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Study on Vector Dynamics of Zika Virus Outbreak in Thiruvananthapuram, Kerala, India

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A B S T R A C T

The first laboratory confirmed case in Kerala was reported in Thiruvananthapuram, on 8th July 2021. Since then, 83 ZIKV positive cases have been reported from Kerala, with majority of cases from Thiruvananthapuram Municipal Corporation (TMC) area. Entomological surveillance was carried out in 19 wards of TMC including 9 micro containment wards. Three species of *Aedes* (*Stegomyia*) mosquitoes-*Ae.aegypti*, *Ae.albopictus* and *Ae.vittatus* could be collected from the survey areas and among this, *Ae.albopictus* was the predominant species. The *Aedes* larval indices such as House index, Container index and Breteau index were found above the critical level in all the surveyed areas. Analysis on immature *Aedes* output in different container types indicated flooded cement floors/water stagnated areas in newly constructing sites had the highest container productivity (43.08) followed by discarded tires (15.68) and plastic containers (11.62). The highest container/breeding site efficiency was noted in the same flooded cement floors (4.0) followed by grinding stones/cement tanks/cement pits (2.97) and in discarded tires (1.92). ZIKV could be detected from *Ae.aegypti*, *Ae.albopictus* and *Ae.vittatus* mosquitoes. The detection of ZIKV from *Ae.albopictus* and *Ae.vittatus* in the present study is the first report from India. Transovarial transmission could be noted in this outbreak investigation. The outbreak of ZIKV in the state capital causes a serious public health concern all over the state. The right approach will certainly rely on intensive source reduction activities and implementation of both traditional and newer vector control interventions with the active participation of the community.

Introduction

Zika virus (ZIKV) is one of the emerging arboviral diseases in India, which is transmitted mainly by *Aedes* mosquitoes (Bhardwaj *et al.,* 2017; Gupta *et al.,* 2019). ZIKV is a single-stranded RNA virus belonging to the family *Flaviviridae* and genus *Flavivirus*, and is closely related to human pathogenic viruses such as Dengue, Chikungunya, Yellow fever, Japanese encephalitis and West Nile viruses (Gubler, 1991). This virus was first identified in a rhesus monkey that was kept as a sentinel animal as a part of a yellow fever study in April 1947. The study was done in the Zika forest located in Entebbe town in Uganda and hence the name Zika virus (Dick, 1952). Subsequent studies carried out in the same forest in the same period showed that *Aedes africanus* mosquitoes carried the ZIKV (Dick *et al.,* 1952).

The first confirmed case of ZIKV was reported in 1954 during the investigation of a jaundice outbreak believed to be caused by yellow fever in Eastern Nigeria (MacNamura, 1954). Since then sporadic cases of ZIKV infections were reported from numerous African countries during 1964-1970 (Moore *et al.,* 1975). A special ZIKV study was carried out during 1970-1975 in Oyo State of Southwestern Nigeria, where patients showed the classical symptoms of the disease such as febrile illness, headache and malaise (Fagbami, 1979).The serological evidence of ZIKV was also reported during this period from various countries in Asia (Marchette *et al.,* 1969; Olsonetal 1981; Olson *et al.,* 1983).

Zika virus was isolated in Africa from *Ae.africanus* mosquitoes collected from Zika forest, Bwamba County, Uganda in 1948, 1958 and 1964 (Dick *et al.,* 1952; Weinbern and Williams, 1958; Haddow *et al.,* 1964). In 1969, ZIKV was detected from *Ae.apicoergenteus* mosquitoes in addition to *Ae. africanus* collected from the Zika forest of Uganda (McCrae and Kriya,1982). The role of *Ae.aegypti* mosquitoes in the transmission of ZIKV in various geographical regions of the world is quite scant. However, during the outbreak study, many investigators showed ZIKV positivity in many field collected mosquitoes, including 20 species from the genus *Aedes* as well assix non-*Aedes* species (*An.coustani*, *An.gambiae*, *Ma.uniformis*, *Cx.perfuscus*, *Eratmapoditesinornatus* and *E.qinquevittatus*) (Faye *et al.,* 2014; Althouse *et al.,* 2015; Diallo *et al.,* 2014). During 1969 ZIKV outbreak in Malaysia, though many pools containing thousands of *Aedes* mosquitoes had been tested, only a single pool of *Ae.aegypti* tested positive for the virus (Marchettee *et al.,* 1969). This was the first isolation of ZIKV outside Africa. During the Zika outbreak in Gabon, the investigators made an attempt to isolate the virus from field collected mosquitoes such as *Ae.aegypti*, *Ae.albopictus*, *Ae.simpsoni* complex, *Cx. quinquefasciatus*, *An.gambiae*, *Ma.africana*, *Ma.uniformis* and *E.quinquevittatus*. However ZIKV positivity could be seenonly in two *Ae.albopictus* pools (Grard *et al.,* 2014).

The first documented ZIKV outbreak occurred in Yap Island in the Federated States of Micronesia in 2007 and this was the first major outbreak identified outside Africa and Asia (Lanciotti *et al.,* 2008; Duffy *et al.,* 2009).This outbreak was followed by outbreaks of ZIKV in French Polynesia during 2013-2014 (Cao-Lormeau *et al.,* 2014). From December 2014 to January 2016, ZIKV disease spread to entire Brazil (Cardoso *et al.,* 2015).

Entomological survey that followed ZIKV outbreak in Yap islands indicated that the most abundant mosquito collected from the field was *Ae.hensilli* followed by *Cx.quinquefasciatus*. None of these mosquitoes were found positive for ZIKV. However, *Ae.hensilli* was experimentally

infected with ZIKV (African lineage) and thus it was assumed that this mosquito might have played a role in the spread of ZIKV in Yap Island (Lenderman *et al.,* 2014).

The earliest indication of ZIKV transmission in India was established in 1954 by the then Virus Research Centre (now ICMR-National Institute of Virology (NIV), Pune) during its active surveillance to identify the viruses of public health importance in India. During the study, ZIKV positivity was seen in Gujarat and Maharashtra (Smithburn *et al.,* 1952). After this prefatory report, no subsequent reports on ZIKV came from India as it was not recognized as a public health threat. Considering the transmission dynamics of this disease, WHO declared ZIKV infection as a Public Health Emergency of International Concern (PHEIC) on 1st February 2016 (Heyman *et al.,* 2016). Meanwhile, WHO advised all countries to initiate surveillance for ZIKV to perceive the extent of disease prevalence throughout the world. Following this, India initiated surveillance for Zika in March 2016.

In India, the first case of Zika was detected by the Virus Research Diagnostic Laboratory (VRDL) at Ahmadabad, Gujarat in November 2016, and subsequently confirmed at NIV, Pune. Three more ZIKV confirmed cases were reported from Ahmadabad, Gujarat state (Sapkal *et al.,* 2018). In July 2017, a 28 year old resident of Krishnagiri district of Tamil Nadu was tested positive for ZIKV and subsequently confirmed by NIV, Pune. This was the first confirmed ZIKV case in Tamil Nadu. About 18,000 mosquitoes were tested for virus detection. But none of them found positive for Zika virus (Bhardwaj *et al.,* 2017; Gupta *et al.,* 2019). ZIKV could not be isolated from mosquitoes during outbreak investigation in Tamil Nadu. ZIKV outbreak was reported in Jaipur, Rajasthan during September-October 2018 and in Madhya Pradesh during October-November 2021. The investigators could detect ZIKV in three pools of *Ae.aegypti*, out of a total of 79 pools with 383 mosquitoes through RT-PCR and further confirmed by DNA sequencing. This was the first report of ZIKV detection in *Ae.aegypti* from India (Singh *et al.,* 2019).

In Kerala, the first confirmed ZIKV case was reported in a 24 year old pregnant woman admitted in a multispecialty hospital in Thiruvananthapuram city on 8th July 2021. Further, as early as mid of May 2021, as many as 14 staff of this hospital had been presented with fever and rash. Nineteen samples collected from the staff of the hospital had been tested negative for measles, rubella, dengue, chikungunya at NIV, Pune when test for Zika has not been conducted. However, after the confirmation of first ZIKV case, the aforesaid 19 archived samples were retrieved and tested for ZIKV, of which 14 were found positive. Nonetheless, on 15th May 2021, two staff of the same hospital, a 30 year old female and 40 year old female, had similar presentations (rash, fever, conjunctivitis) and confirmed for Zika positive along with the results of 8th July 2021from NIV, Pune. Since then, a total of 83 Zika positive cases have been reported from Kerala. Zika virus is transmitted either through different species of mosquitoes or through sexual route.

Consequently, vector incrimination study forms quite crucial in ZIKV transmission dynamics (Gregory *et al.,* 2017). Hence extensive vector surveillance has been carried out in the disease affected areas in Thiruvananthapuram district. Present study corresponds to an interim entomological investigation including virus detection carried out on the field collected mosquitoes.

Materials and Methods

Study area

Thiruvananthapuram Corporation is consisted

of 100 Wards of which 19 Wards were selected for the present study. The district gets an average of 1835 mm of rainfall per year or 152.9mm per month. The population density is high; with 5400 persons/Sq. Km. *Aedes* mosquito abundance and tropical climate are favorable conditions for the outbreak of *Aedes*-borne diseases in the urban area.

For the control of vector-borne diseases, vector surveillance is an essential tool to assess entomological data required for the implementation of appropriate vector control strategy. Hence it has been planned to carry out a detailed investigation covering entomology, ecology of disease vectors, vector incrimination and plan of action for disease prevention.

Entomological survey

An entomological survey for ZIKV was carried out to detect immature stages of *Aedes* mosquitoes from 8.7.2021 to 1.9.2021.All the accessible water holding containers/habitats in and around the house/premise were searched for the presence of larvae/pupae of mosquitoes and recorded.

This was done mainly to identify the most productive and efficient container types of vector mosquitoes. Larvae/pupae from each container were collected separately. The immature stages of mosquitoes from small containers (less than 10L capacity) were collected using appropriate Steiner. Larvae and pupae from large containers were collected using modified dipper and from flooded floor, roof gutters, etc. were collected using ladle or scoop. The larvae/pupae collected from each container/source were kept in separate vials labeled with date of collection, name of the locality, house number and breeding source (container type/habitat). The breeding habitats of the collected mosquitoes were entered in a pre-designed survey form. The immature kept in separate vials were placed in rearing jars filled with 150 ml fresh water and were covered with fine piece of mosquito net. The larvae were fed with larval diet (prepared with 12.5 gm of tuna meal,9 gm of bovine liver powder and 3.5 gm of yeast, in 100 ml of distilled water). House/Premise index (HI/PI), Container index (CI), Breteau index (BI), and pupal index (PI) of each area were calculated to know about the transmission potential of Zika infection in Kerala.

Selection of localities for vector surveillance

The staff of the multispecialty hospital, whose samples turned positive for ZIKV, were mostly the residents of different wards of Thiruvananthapuram city Corporation viz, Anamugham (Ward 95), Kadakampally (Ward 92), Nanthancode (Ward 25), Vallakkadavu (Ward 88), Medical College (Ward 16), Pangappara (Ward 4), Attukal (Ward 70). The preliminary investigation evidenced that the epicenter of disease outbreak is Anamugham where the hospital is located. A cluster of cases could be seen in a zone comprising the wards- Anamugham, Kadakampally, Kannanmoola, Medical College and part of Sreekandeswaram. From this zone the disease is spread to neighboring wards. Hence entomological surveillance was done in 19 wards of Thiruvananthapuram Corporation area including the micro containment wards (Map-1). The residential, commercial and public place premises were surveyed in randomly selected areas to assess vector breeding habitats.

Categorization of containers noted as larval breeding sites

For the convenience of analysis and exposition, the containers were divided into 14 categories. 1) Plastic containers (broken trays, buckets, mugs, cups, chairs, bottles, etc.) 2) Plant pot trays (Plastic) 3) Tarpaulin sheets 4) Metal containers (paint tin, steel & aluminum

utensils) 5) Glass bottles (beer bottles, soda bottles, normal bottles) 6) Money plants of species *Scindapsus aureus* and *Epipremnumaureum, the* Devil's Ivy plant (family Araceae), etc. kept in glass vases 7) Discarded tires 8) flooded cement floors (newly constructing buildings 9) coconut shells 10) Drums/barrels/synthetic tanks 11) Earthen 12) Grinding stones/cement tanks/ cement pits 13) Tree holes/stumps/ leaf axils 14) Clay wares (broken wash basins, closets, flush tanks, etc.). The role of these different container types in facilitating the production of vector mosquito was estimated by calculating Container productivity (CP) and Container efficiency (CE) (Hammond *et al.*, 2007).

Identification of mosquitoes

The field collected larvae and pupae, kept in separate vials with requisite details, were brought to the laboratory of State Entomology Unit (SEU) attached within the office of Directorate of Health Services (DHS), Thiruvananthapuram. About ten percentages of the larvae were identified using microscopic examination. Pupae and remaining larvae were reared in separate rearing cages and the emerged mosquitoes were identified using standard key (Rueda, 2004; WHO, 2020).

Detection of ZIKV from mosquitoes

The field collected adult mosquitoes/adult mosquitoes emerged from immature were identified. The identified male and female mosquitoes kept in separate pools. The mosquitoes in different pools with details such as place of collection, male/female mosquito species, number of mosquitoes in each pool, etc. were sent to Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram for virus detection using RT-PCR/virus isolation protocols.

Results and Discussion

During the Zika outbreak, vector surveillance was done in 19 Wards of Thiruvananthapuram Corporation area including 9 micro containment Wards. A total of 794 building/premises (residential, commercial and public place) were randomly checked for *Aedes* breeding, of which breeding could be detected in 217 houses/premises (HI/PI-27.33%). In all these edifices, 1571 water holding containers were checked, out of which 443 (CI- 28.19%) were found positive for *Aedes* mosquito breeding (indoor and outdoor). Thus the overall Breteau index (BI) has been 55.79. A total of 9380 immature *Aedes* could be collected during this survey, of which 8549 (91.14%) were in various stages of larvae and the remaining 831 (8.86%) were pupae. Among the 19 survey areas, the lowest HI (11.5%) was noted in Karamana (Ward no. 45) and the highest (62.7%) in Anamugham (Ward no. 95). Similarly, the lowest CI (7%) was noted in Akkulam (Ward no. 96) and highest CI was in Anamugham Ward (61.7%). The lowest and highest BI in the present survey was detected in Medical College Ward (19.4) and Anamugham Ward (183.3) respectively (Table 1)

The HI has been generally used for observing infestation levels of *Aedes* mosquitoes, but it neither considers the number of positive containers nor the productivity of those containers. Furthermore, the CI only brings forth information on the proportion of water-holding containers that were infested with *Aedes* immature. On the other hand, BI signifies a relationship between *Aedes* positive containers and the houses infested and is considered to be the most useful single index for estimating *Aedes* density in a locality. The HI and BI are commonly used for the determination of risk areas for the exertion of appropriate vector control interventions.

The rate of contribution of newly emerged adult *Aedes* mosquito population from different containers/ breeding sites can vary widely. The approximation of relative *Aedes* adult mosquito production may be based on pupal counts (WHO, 1995) and the designated index is Pupal index (PI). Generally, a HI greater than 5% and or, a BI greater than 20 for any locality is an evidence to ascribe the area is dengue sensitive. Earlier, *Aedes* larval indices were advanced to avert outbreaks of yellow fever (YF). The same criteria can be made use for the initiation of preventive measures against the vectors of Zika virus infection.

The entomological survey, during the outbreak of ZIKV in Thiruvananthapuram showed that in all the 19 wards of the disease affected areas, the HI and BI were found above the critical level (Fig 1). Moreover, the PI was also high in Sreekanteswaram Ward (318.8%) followed by Akkulam (306.5%), Anamugham (238.2) and Karikkakam (194) Wards. While studying the prevalence of *Aedes* mosquitoes during the first outbreak of Zika in Jaipur city, the investigators reported that all the larval indices were found above the critical level (Singh and Singh, 2019). The interrelation between high level of HI and ZIKV infection was reported recently from Ahmedabad, Gujarat (Kumavat *et al.,* 2019).

The confirmation of thirteen positive cases from a multispecialty hospital located in Anamugham (Ward no.95) of Thiruvananthapuram Corporation area and the confirmation of ZIKV infection in a pregnant woman who was treated in the same hospital tends to presume that the recent ZIKV infection is in one way or other related to the hospital premise or the epicenter of the disease is around the hospital demesne. All the *Aedes* larval indices such as House index, Container index, Breteau index and Pupal index in Anamugham were found above the critical level (Table 1, Fig1&2) during the survey carried out in this area from 9th to 14th July 2021.The highest positive containers were recorded in Anamugham (61.7%) followed by Nanthancode (57.1%), Medical college (35.1%) and Ulloor (31.7%) Wards (Fig. 3). This substantiate that the focal area of the ongoing Zika outbreak is Anamugham Ward, especially the locality around the multispecialty hospital.

Of the total 443 *Aedes* breeding habitats, flooded cement floors especially of the newly constructing buildings constitute 32.05% followed by plastic containers (trays, buckets, mugs, cups, and bottles) including plant pot trays (25.3%), discarded tires (9.71%), money plant glass pots (6.77%), metal (tin can, steel and aluminum utensils, paint tin) containers (6.09%) and the details of which is given in Table 2.

The existence of immature *Aedes* in a container depends on many factors and further the abundance of larvae and pupae in each container type assumes significance in entomological investigations of any vector-borne disease outbreak. The key containers are identified by establishing the relative contribution that a specific container/ breeding site contributes to the gross production of *Aedes* immature, especially pupa that are frequently called the most productive containers (WHO, 2011). In the present study, it has been noted that the distribution pattern of *Aedes* larvae and pupae by container/ breeding sites are similar. According to the immature *Aedes* output in different container types, flooded cement floors/water stagnated areas in newly constructing sites had the highest container productivity (43.08), followed by discarded tires (15.68), and plastic containers (11.62). Similarly, the highest container/breeding site efficiency was noted in the same flooded cement floors (4.0) followed by grinding stones/cement

tanks/cement pits (2.97) and in discarded tires (1.96) (Table 2). Entomological survey carried out by Rahman *et al.,* (2021) in Bangladesh revealed that plastic containers had the highest container productivity followed by vehicle and machinery items (discarded tires, vehicle parts, etc.). In a study in Dhaka, Bangladesh also observed various types of plastic containers as the most productive breeding sites of *Aedes* mosquitoes (Dhar-Chowdhury *et al.,* 2016). Discarded tires were found as most productive *Ae. aegypti* pupal production sites in Cains, Queensland, Australia (Williams *et al.,*2013). A large number of constructions are progressing around the multispecialty hospital in Anamugham Ward, where more number of Zika cases was initially reported. The flooded cement floors of the newly construction buildings have been identified as one of the major sources of *Aedes* breeding in the present investigation.

A total of 8549 different stages of larvae and 831 pupae were collected from 443 *Aedes* positive containers spread over nineteen localities of Thiruvananthapuram Corporation area. Of the total 8549 larvae, 5809 (67.95%) larvae were of *Ae.albopictus* followed by 1442 (16.87%) larvae belongs to *Ae.aegypti* and the remaining 1298 (15.18%) were of *Ae. vittatus* mosquitoes. Similarly, of the total 831 pupae, 352 (42.36%) were of *Ae.aegypti*. The number of pupae of *Ae.albopictus* and *Ae.vittatus* were 372(44.76%) and 107 (12.88%) respectively. Thus it is evident from the present observation that among the *Aedes* mosquitoes, the predominant mosquito species is *Ae.albopictus* followed by *Ae.aegypti* and *Ae.vittatus*. The study related to the entomological surveillance of vectors of dengue in and around International Airport, Thiruvananthapuram during pre monsoon, Sharma *et.al*. (2004) noted the prevalence of both *Ae.aegypti* and *Ae.albopictis* in 4 wards of Thiruvananthapuram Corporation ie, Chakka, Sankummugham, Valiyathura and Vettukade with BI ranging from 23.5% to 51.9%.The prevalence of *Ae.aegypti*, *Ae.albopictus* and *Ae. vittatus* mosquitoes and anthropophilc behavior of *Ae.albopictus* were studied in Thiruvananthapuram by Samuel *et al.,* 2014 & 2016. The prevalence of dengue vector mosquitoes in different localities of Thiruvananthapuram was also reported by Vijayakumar *et al.,* 2014; Anish *et al.,* 2011; Pradeepkumar *et al.,* 2019). While studying the distribution of vectors of dengue in different zones of Thiruvananthapuram district, Sunil Kumar *et al.,* (2018) noted that *Ae. aegpyti* was the most predominant vector inthe coastal zone; whereas *Ae.albopictus* was the dominant species in hilly and sub urban zones and equal distribution of both the species in urban area. However, the present study indicated that *Ae.albopictus* has been the predominant species among the *Aedes* mosquitoes in the survey area. Among the 443 *Aedes* positive containers, *Ae.albopictus* breeding could be noted in 200 (45.15%) containers, *Ae. aegypti* in 71 (16.03%) and *Ae.vittatus* in 10 (2.26%) habitats. Moreover, *Ae.albopictus* co-existed with *Ae.vittatus* was noted in 135(30.47%), whereas co-existence of *Ae.aegypti* and *Ae.albopictus* was found only in 25 (5.64%) containers/habitats.

Among the individual containers, flooded cement floors were found to be most productive habitat (84.1%) for *Aedes* larval breeding, followed by grinding stones/cement tanks/cement pits (45.5%), Tarpaulin sheet (35.6%), and discarded tires (34.1%) (Fig. 4).

Table.1 *Aedes* larval indices in Zika affected areas in Thiruvananthapuram Corporation area.

Localities/Divisions searched	Houses/ Premises visited	Houses/ Premises positive	Containers searched	Containers positive	HI (%)	CI (%)	BI
Nanthancode / 25	63	14	28	16	22.2	57.1	25.4
Anamugham/95	102	64	303	187	62.7	61.7	183.3
Kadakampally/92	36	07	49	14	19.4	28.6	38.8
Kunnukuzhi/17	40	13	67	20	32.5	29.9	50.0
Pettah/93	24	03	34	5	12.5	14.7	20.8
Karikkakom/91	50	16	108	19	32.0	17.6	38.0
Medical College/16	67	09	37	13	13.4	35.1	19.4
Akkulam/96	31	05	128	9	16.1	7.0	29.0
Pattom/17	57	08	115	16	14.0	13.9	28.1
Kumarapuram/16	52	10	112	25	19.2	22.3	48.0
Balaramapuram/19	30	04	72	8	13.3	11.1	26.6
Pangappara/04	30	17	112	33	56.7	29.5	110.0
Kazhakoottom/98	24	05	79	06	20.8	7.6	25.0
Karamana/54	26	03	37	04	11.5	10.8	15.4
Uloor/06	34	08	60	19	23.5	31.7	55.9
Sreekandeswaram/83	32	08	47	10	25.0	21.3	31.3
Vellayambalam	27	07	50	07	25.9	14.0	29.2
Shankhummugham/89	39	07	72	19	17.9	26.4	48.7
Vallakkadavu/88	30	09	61	13	30.0	21.3	43.3
Total	794	217	1571	443	27.3	28.2	55.8

Fig.1

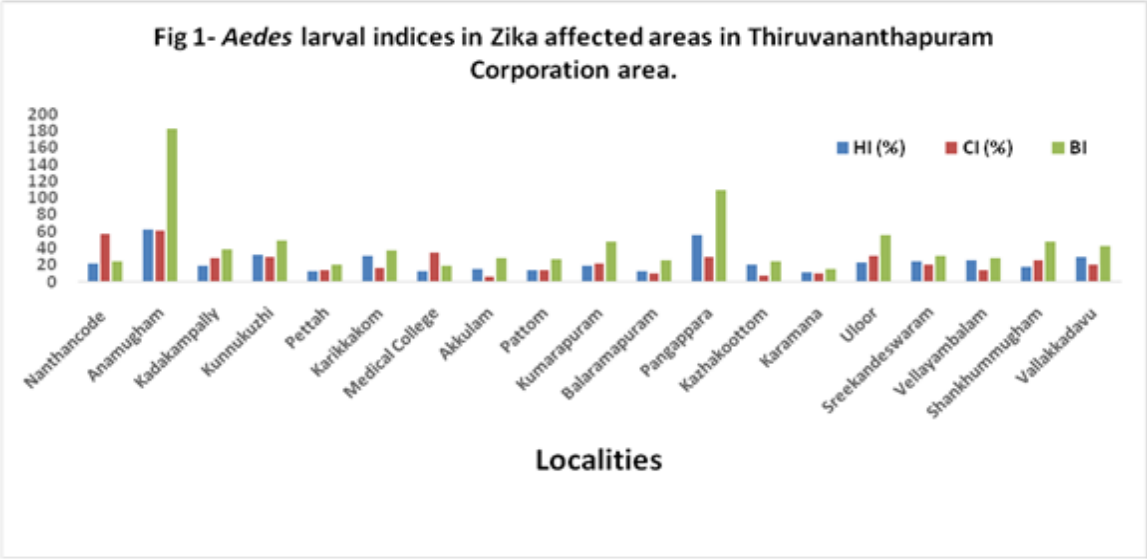


Table.2 The efficiency of different containers types or the production of *Aedes* Larvae

Container types	Frequency (%)	Positive containers (%)	Immature *Aedes*	CP	CE
Plastic trays, plastic buckets, plastic mug, plastic bottles, plastic bag, plastic cups	226 (14.39)	65 (14.67)	1090	11.62	0.81
Plant pot trays (Plastics)	201 (12.79)	47 (10.61)	293	3.12	0.24
Tarpaulin Sheets	59 (3.76)	21 (4.74)	234	2.49	0.66
Metal / tin can, steel and aluminum utensils, paint tin	101 (6.43)	27 (6.09)	257	2.74	0.43
Glass bottles (Beer bottles, soda bottles, glass wares)	251 (15.98)	20 (4.51)	364	3.88	0.24
Money plant pots (Glass)	147 (9.36)	30 (6.77)	308	3.28	0.35
Discarded tires	126 (8.02)	43 (9.71)	1471	15.68	1.96
Flooded cement floors water/ stagnated areas in newly constructing buildings	169 (10.76)	142 (32.05)	4041	43.08	4.0
Coconut shells	86 (5.47)	6 (1.35)	115	1.23	0.22
Drums / Barrels / Synthetic tanks	62 (3.95)	16 (3.61)	404	4.31	1.09
Earthen pot, mud pot, flowers pots /jars	63 (4.01)	6 (1.35)	181	1.93	0.48
Grinding stones / cement tanks/ cement pits	22 (1.40)	10 (2.26)	390	4.16	2.97
Tree holes / tree stumps / leaf axils	30 (1.91)	5 (1.13)	112	1.19	0.62
Clay containers, broken closets wash basin	28 (1.78)	5 (1.13)	120	1.28	0.72

CP – Container productivity: (no. of immature collected from the respective container type X 100 / all immature collected from all types of containers)
CE - Containers efficiency: (Container productivity / prevalence of containers)
Prevalence of containers = no. of water holding containers in the respective container type X 100 / all water holding containers

Fig.2

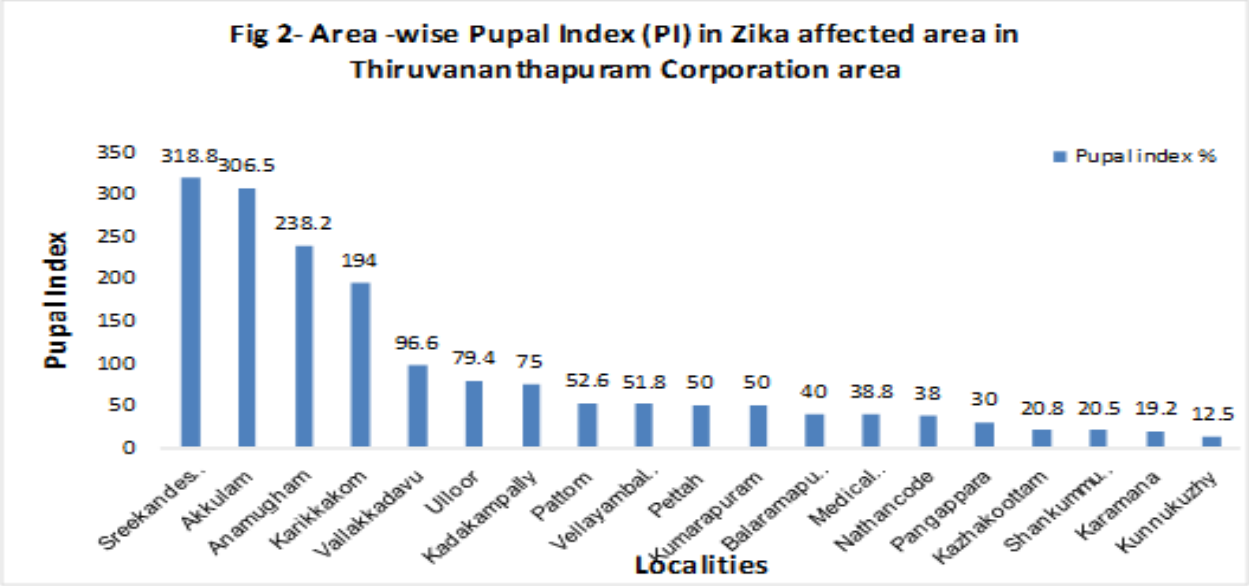


Fig 2- Area -wise Pupal Index (PI) in Zika affected area in Thiruvananthapuram Corporation area
Pupal index %
Pupal Index
Localities
350
300
250
200
150
100
50
0
318.8
306.5
238.2
194
96.6
79.4
75
52.6
51.8
50
50
40
38.8
38
30
20.8
20.5
19.2
12.5
Sreekandes..
Akkulam
Anamugham
Karikkakom
Vallakkadavu
Ulloor
Kadakampally
Pattom
Vellayambal..
Pettah
Kumarapuram
Balaramapu..
Medical..
Nathancode
Pangappara
Kazhakoottam
Shankummu..
Karamana
Kunnukuzhy

Fig.3

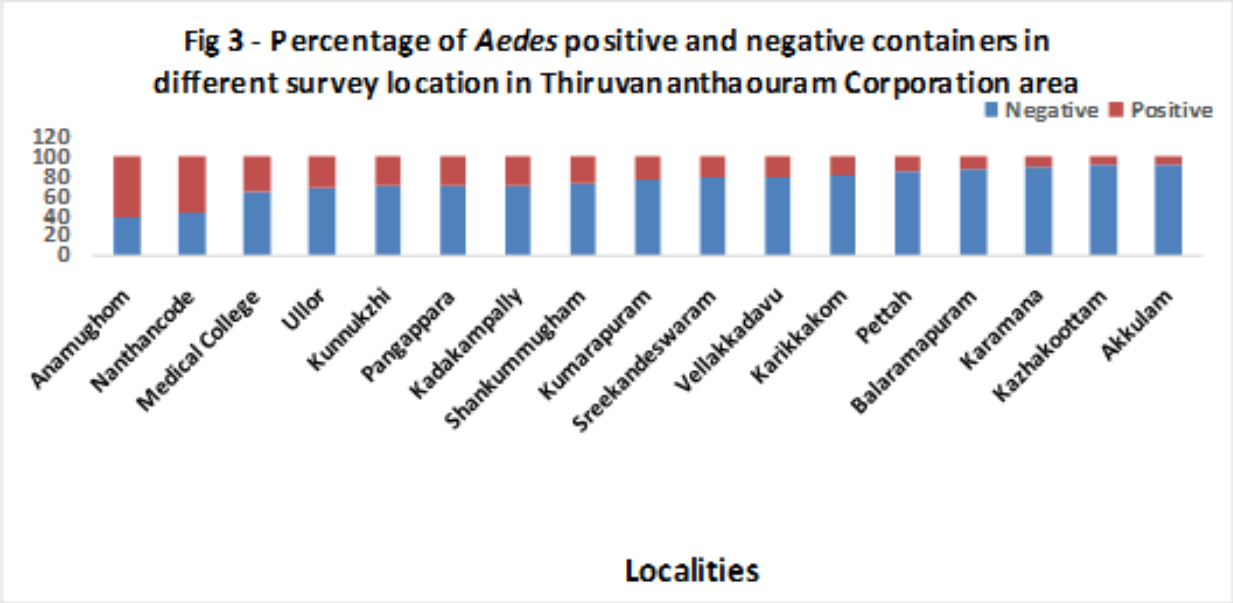


Fig 3 - Percentage of Aedes positive and negative containers in different survey location in Thiruvananthaouram Corporation area
Negative
Positive
120
100
80
60
40
20
0
Anamughom
Nanthancode
Medical College
Ullor
Kunnukzhi
Pangappara
Kadakampally
Shankummugham
Kumarapuram
Sreekandeswaram
Vellakkadavu
Karikkakom
Pettah
Balaramapuram
Karamana
Kazhakoottam
Akkulam
Localities

Fig.4

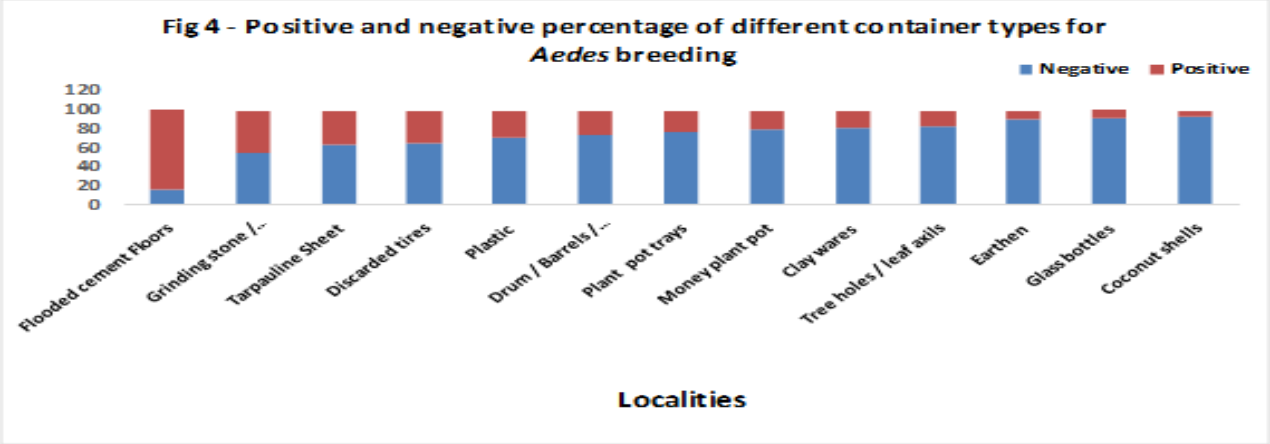


Fig 4 - Positive and negative percentage of different container types for Aedes breeding
Negative
Positive
120
100
80
60
40
20
0
Flooded cement floors
Grinding stone /...
Tarpauline Sheet
Discarded tires
Plastic
Drum / Barrels /...
Plant pot trays
Money plant pot
Clay wares
Tree holes / leaf axils
Earthen
Glass bottles
Coconut shells
Localities

Fig.5A

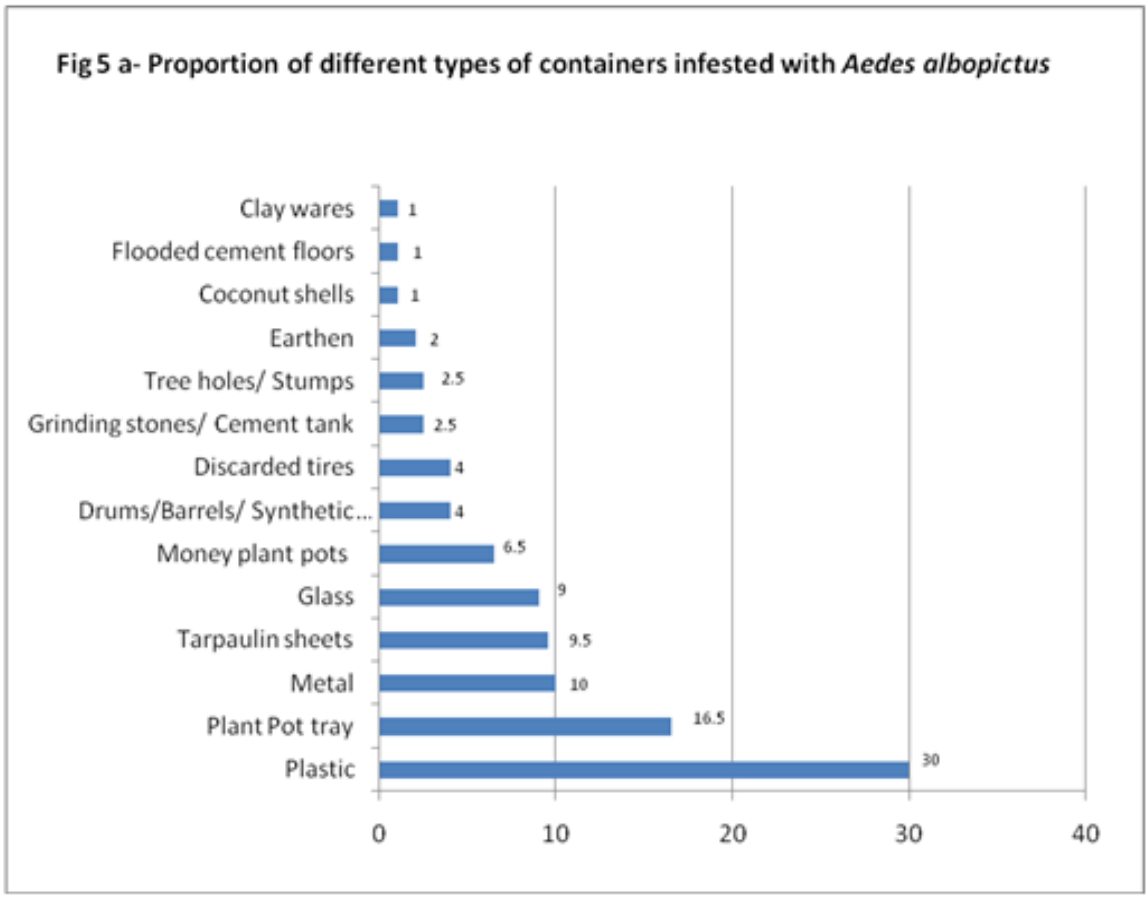


Fig 5 a- Proportion of different types of containers infested with Aedes albopictus
Clay wares
Flooded cement floors
Coconut shells
Earthen
Tree holes/ Stumps
Grinding stones/ Cement tank
Discarded tires
Drums/Barrels/ Synthetic...
Money plant pots
Glass
Tarpaulin sheets
Metal
Plant Pot tray
Plastic
1
1
1
2
2.5
2.5
4
4
6.5
9
9.5
10
16.5
30
0
10
20
30
40

Fig.5B

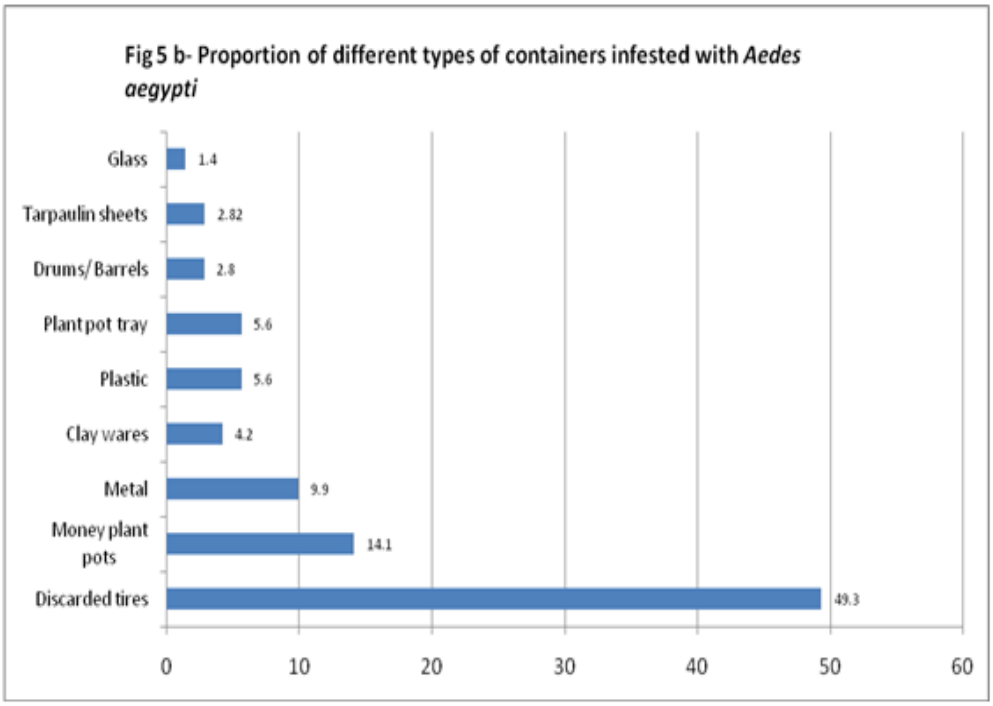


Fig 5 b- Proportion of different types of containers infested with *Aedes aegypti*
Glass
1.4
Tarpaulin sheets
2.82
Drums/Barrels
2.8
Plant pot tray
5.6
Plastic
5.6
Clay wares
4.2
Metal
9.9
Money plant pots
14.1
Discarded tires
49.3
0
10
20
30
40
50
60

Fig.5C

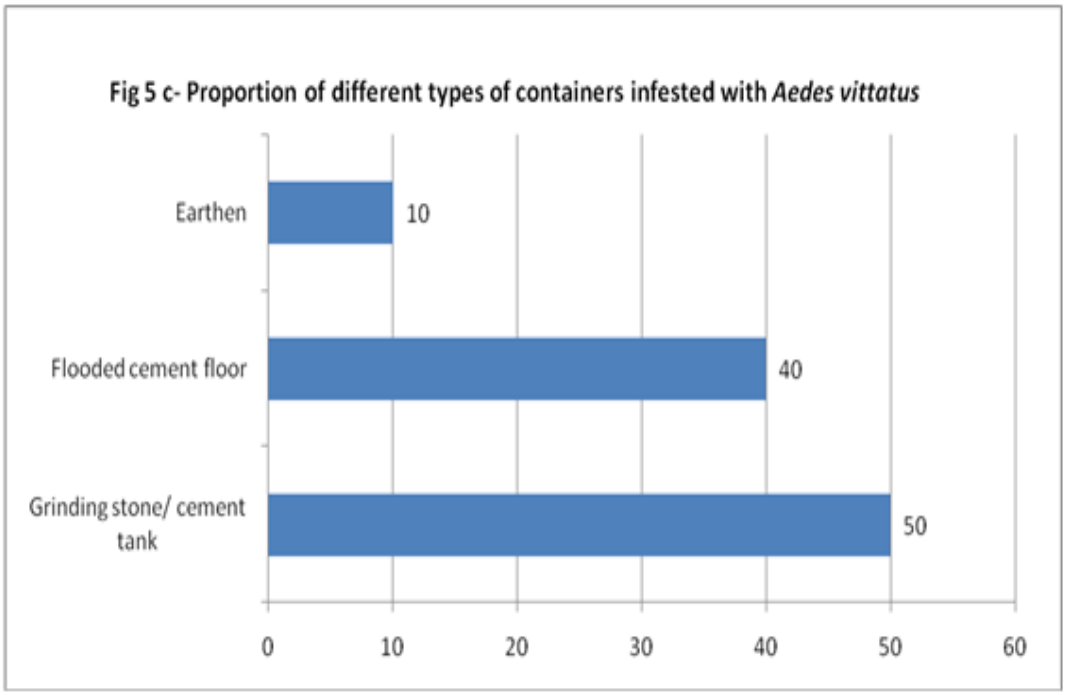
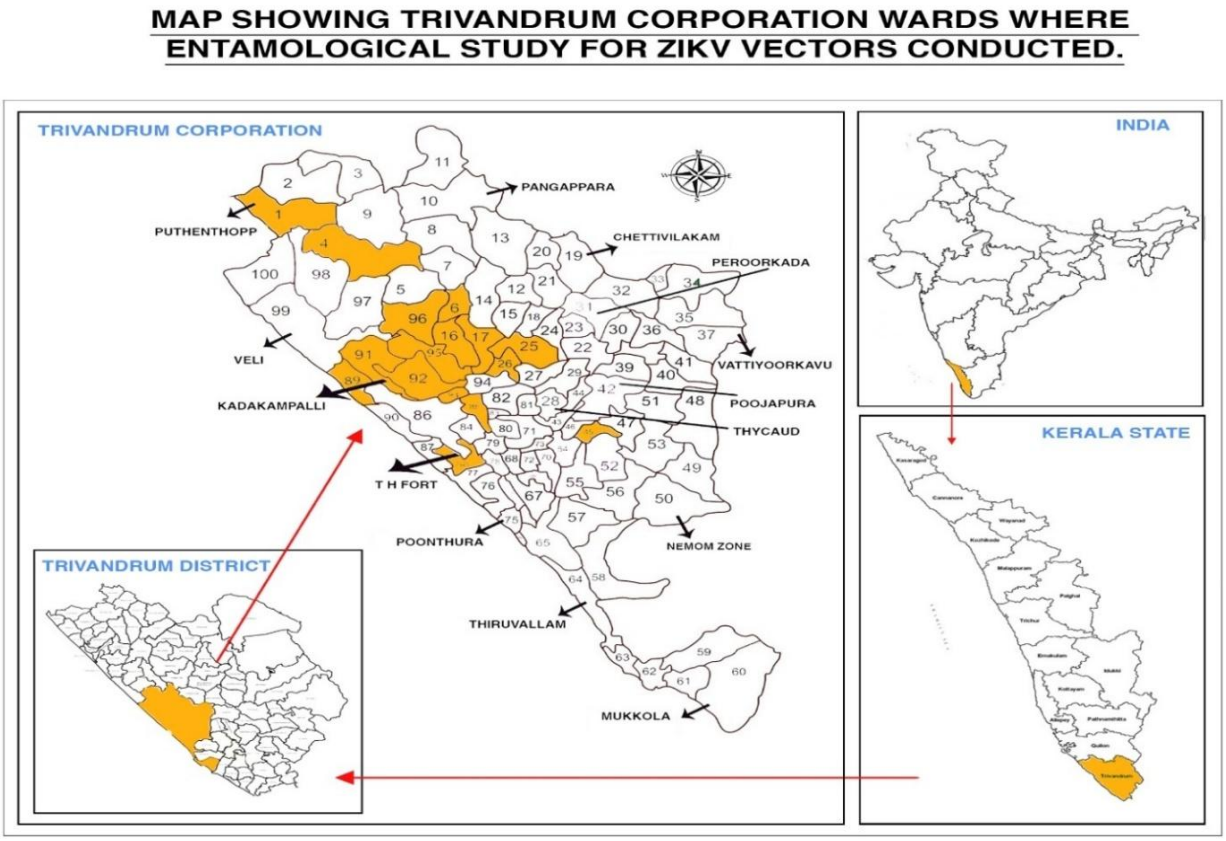


Fig 5 c- Proportion of different types of containers infested with *Aedes vittatus*
Earthen
10
Flooded cement floor
40
Grinding stone/ cement tank
50
0
10
20
30
40
50
60

Map.1



An attempt was also done to find out the proportion of different containers infested with *Ae.albopictus*, *Ae.aegypti* and *Ae.vittatus*. It has been noted that the immature *Ae.albopictus* mosquitoes were found more in plastic containers (30%) followed by plant pot tray (16.5%), metal (10%), tarpaulin sheets (9.5%), glass (6.5%), and money plant pots (6.5%) (Fig. 5a). However *Ae.aegypti* mosquitoes prefer to breed in discarded tires (49.3%) followed by money plant pots (14.1%), and metal (9.9%) containers (Fig 5b). In this area, a prevailing local belief is that if money plants are grown in houses, one would be never be in shortage of money and hence it has got the name money plants. Due to Covid-19 pandemic and subsequent lock down, people got enough time to grow such money plants in and around their houses. This is one of the important habitat for *Aedes* mosquitoes preferably *Ae.aegypti* mosquitoes. *Ae.vittatus* mosquitoes prefer to breed in grinding stones/cement tanks/cement pits (50%) followed by flooded cement floors (newly constructing buildings) (40%) and earthen (10%) containers (Fig 5c). The study on *Aedes* (*Stegomyia*) mosquitoes in Thiruvananthapuram by Vijayakumar *et al.*, (2014) reported that tires were the most preferred breeding sites of *Aedes* mosquitoes.

Detection of ZIKV from mosquitoes

As a part of ZIKV outbreak investigation, all the mosquitoes collected from the disease affected areas of Thiruvananthapuram were processed for PCR analysis to detect the virus. The results are given in Table 3 & 3a. It has

been noted that both male and female *Ae.aegypti*(pool consisting of 3 males and 3 females) mosquitoes collected from Anamugham(ward no.95) showed ZIKV positivity. Moreover, one pool (3 males and 5 females) of *Ae.albopictus* mosquitoes collected from Nandancode (ward no.25) and one pool (7 males) of *Ae.vittatus* were also showed the presence of ZIKV.

The detection of ZIKV from all the three *Aedes* mosquitoes viz, *Ae.aegypti*, *Ae.albopictus* and *Ae.vittatus* during the ZIKV outbreak investigation in Thiruvananthapuram, Kerala were reported earlier (Sasi *et al.*, 2021). The detection of ZIKV from *Ae.albopictus* and *Ae.vittatus* mosquitoes is the first report from India. However, ZIKV was confirmed to be present in the indigenous *Ae.aegypti* by PCR assay during the Zika outbreak in Rajasthan (Singh *et.al*.2019). This was the first report of the detection of ZIKV from *Ae.aegypti* mosquitoes in India. ZIKV infection in male specimens of *Ae.aegypti*, *Ae.albopictus* and *Ae.vittatus* indicate the transovarial transmission of the virus as reported by Lai *et al.*, (2020).

An attempt was also made to detect ZIKV from non-aedine mosquitoes collected from the disease affected areas such as *Ma.uniformis*, *Cx.tritaeniorhynchus*, *Cx.gelidus* mosquitoes, but none of them were found positive for ZIKV. It is interesting to note that ZIKV was isolated from *Ma.uniformis*, *Cx.perfuscus* and *An.coustani* mosquitoes in 2011 from South-eastern Nigeria (Diallo *et al.*, 2014).

Various outbreak investigation reports indicate that 15 species of *Aedes*, 3 species of *Anopheles*, 2 species of *Culex* and one species of *Mansonia* are responsible for spreading ZIKV through both the Asian and African lineages (Bhattacharya *et al.*, 2019). The present vector surveillance was done according to existing guidelines (NVBDCP, GOI, 2016). However, the integrated remote sensing and GIS for mapping of potential vector breeding habitats, and the internet GIS surveillance for epidemic transmission control, and management is under consideration as part of the forthcoming surveillance activities, especially during outbreak investigations of vector-borne diseases. This is intended to be done with the approval from the concerned authorities.

ZIKV infection is an emerging public health threat in India. Though, there are major issues regarding differential diagnosis and strain in managing ZIKV outbreak, the emergency response of the public health, strong health system, and perusal of global norms would definitely help in fighting and restraining such outbreaks. It is essential to continue human, veterinary, entomological and environmental surveillance to ascertain the prevalence and geographical distribution of virus, host and vector range and also the transmission potential of circulating virus strain. Vector control efforts mainly focusing on source reduction activities with the active participation of the community should be continued to prevent future outbreaks. If we fail to control the vectors effectively, in the forthcoming monsoon, ZIKV infection will definitely become a major public health issue in Kerala. Hence there is a need for continued efforts towards vector surveillance and vector management to prevent further large scale outbreaks in Kerala.

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