	22	0.8	0.2	0.8	0
05/06/12	2836	37	9723	41	1.3	0.4	1.4	0
06/06/12	2513	19	8887	19	0.8	0.2	0.8	0
07/06/12	2922	26	12922	26	0.9	0.2	0.9	0
08/06/12	2804	23	11499	23	0.8	0.2	0.8	0
09/06/12	2286	15	8022	15	0.7	0.2	0.7	0
				Mean	1.34	0.36	1.54	

- Supportive Data for unconventional Breeding habitats of *Aedes aegypti* and *Aedes albopictus*

Preferred breeding site for *Ae.aegypti*



Showing the hidden habitats of *Aedes species* in some rural and urban of Cuddalore District those contributed dengue positives along with the permissible *Aedes* Indices

S.No	Name of the Block	Name of the villages those extended more than 15 days to stop Dengue	No of cases reported from NS1 tool	Occult habitats of Dengue and its index	NS1	Ig-M	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			

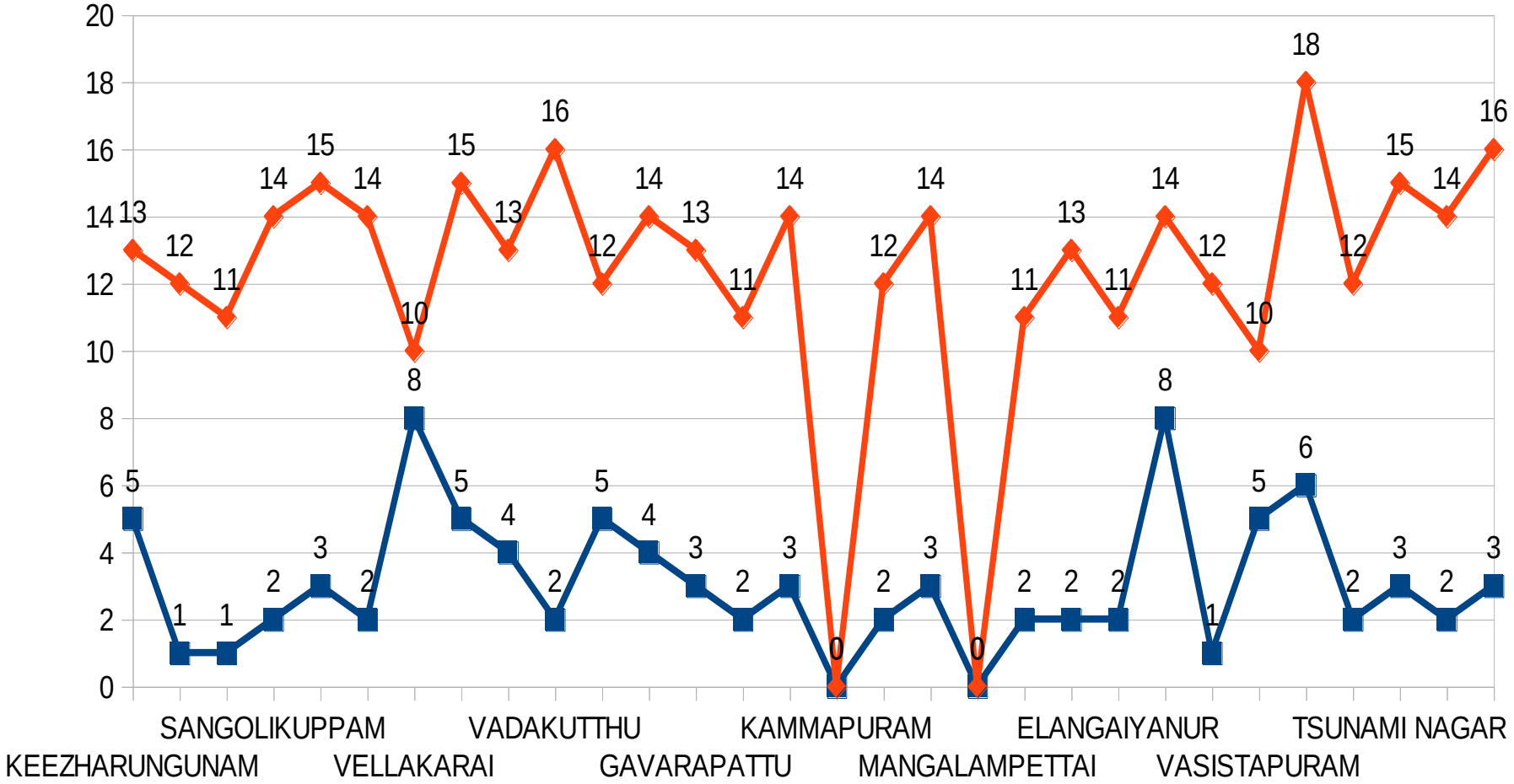
S.No	Name of the Block	Name of the villages those extended more than 15 days to stop Dengue	No of cases reported from NS1 tool	Occult habitats of Dengue and its index	NS1	Ig-M	Total
3	PANRUTI	SILAMBINATHANPETTAI	23	REFRIGERATOR	103	15	118
				PLASTIC CANS			
4	VADALUR	KURINJIPADI	14	REFRIGERATOR	112	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			

S.No	Name of the Block	Name of the villages those extended more than 15 days to stop Dengue	No of cases reported from NS1 tool	Occult habitats of Dengue and its index	NS1	Ig-M	Total
		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			
		VANDIPALAYAM	16	REFRIGERATOR			
		SEMMANDALAM	10	REFRIGERATOR			
		MANJAKUPPAM	19	REFRIGERATOR			

S.No	Name of the Block	Name of the villages those extended more than 15 days to stop Dengue	No of cases reported from NS1 tool	Occult habitats of Dengue and its index	NS1	Ig-M	Total
		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

S.No	Name of the Block	Name of the villages those extended more than 15 days to stop Dengue	No of cases reported from NS1 tool	Occult habitats of Dengue and its index	NS1	Ig-M	Total
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

Time taken in days to halt the dengue outbreak



The declining trends of the House Index (HI) and Container Index (CI) from the day of intervention to the halt of the dengue outbreaks in the urban areas of the Nagapattinam Dstrict of Tamil Nadu, India.

SevaBharathi			Old Nambiyar Nagar		New Nambiyar Nagar		TATA Nagar	
Days from the intervention	*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

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 the Nagapattinam District of Tamil Nadu, India.

SevaBharathi			Old Nambiyar Nagar		New Nambiyar Nagar		TATA Nagar	
Days from the intervention	*HI*	*CI*	*HI*	*CI*	*HI*	*CI*	*HI*	*CI*
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**

The declining trend of larval indices from the day of intervention to the halt the dengue outbreak in SevaBharathy of Nagapattinam District

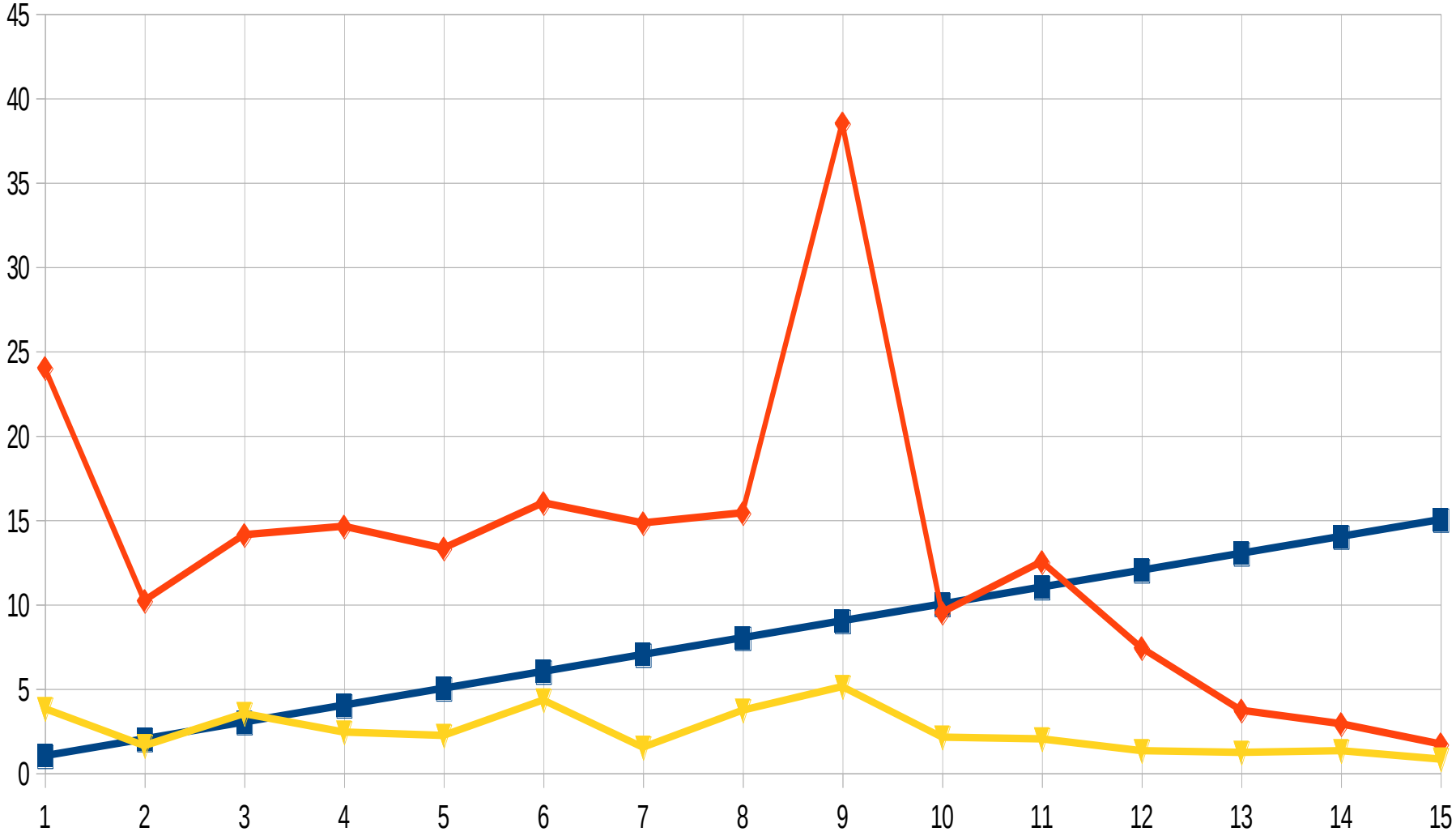
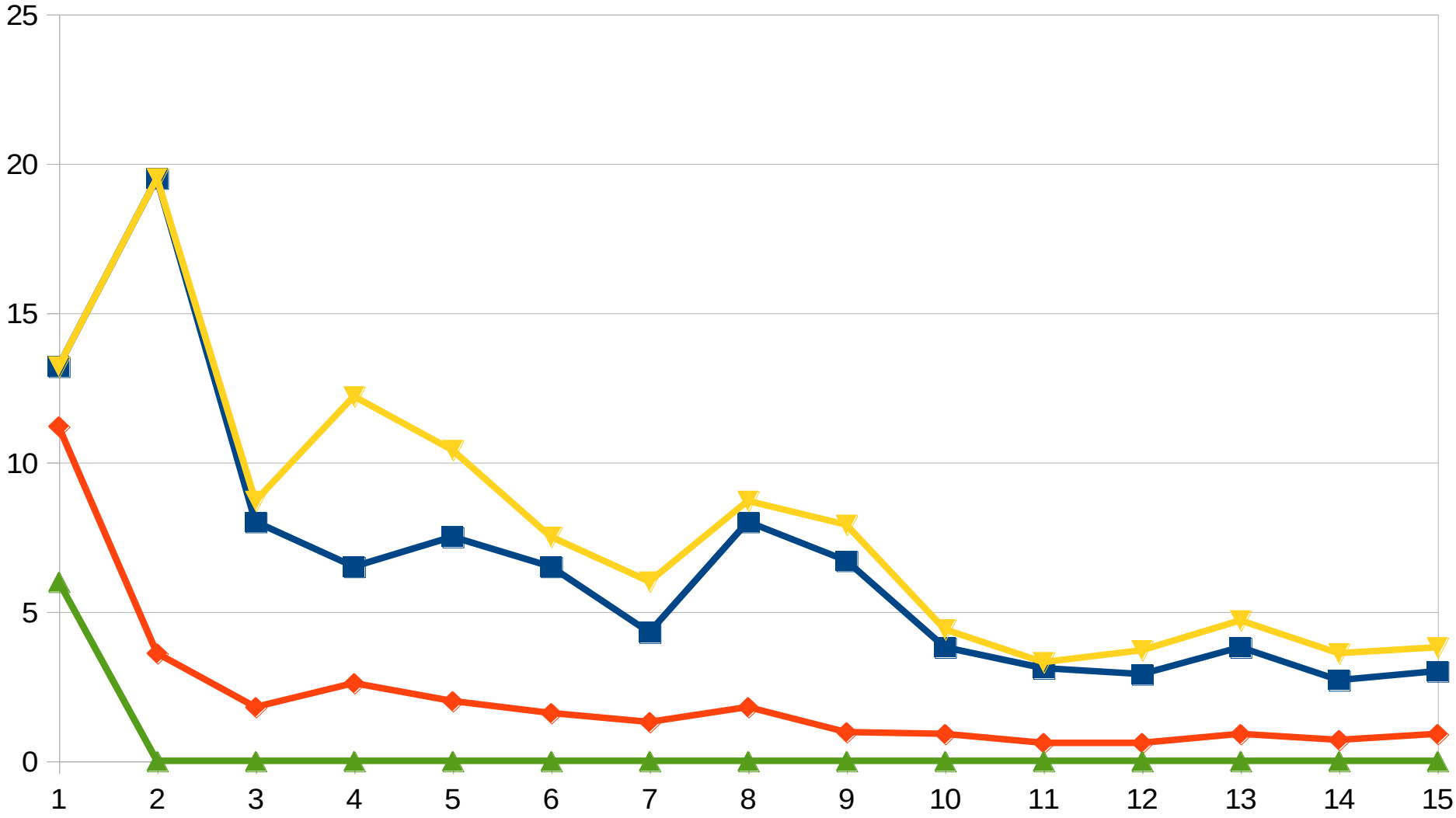


Figure-10 showing the declining trend of indices HI,CI and BI during the outbreak in Mukkudal PHC of Thirunelveli District of Tamil Nadu, India



Comparison of outbreak and non-outbreak area with reference to *Aedes larval* Indices

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 Ig-M ELISA
25.5.2012	5905	101	11321	244	1.7	2.2	4.1	0
26.05.2012	3553	78	7644	114	2.1	1.5	3.2	0
27.05.2012	3338	48	6215	56	1.6	0.9	1.7	0
28.05.2012	4929	89	10162	101	1.8	1.0	2.0	0
29.05.2012	4523	85	9487	105	1.8	1.1	2.3	0
30.05.2012	3945	71	8326	87	1.7	1.0	2.2	0
31.05.2012	4024	78	9280	97	1.9	1.0	2.4	0
01/06/12	3744	70	8285	106	2.1	1.3	2.8	0
02/06/12	3766	86	8366	127	2.2	1.5	3.4	0

Aedes indices within permissible level in Nangunery where Dengue positives had not been reported in May -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 Ig-M ELISA
03/06/12	3797	80	9374	114	2.1	1.2	3.0	0
04/06/12	4935	71	10931	85	1.4	0.8	1.7	0
05/06/12	5587	65	10645	85	1.2	0.8	1.5	0
06/06/12	4024	56	8837	86	1.4	1.0	2.1	0
07/06/12	4242	86	10343	118	2.0	1.1	2.8	0
08/06/12	4384	66	10180	91	1.5	0.9	2.1	0
09/06/12	4156	50	9379	73	1.2	0.8	1.8	0
10/06/12	4290	58	8437	76	1.4	0.9	1.8	0
11/06/12	4989	64	11531	88	1.3	0.8	1.8	0
12/06/12	4587	65	9843	65	1.4	0.7	1.4	0
13.06.2912	3994	46	8841	66	1.2	0.7	1.7	0
14.06.2012	4279	62	9710	52	1.4	0.5	1.2	0
15.06.2012	4282	60	4469	73	1.4	1.6	1.7	0
Mean					**1.63**	**1.06**	**2.22**	

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 Ig-M ELISA
25.5.2012	5984	93	20593	93	1.6	0.5	1.6	0
26.05.2012	5409	41	20900	41	0.8	0.2	0.8	0
27.05.2012	60	2	136	2	3.3	1.5	0	0
28.05.2012	5589	27	26498	27	0.5	0.1	0.5	0
29.05.2012	6240	108	27215	108	1.7	0.4	1.7	0
30.05.2012	5411	21	22569	21	0.4	0.1	0.4	0
31.05.2012	5911	15	23579	17	0.3	0.1	0.3	0
01/06/12	5139	12	20381	15	0.2	0.1	0.3	0
02/06/12	4626	21	19758	25	0.5	0.1	0.5	0
03/06/12	3094	10	11145	12	0.3	0.1	0.4	0

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 Ig-M ELISA
04/06/12	5314	13	23097	13	0.2	0.1	0.2	0
05/06/12	5312	9	22613	10	0.2	0.0	0.2	0
06/06/12	5524	3	20880	3	0.1	0.0	0.1	0
07/06/12	5569	11	17548	13	0.2	0.1	0.2	0
08/06/12	5073	12	19772	12	0.2	0.1	0.2	0
09/06/12	4310	9	15811	10	0.2	0.1	0.2	0
10/06/12	2947	2	10433	2	0.1	0.0	0.1	0
11/06/12	5577	8	20929	8	0.1	0.0	0.1	0
12/06/12	5546	8	22586	8	0.1	0.0	0.1	0
13.06.2012	5381	9	21861	9	0.2	0.0	0.2	0
14.06.2012	5233	10	21395	11	0.2	0.1	0.2	0
15.06.2012	4897	8	19089	8	0.2	0.0	0.2	0
16.06.2012	5215	7	2066	7	0.1	0.3	0.1	0

Summary of the Study

- 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.

Further research Avenues from this study

- 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 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 flavi viruses during outbreaks, it is confirmed that this may give a new dimension to dengue diagnosis.

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