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Assessment of dengue mosquito breeding sources at Pulicat, Tamil Nadu, India

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Abstract
Dengue Fever (DF) is a viral disease transmitted by female adult mosquitoes, *Aedes aegypti* and *Aedes albopictus*. Until a vaccine becomes available for public health use, primary prevention of transmission is crucial to decrease the burden of dengue, and the control of *Aedes* species is the only available stratagem. A cross sectional study was conducted at Pulicat village, Tamil Nadu, India. A survey was conducted in a total of sixty nine houses in order to assess mosquito breeding sources for the presence of dengue vector species. Presence of mosquito breeding was confirmed in 56% of the surveyed houses. Both the vector species for dengue transmission were present, with *Aedes aegypti* as the majority species. This study focused on the prevalence of these vectors in the villages of Pulicat and Vairavan Kuppam as an indicator for the risk of a DF outbreak. However, further studies should consider analyzing other variables that can affect breeding (salinity, disturbance, population density of juveniles), as well as the geographical distribution of houses where breeding is present.

Keywords: Pulicat, dengue, *Aedes aegypti*, breeding

Introduction

Vector-borne diseases are responsible for a large proportion of worldwide human mortality and morbidity [1, 2]. Among these diseases, more than 90% of infection-associated Disability-Adjusted Life Year (DALY) is caused by mosquito agents [3]. *Aedes aegypti* and *Aedes albopictus* female mosquitoes are responsible for transmitting dengue virus to human. *Aedes aegypti* is considered the principal vector for dengue due to its behavioural characteristics, and *Aedes albopictus* as the secondary vector. *Aedes* species are found in tropical and subtropical regions, usually between latitudes 35°N and 35°S [4], and are day biters, anthropophilic, and has evolved in close proximity to humans, developing traits such as oviposition in man-used natural and artificial containers [5]. *Aedes aegypti* breeds and develops in artificial containers of small volume, *viz*., flasks, bottles, flower vases, tin cans, jars, discarded automobile tyres, unused water closets, cisterns, rain barrels, sagging roof gutters; whereas *Aedes albopictus* breeds in coconut shells, snail shells, leaf axils and tree holes [6]. Optimal temperature for development of juvenile *Aedes* species mosquito ranges between 20 and 34°C and eggs can resist drought, remaining viable for several months [4].

Dengue Fever (DF) is an old disease that spread through commerce and shipping during the seventeenth, eighteenth and nineteenth centuries [7]. Due to its fast emergence, DF constitutes one of the main burdens in more than 100 countries worldwide, particularly in tropical and subtropical regions [8]. Frequency rate of dengue has increased dramatically over the years. Worldwide, more than 100 countries with 100 million annual dengue cases approximately have reported dengue as a critical re-emerging arboviral disease [9]. It is estimated that billions of people living in Southeast Asia are at threat of dengue infection and India has been identified as endemic for dengue. Dengue is prevalent and has been known in India since 1945 [10], and has witnessed massive loss of life and property. Delhi, Punjab and Haryana were the highly affected states in the north region. Tamil Nadu, Kerala, Karnataka and Andhra Pradesh were more affected in south region. West Bengal and Orissa in east; Maharashtra, Gujarat, Rajasthan in west; Assam and Arunachal Pradesh in northeast and Madhya Pradesh in central region [11]. In Southern India, dengue cases were reported during 1960's and 1970's [12]. The first evidence of occurrence of dengue fever in Tamil Nadu was reported in 1956. According to Samuel *et al*. [8], dengue have been observed in Tamil Nadu, and cases of DF were reported as early as in 1960 in Vellore. Among the 32 districts of Tamil Nadu, 29 districts were found to be affected with dengue infections, which included outbreaks in Chennai, Dharmapuri, Tiruchirappalli, and Virudhunagar districts [13-17]. In the last five decades, the number of DF and Dengue Hemorrhagic Fever (DHF) cases reported to the World Health Organization (WHO) increased by 30-fold [4], mainly due to uncontrolled urbanization, population growth, frontier agricultural expansion, and an increment in human migrations [18]. Dengue Virus (DENV) can cause a wide range of illness in humans, from unapparent infection, to classic DF and to fatal DHF [7]. Until a vaccine becomes available for public health use, primary prevention of transmission is crucial to decrease the burden of dengue, and control of *Aedes* is the only available strategy [19]. Elimination of the breeding sites of *Aedes aegypti* from the human habitat is the most effective way to manage this vector [20, 21], hence social and behavioural interventions at household level are thought to be the most viable measures for reducing the dengue vector [22]. Dengue vector control is effective in reducing vector populations, particularly when interventions use a community-based, integrated approach, which is tailored to local eco-epidemiological and sociocultural settings and combined with educational programmes to increase knowledge and understanding of best practice [23]. Hence, vector control stratagem still remains the most used and reliable method against mosquito transmitted diseases. Vector control presents several difficulties that range from behavioural aspects of the vector, to the particular environmental conditions of an endemic geographic area, to the economy of the affected human population and its cultural practices. Ideally, a better understanding of both the vector species and the current vector control measures taken in a particular area can help improve protection against possible dengue outbreaks. Therefore, the present observational and cross-sectional study focuses on the assessment of preferential mosquito breeding sources, especially *Aedes* species as a measure for the potential of a dengue outbreak in the area of Pulicat. In assessing the potential for a dengue outbreak, two aspects were taken into consideration: the presence of containers in which mosquitoes can breed and the primary vector mosquito species for dengue present in the study area and also the availability of breeding sites within households which provide mosquitoes with the chance of completing their life cycle in close proximity to humans; thereby having every chance of transmitting dengue.

Materials and Methods

Located at the north of Tamil Nadu and bordering on Andhra Pradesh, the town of Pulicat ('Pazhaverkadu' in Tamil) part of the Thiruvallur district (Figure 1 and 2) was founded in 1609 by the Dutch, and later ceded to the British from 1825 until India became independent [24]. Pulicat is a coastal area, which lies 60km away from Chennai. Pulicat lake, with an average water spread area of 350sq. km. is the second largest lagoon (bay) in India. Pulicat is endowed with diverse natural resources, which includes both aquatic and terrestrial flora and fauna. Fishing is the oldest and most important livelihood option for the inhabitants since time immemorial. Some villages in and around Pulicat are agriculture based. Besides fishing and agricultural labour, people are involved in other livelihood activities (cattle rearing, construction, etc.) [25]. Vairavan Kuppam village is situated in Pulicat at 1.5km southern part of Light house and houses 215 families comprising of fishermen community. Fishing comprises the economic status of the community dwelling in this village [26]. According to the 2011 census, the Pulicat village had a population of 17,925 inhabitants, of which 8,915 are males and 9,010 are females. Literacy rate in the village is 66.8%, almost equally distributed between males and females (52.8% male literacy versus 47.2% female literacy) [24]. Being one among many rural villages in India, Pulicat's economy depends primarily on fisheries, both from its brackish water estuary and from the sea. The presence of mosquito species that can act as vectors for DENV constitutes a risk for a local outbreak of DF.

A survey was developed to assess dengue mosquito breeding sources in households in the area of Pulicat, particularly in the villages of Pulicat and Vairavan Kuppam from July to October, 2015. The survey was performed by selecting houses where freshwater was available. At each house, water sources and containers *viz*., cement cisterns, coconut shells, discarded containers, flower pots, grinding stones, metal containers, metal drums, mud pots, overhead tanks, plastic containers, plastic drums, tyres and wells that were present inside and outside the houses were observed. For each household, the presence or absence of possible breeding sources were recorded, as well as the presence or absence of mosquito larvae and/or pupae. For containers in which there was breeding activity, air and water temperature, as well as water quantity and its intended use were recorded. A total of sixty nine houses were surveyed. Breeding was also observed in open, public areas, like pools of stagnant rain water in the streets, or community wells. For these breeding sites, water and air temperature were recorded. Water samples containing larvae and/or pupae were collected at study sites and transported to the laboratory for adult emergence. Then, species and sex of adult mosquitoes were determined based on morphological features with the help of mosquito identification key [27-29]. Data were processed and graphed. Histograms were used to show the occurrence of breeding indoors and outdoors, as well as the presence or absence of breeding containers in each household. Sex and species of the adult mosquitoes that emerged from the collected larvae and/or pupae were recorded.

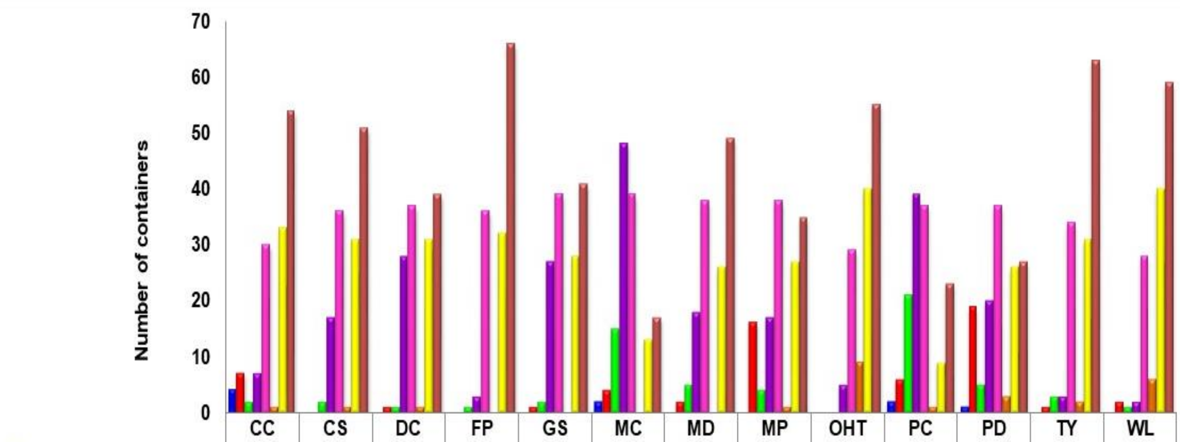
Fig 1: Map from the region of Pulicat lake showing the distribution of main streets in Pulicat (left side of Pulicat bridge) and Vairavan Kuppam (right side of Pulicat bridge) © Google Maps, 2016

Fig 2: Satellite view of Pulicat. Image shows distribution of water masses, households and forest in Pulicat and Vairavan Kuppam © Google Maps, 2015

Results

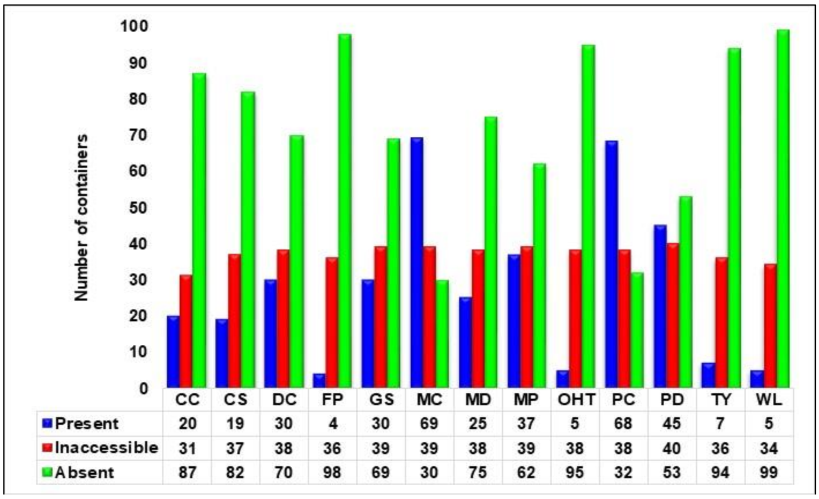
All types of containers were found among the sixty nine surveyed houses. Metal containers, plastic containers and plastic drums were the most often found; whereas flower pots, over-head tanks, and wells were the least frequently observed water storage areas (Figure 3 and 4). However, not all of these sources showed breeding. Mosquito larvae and/or pupae were most often found in plastic drums and mud pots than metal containers and plastic containers which were less frequently used as breeding sites (Figure 5). Among containers in which larvae and/or pupae were present, cement cisterns showed the largest frequency of indoor breeding, whereas outdoor breeding was most frequent in mud pots and plastic drums. For some container types, breeding was only present when the container was found outdoors. This is the case for discarded containers, grinding stones, metal drums, mud pots, tyres and wells (Figure 6). Contrarily, indoor breeding was not exclusive to any container type. Overall, 81.3% of surveyed containers showed no breeding. Of the remaining containers with larvae and/or pupae, only 2.5% of breeding occurred indoors, and 16.2% took place in containers found outdoors (Figure 7).

Average water temperature for containers showing breeding was 31.1±1.9°C, while average air temperature was 31.0±1.6°C. Water volumes varied greatly among the containers in which juvenile mosquitoes were present; from approximately 0.1L in containers that were left outside to hold rain water, to 700L in cement cisterns. Washing and drinking were the most commonly reported uses for water in which breeding was present. Mosquitoes successfully bred in rain water, well water, domestic water and water used for commercial establishments. The total juvenile mosquitoes collected at the study site constituted a population of seventy four individuals, of which, only thirty nine emerged as adults. The population of adult mosquitoes was composed mainly by *Aedes aegypti* species and the male to female ratio was approximately one male for every 1.5 females (Figure 8).



	CC	CS	DC	FP	GS	MC	MD	MP	OHT	PC	PD	TY	WL
Positive breeding I	4	0	0	0	0	2	0	0	0	2	1	0	0
Positive breeding O	7	0	1	0	1	4	2	16	0	6	19	1	2
Negative breeding I	2	2	1	1	2	15	5	4	0	21	5	3	1
Negative breeding O	7	17	28	3	27	48	18	17	5	39	20	3	2
Inaccessible I	30	36	37	36	39	39	38	38	29	37	37	34	28
Inaccessible O	1	1	1	0	0	0	0	1	9	1	3	2	6
Absence of container I	33	31	31	32	28	13	26	27	40	9	26	31	40
Absence of container O	54	51	39	66	41	17	49	35	55	23	27	63	59

Fig 3: Number of larvae and/or pupae present, absent and inaccessible in containers in the 69 surveyed households. Each container type shows the result of indoors (I) and outdoors (O) survey. Container type: CC- cement cistern; CS- coconut shell; DC- discarded container; FP- flower pot; GS- grinding stone; MC- metal container; MD- metal drum; MP- mud pot; OHT- overhead tank; PC- plastic container; PD- plastic drum; TY- tyre; and WL-well



	CC	CS	DC	FP	GS	MC	MD	MP	OHT	PC	PD	TY	WL
Present	20	19	30	4	30	69	25	37	5	68	45	7	5
Inaccessible	31	37	38	36	39	39	38	39	38	38	40	36	34
Absent	87	82	70	98	69	30	75	62	95	32	53	94	99

Fig 4: Number of larvae and/or pupae present, absent and inaccessible in containers in the 69 surveyed households. Each container type shows the combined result of indoors and outdoors survey. Container type: CC- cement cistern; CS- coconut shell; DC- discarded container; FP- flower pot; GS- grinding stone; MC- metal container; MD- metal drum; MP- mud pot; OHT- overhead tank; PC- plastic container; PD- plastic drum; TY- tyre; and WL-well

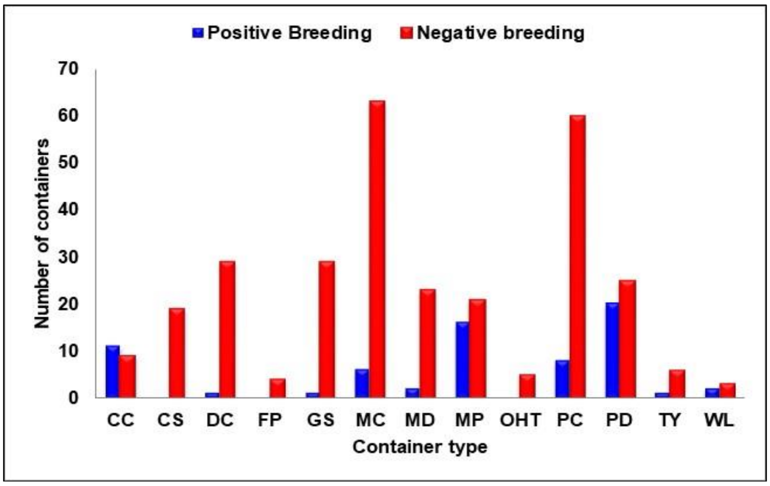


Fig 5: Number of containers in which larvae and/or pupae were present, and number of containers in which breeding was not found (results for the total 69 surveyed households). Container type: CC- cement cistern; CS- coconut shell; DC- discarded container; FP- flower pot; GS- grinding stone; MC- metal container; MD- metal drum; MP- mud pot; OHT- overhead tank; PC- plastic container; PD- plastic drum; TY- tyre; and WL-well

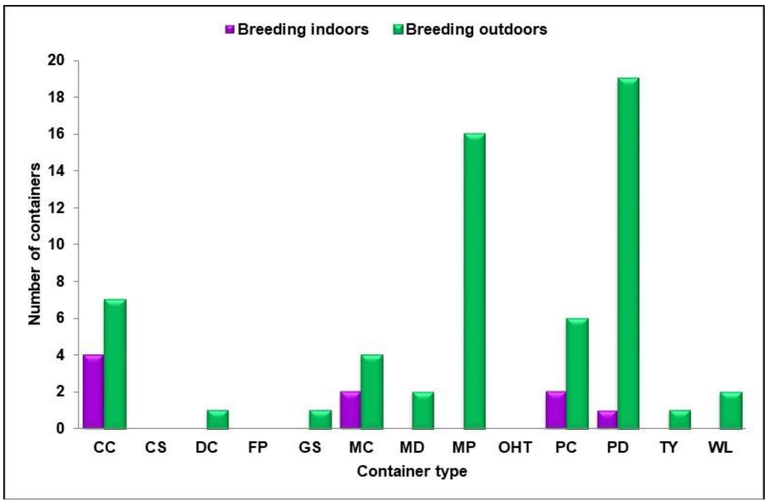


Fig 6: Number of containers showing presence of larvae and/or pupae. Comparison between breeding indoors and outdoors. Container type: CC- cement cistern; CS- coconut shell; DC- discarded container; FP- flower pot; GS- grinding stone; MC- metal container; MD- metal drum; MP- mud pot; OHT- overhead tank; PC- plastic container; PD- plastic drum; TY- tyre; and WL-well

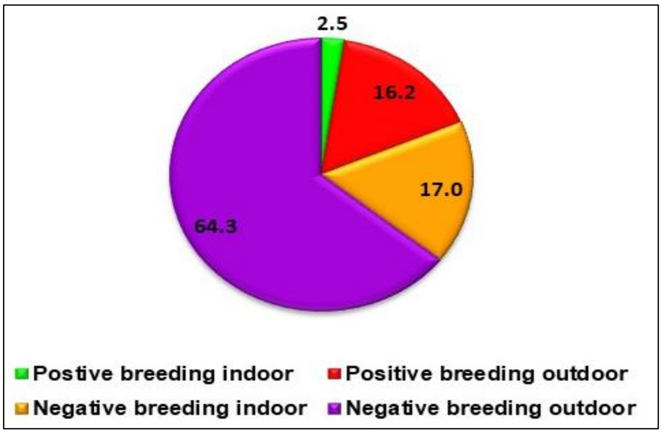


Fig 7: Per cent distribution of breeding in observed water containers, with distinction between indoors and outdoors (total positive = 18.7%). Values correspond to the total number of containers with larval/pupal presence in the 69 households

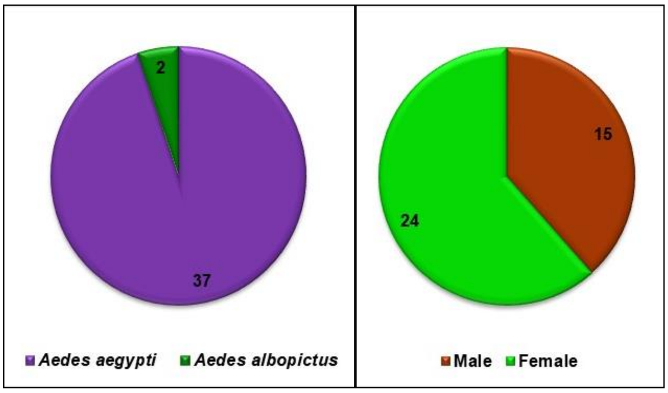


Fig 8: Species and sex composition of sampled adult mosquitoes in the study area

Discussion

Dengue, a fast spreading vector-borne disease is endemic in more than 100 countries with half of world's population living in areas at risk of this disease [30]. Heaviest burden of dengue was reported by Asia Pacific countries including India with 1,800 million people at risk of dengue infection [31]. World Health Organization-South East Asia (WHO-SEA) has placed India in `Category A' in terms of dengue endemicity as being a major public health problem, population growth, unplanned urbanization and poor water management systems leading to frequent water shortages and storage practices which have promoted breeding sites for the *Aedes* mosquito [32] which primarily breeds in domestic water storage containers in and around human dwellings [33].

In the present study, containers that could potentially serve as breeding sites for mosquitoes were present in all of the houses surveyed. Breeding was confirmed in 56% of houses. Nonetheless, some water containers were impossible to observe (either due to inaccessibility of a container or lack of willingness from the household owners); thus the percentage for breeding activity might be higher. Further in the present study, the majority of containers in which breeding was observed was found outside the households. This result seems to suggest that breeding occurs more frequently outdoors than indoors. Frequent outdoors breeding could be explained by several factors: presence of organic substrate in containers found outside, higher cleaning frequency in indoors containers (hence higher disturbance), or more chances for mosquitoes to mate outdoors and quickly start oviposition. Furthermore, breeding was observed most frequently in cement cisterns placed indoors and in mud pots and plastic drums placed outdoors. Among these three container types, mud pots exclusively showed breeding when they were placed outdoors, whereas the other two types of containers showed breeding activity regardless of the location in which they were found. This pattern might point to a difference in the use of mud pots according to their physical location in the household. Although the mechanisms underlying container suitability are beyond the scope of the present study, two distinct components may be involved: (1) the female selection for oviposition, and (2) the impact of the container quality on egg hatching and development of immature mosquitoes [34].

Breeding sites of *Aedes* are closely related to macro and micro ecological factors, and ecological factors relate to climate (rainfall, humidity, temperature etc.) [35, 36]. In the present study, average water and air temperature recorded in the field correspond to the range of optimal temperatures in which juvenile mosquitoes develop. The large variation in the amounts of water that served as breeding sources seems to indicate that water volume is not a limitation for breeding. Water conditions also differed, with juveniles found both in clean water mainly used for drinking and washing purposes and also in stagnant pools. These observations seemed to suggest that population density in stagnant pools was higher than in any of the water containers found in the surveyed households. The causes for a higher density could be related to the existence of organic substrate in stagnation pools that serves the feeding purposes of larvae, but these observations were not numerically confirmed. There were no juvenile mosquitoes in a few pools of brackish water that were observed, which could reveal that salinity was an important limiting factor to oviposition, growth and development of larvae and pupae. However, in the dry season the water holding containers found to support *Aedes* breeding will certainly shoot up [37], because storage of water in different kinds of containers in indoors are practiced.

Sex distribution of adult mosquitoes is important because only adult females can transmit dengue to humans. From the seventy four juvenile mosquitoes that were collected at sites, only thirty nine emerged as adults under laboratory conditions. However, laboratory observations might differ from field observations, which could affect the actual number of mosquitoes that reach adulthood in the field. In the present study, among the thirty nine adults obtained under laboratory conditions, thirty seven were *Aedes aegypti*, and the sex ratio was approximately 1.5 females for every male. Sampling errors collecting larvae and pupae might indicate that the actual per cent distribution of sex and species could be different in the field than what was measured. Nonetheless, results support the existence of the optimal vector species for dengue in the households of Pulicat and Vairavan Kuppam. Furthermore, since a single male mosquito can mate with more than one female, the sex ratio obtained in laboratory observations would support the dynamics of a growing mosquito population.

The occurrence of *Aedes aegypti* in the Andaman archipelago was reported in 1983 [38]. During the past decade, rapid urbanization in Port Blair [39] has led to the proliferation of *Aedes aegypti* breeding sites resulting in an increase in prevalence and high infestation levels. The lack of infrastructure such as piped water and intermittent water supply necessitates storage of water in containers, and inadequate solid waste management has led to an accumulation of discarded containers [20]. *Aedes aegypti* also showed signs of infiltration into rural areas [40] adjoining urban Port Blair. The villages of Pulicat where the present study was performed could be facing a similar situation to that of Andaman islands. Active source reduction involving elimination of all possible breeding grounds for vector mosquitoes has an important role in the control of dengue. Since, *Aedes aegypti* and *Aedes albopictus* develop in small water holding containers, active source reduction is carried out in almost all dengue affected countries to reduce vector populations and consequently disease transmission. However, it can be argued from an adaptive evolutionary point of view that the elimination of natural breeding grounds might have given opportunities to vector populations to exploit other unexploited and perhaps less desirable habitats for survival [41].

The availability of aqueous habitats where mosquitoes oviposit, the eggs hatch and then develop through various larval instars to pupae, strongly influence the density of the resulting adult mosquito populations. Such habitats may have natural or anthropogenic origins. Their productivity are influenced by abiotic (hydrology, temperature, sunlight, pH, chemicals, salinity, nutrient availability, turbidity) and biotic (predation, competition) factors and the interactions between the different factors [42]. *Aedes aegypti* and *Aedes albopictus*, the vectors of dengue, normally lay eggs within 1km from the site of a blood meal. The selection of oviposition sites are influenced by the distance that adult females can fly after a blood meal [43, 44]. Anthropogenic activities can create suitable new habitats within the natural range of the vectors. A combination of the new and pre-existing habitats can then lead to an increase in the vector population and, therefore, disease transmission. Furthermore, anthropogenic activities that eliminate or greatly minimise the preimaginal habitats preferred by vector mosquitoes can be the driving force for their adaptation to less optimal and underutilized habitats within their normal range close to human habitation [41].

Presence of an anthropophilic mosquito species such as *Aedes aegypti* in the region of Pulicat confirms the risk for a dengue outbreak in the present study. Due to the behavioural characteristics of *Aedes aegypti* (flying distance, breeding in close proximity to humans), households in which breeding was not found at the time of the present study could show breeding activity later on. The WHO's dengue guidelines for diagnosis, treatment, prevention, and control state that the average lifetime flying distance of *Aedes aegypti* is typically within 100 meters from the place of emergence from the egg [4]. This infers that, although their range of displacement is limited, female mosquitoes that developed in one household could fly to a neighbouring house to lay their eggs or to feed. Anthropophagic mosquitoes have additionally evolved behavioural traits and ecological preferences that facilitate feeding on humans and have therefore evolved over a long time period to take advantage of increasing human populations, their geographical expansion, societal features like agriculture and urbanization as it was observed in Pulicat. Major disturbances in human ecology and deterioration in the environment and standards of sanitation have helped in the amplification of the vectors of arboviral diseases in urban and rural areas. Urbanization is a continuous process in developing countries like India and this has naturally led to aggregation of population. Further, due to rapid industrialization, large numbers of labourers migrate from rural to urban areas in search of job opportunities. This has resulted in the development of many slums with no proper sanitary and waste water disposal arrangements. Due to unplanned town expansion, the peripheral areas of towns bordering villages have become semi-urbanized and this process continues unchecked. The results are environmental changes including the creation of water bodies highly conducive for the breeding of mosquitoes. The increasing breeding potential for these ubiquitous mosquitoes can thus be attributed to the development process [45,46]. Water scarcity was the main reason for the storing of water in the households which supported the profuse breeding of vector species [47,48]. Urban areas with high-density of water storage receptacles are suitable for breeding of *Aedes* mosquitoes [32]. In most of these areas, small number of *Aedes* breeding habitats exist even during the adverse months of the year and consistently serve as the primary producers of *Aedes aegypti*, referred as 'Key Containers' [49] which are region specific for *Aedes* breeding [50].

Human migration has led to increasing movement of disease vectors including *Aedes* mosquitoes and the pathogens they carry. The transport of mosquitoes beyond their native range, *via* shipping, aircraft and transport has been well documented [51]. As a result of rapid transport of *Aedes* mosquitoes, dengue has become one of the fastest growing mosquito-borne diseases in the world with about twenty thousand deaths every year. Dengue has been rampant in parts of Tamil Nadu in the past two decades. The prevalence of dengue vector and silent circulation of dengue viruses have been detected in rural and urban Tamil Nadu, which is ever increasing [52]. Deforestation resulting from expanding agriculture and housing, logging, etc., contributes in a major way to changing local ecosystems that affect local flora and fauna including disease vectors. Ecological divergence resulting from the deforestation creates novel habitats and ecological heterogeneities that provide new adaptive opportunities to mosquito vectors that are in turn capable of influencing disease transmission [42].

In managing an effective dengue control program, it is necessary to assess the population at risk, vector ecology and the virus surveillance of the area. The concept of epidemiological triangle of disease is well known by public health practitioners. The host, agent and environment need to co-exist in order to facilitate disease transmission. In the absence of any of these three elements, the transmission cannot take place. As it relates to dengue transmission, the host is human, the agent is the dengue virus and the environment is represented by the vectors and climatic parameters. All three elements are required for the transmission of dengue. Endemic area of DF/DHF would expand in both time (length of season) and space (geographic area) under socio-environmental condition (optimal climate, inadequate urban planning, and ecosystem change) [53].

However, from entomological point of view, dengue is hard to control due to prevalence of *Aedes* species. Community involvement plays a fundamental role in vector control. Community participation or local action is central to this approach. Since humans have contributed to the breeding of *Aedes* species, the most economical way is to develop cooperation between neighbours in order to identify breeding sources and to keep mosquito populations under control. Similarly, community involvement provides a great opportunity for an informal educational instance, in which neighbours can discuss and exchange information about DF and its vector. Ideally, everyone in the villages should be aware that the sum of individual efforts towards eliminating mosquito breeding sources results in a larger benefit for the community. When analyzed in detail, assessing the risk for an outbreak of DF is much broader and complicated than just observing the presence of a vector species in a community. Due to constraints, the present study focused only on the existence of the dengue vector in the villages of Pulicat and Vairavan Kuppam as an indicator for the risk of a DF outbreak. However, further studies should consider analyzing other variables that can affect breeding (salinity, disturbance, population density of juveniles), as well as the geographical distribution of houses where breeding is present. Close monitoring of this approach keeping long term sustainability is warranted. Since there is no regular vector surveillance or a control program in Pulicat and its surrounding villages for the control of dengue, continued risk of dengue in Pulicat and its adjoining villages cannot be ignored.

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