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Entomological investigations with special attention to pupal indicators of *Aedes* vectors during outbreaks of dengue in coastal Odisha, India

Biswadeep Das & Rupenangshu K. Hazra

Department of Medical Entomology, Regional Medical Research Centre, Bhubaneswar, India

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Dengue, a major public health problem in India is caused by the dengue virus (DENV) (Family: *Flaviviridae*) comprising four serotypes and is transmitted by the bite of female *Aedes* mosquito, mainly *Ae. aegypti* and *Aedes albopictus*[1, 2]. Spread of the mosquito vector and the virus has led to the resurgence of dengue epidemics and the emergence of dengue in new areas. Due to unavailability of any vaccine/drug against DENV, vector control currently is the only way to curb the spread of dengue[3]. Vector control is presently evaluated using surveillance techniques based on larval indices to determine risk and to guide mosquito control activities[4]. However, recently there has been a movement towards pupal indices, considering early instars as too immature to be the representative of true mosquito productivity, because survival from early stage larvae to pupae is variable and a majority of larvae do not survive to adulthood, whereas most pupae survive to emerge as adults[5]. Moreover, pupal surveys provide an accurate estimate of adult population density resulting in more useful infestation indices, such as pupae per person (PPP) and pupae per house (PPH)[6, 7]. During epidemics, some containers produce more pupae than others. Hence, targeting and eliminating such containers will greatly aid in source reduction during epidemics[8]. Therefore, entomological surveys targeting the most productive containers that contribute maximum pupae during outbreaks can rapidly alleviate the vector density thereby reducing the force of transmission[9].

Odisha state of India is divided into four distinct physiogeographical regions; northern plateau, central tableland, coastal plains and eastern ghats. As per the State Health Department, most parts of Odisha had been affected by periodic outbreaks of dengue since 2011, out of which Bhograi block in Balasore district of coastal region has been the most affected and reported >70 dengue cases from June to August 2012. Bhograi block is a suburban area, located besides the sea, having a rich vegetation of forests with a population of about 40,521 (Census 2011). Due to its proximity to sea, people from nearby Bangladesh and other places often migrate to this region, thus increasing the risk of transmission of vector-borne diseases. The local people are mostly involved in pottery work and manufacture a variety of earthen pots for drinking and commercial purposes.

At the request of the State Health Department, detailed entomological investigations focusing mainly on *Aedes* pupae were carried out from July to August 2012 in the most affected villages, i.e. Nimatpur, Kaubani and Kakada of the Bhograi block. Around 300 houses were inspected in each of the villages after stratifying the areas by attack rates. Adult mosquitoes were collected using battery operated aspirators in the houses surveyed. All water containing indoor and outdoor breeding containers were thoroughly searched for the presence of *Aedes* pupae, which were collected by using pipettes and dippers. Adult mosquitoes and pupae collected from field were brought to the laboratory. Pupae were left to emerge as adults for species identification. Emerged adults from the collected samples were identified as *Aedes* species[10] and pooled according to species, sex and the type of container habitat. The data on pupal survey were analyzed and calculated in terms of different pupal indices, i.e. pupal container index (PCI), PPP and PPH [6,11]. Abundance of indoor and outdoor containers with *Aedes* pupae at the collection sites was assessed in the study to know the most productive container in the areas surveyed. Productivity of a container type (the number of pupae in the container type divided by the total number of pupae in all the containers)[6] was estimated for each container that harboured *Aedes* pupae. Each mosquito pool (≤10 mosquitoes), i.e. field collected adults and those emerged from pupae was subjected to laboratory processing for DENV identification by reverse transcription polymerase chain reaction (RT-PCR)[12]. The infection rate of each RT-PCR positive DENV mosquito pool was estimated using a maximum likelihood estimate (MLE) statistical method for unequal pool sizes that calculated 95% confidence interval (CI) per 1000 mosquitoes[13]. The non-parametric Mann- Whitney U-test was used to calculate the two-tailed *p*-values by comparing the abundance of indoor and out-

door containers harbouring *Aedes* pupae in the affected areas. The relative abundance of all indoor and outdoor containers harbouring *Aedes* pupae in the affected areas was analyzed by the non-parametric Kruskal Wallis one way analysis of variance (ANNOVA) test. The Fisher Exact test was employed to compare the occurrence of DENV infected *Aedes* pool and to calculate the odds ratio in indoor and outdoor containers. A p-value <0.05 was considered to be statistically significant for all the tests. All the statistical tests were performed by using the Graphpad Prism (version 5.01) software.

A total of 948 *Aedes* mosquitoes [132 (25 males and 107 females) field caught and 816 pupae reared] were collected from the affected areas and processed in 95 pools. The collection comprised 60.2% *Ae. albopictus,* 29.9% *Ae. aegypti* and 9.8% *Ae. vittatus* (Table 1). Indoor containers (earthen pots, buckets and plastic drums) were the most abundant (68.2%) and contributed maximum *Aedes* pupae in the affected areas. The average productivity of indoor containers was high (30.77) in comparison to outdoor containers (11.71), with earthen pots registering the highest value (42.6) (Table 2). DENV RNA was detected in 5 of the 59 *Ae. albopictus* pools and 1 of the 31 *Ae. aegypti* pools by RT-PCR. One field caught mosquito pool and four adult pools (reared from pupa) of *Ae. albopictus* and one adult pool (reared from pupa) of *Ae. aegypti* were found to be positive for DENV RNA. DENV-2 was detected in four *Ae. albopictus* pools and one *Ae. aegypti* pool obtained from the affected areas. DENV-2 and DENV-3 was detected in one *Ae. albopictus* pool collected from the affected areas. Out of 6 DENV positive mosquito pools (reared from pupa), 5 (85.7%) pools were detected in indoor breeding *Aedes* species, mainly in earthen pots and 1 (14.3%) pool was detected in outdoor breeding *Ae. albopictus*, i.e in discarded tires. MLE infection rate was high for indoor breeding *Aedes* species (5.02; 95% CI: 1.63, 12.04) in comparison to outdoor breeding *Aedes* species (1.23; 95% CI: 0.07, 5.97). Overall MLE of *Ae. albopictus* was high (8.75; 95% CI: 3.27,19.28) in comparison to *Ae. aegypti* (3.26; 95% CI: 0.19, 15.77), which indicated that *Ae. albopictus* was the principal vector responsible for the outbreaks (Table 1). Furthermore, maximum *Aedes* pupae were obtained from indoor containers, having high pupal indices (PCI, PPP and PPH) in Nimatpur which reported maximum dengue cases, followed by Kaubani and Kakada (Table 2). Earthen pots proved to be the chief containers that contributed maximum pupae. The local tradition of storing water for long periods in earthen pots for several purposes within houses correlated with high pupal productivity in such containers. The Mann-Whitney U-test showed that *Aedes* pupae preferably bred in indoor containers than outdoor containers in the affected areas (sum of ranks = 40, 15, U = 1.00, p = 0.011). Kruskal Wallis ANNOVA depicted that earthen pots (indoors) were the most abundant containers harbouring *Aedes* pupae (Kruskal Wallis statistic, K= 33.6, p <0.001). Fisher exact test revealed that there was significant differences in the occurrence of DENV infected *Aedes* pools at indoor containers in comparison to outdoor containers and showed that the odd ratio of the occurrence of DENV infected *Aedes* pool was significantly high in indoor containers as compared with outdoor containers (OR = 6.0, p = 0.042, 95% CI = 1.12, 31.89).

Table 1. *Aedes* mosquito species collected, total number of pools, dengue virus positive pools and MLE after RT-PCR analysis in the affected villages of Bhograi block, Odisha

Mosquitoes collected from the field	No. of specimens (Pools)	DENV positive pools	Infection rate MLE (95% CI)	DENV serotypes detected
Ae. albopictus female	92 (9)	1	10.87 (0.64, 53.05)	2
Ae. albopictus male	17 (2)	0	0	–
Ae. aegypti female	11 (1)	0	0	–
Ae. aegypti male	8 (1)	0	0	–
Ae. vittatus female	4 (1)	0	0	–
Ae. vittatus male	0 (0)	0	0	–
Total	132 (14)	1	0	–
Pupae reared mosquitoes				
Ae. albopictus	485 (48)	4	8.49 (2.77, 20.36)	2*, 2+, 2*, 2&3*
Ae. aegypti	290 (29)	1	3.45 (0.20, 16.69)	2*
Ae. vittatus	41 (4)	0	0	–
Total	816 (81)	5	0	–

*DENV positive pools in indoor breeding spots; +DENV positive pools in outdoor breeding spots; DENV–Dengue virus; MLE–Maximum likelihood estimate.

Table 2. Abundance of indoor and outdoor containers with *Aedes* pupae alongwith their productvity in the affected villages of Bhograi block, Odisha

Villages surveyed	Distribution	Container type	No. of water filled containers	No. of containers with pupae	No. of pupae	Productivity of container	PCI	PPH	PPP
Nimatpur	Indoors	Earthen pots (1–3 L)	210	188	272	40.17	0.89		
(n = 300)		Buckets	156	96	112	16.54	0.61		
(p = 1453)		Plastic drums	110	69	101	14.91	0.62		
	Outdoors	Discarded tires	124	68	88	12.99	0.54		
		Tree holes	65	28	37	5.46	0.43	2.25	0.46
		Cement tanks (20–100 L)	55	21	34	5.02	0.38		
		Discarded small wates (<2 L)	35	16	18	2.65	0.45		
		Discarded large wastes (>5 L)	23	11	15	2.21	0.47		
					677				
Kaubani	Indoors	Earthen pots (1–3 L)	115	76	90	39.3	0.66		
(n = 275)		Plastic drums	75	35	49	21.39	0.46		
(p = 1012)	Outdoors	Cement tanks (20–100 L)	75	31	41	17.9	0.41		
		Tree holes	38	17	34	14.84	0.44	0.83	0.22
		Discarded small wastes (<2 L)	23	10	15	6.55	0.43		
					229				
Kakada	Indoors	Earthen pots (1–3 L)	108	66	78	47.85	0.61		
(n = 300)	Outdoors	Cement tanks (20–100 L)	75	30	41	25.15	0.4		
(p = 1512)		Tree holes	32	14	23	14.11	0.43	0.54	0.1
		Discarded small wastes (<2 L)	17	7	13	7.97	0.41		
		Tires	16	6	8	4.9	0.37		
					163				

n=Number of houses inspected; p=Number of people in inspected houses; Productivity of container = Number of pupae in the container type × 100/ Total number of pupae; PPH (Pupae per house) = Number of pupae/Number of houses inspected; PPP (pupae per person) = Number of pupae/Number of people in inspected houses; Pupal container index (PCI) = Number of pupal positive containers/Number of containers searched; Numbers in bold indicate the total number of pupae collected from the respective villages.

From the present study it can be concluded that the recent outbreaks of dengue in Bhograi block of Odisha were caused due to the circulation of DENV-2 and DENV-3, DENV-2 being the predominant serotype. DENV RNA was detected in pupae reared mosquitoes, which confirmed the vertical transmission of DENV, which is a major factor responsible for virus persistence and survival in nature for long periods, especially during adverse climatic conditions and inter-epidemic periods when the vector density is low and has an important role in the re-emergence of DENV[14]. High DENV infection rate in *Ae. albopictus*, the most abundant vector in the areas surveyed, rendered it to be the main arboviral vector in this region, also documented to be the major arboviral vector in Odisha in our previous studies[15, 16]. *Aedes* species, particularly *Ae. albopictus* was found mainly breeding indoors in all the affected areas, which was quite interesting finding in the study. Since, *Ae. albopictus* prefer to breed outdoors, hence more indoor breeding *Ae. albopictus* during outbreaks suggested change in breeding behaviour of *Ae. albopictus*. Earthen pots proved to be the most ideal indoor breeding spots in all the areas since most pupae were obtained from earthen pots, thus having high productivity. During the outbreak, vector control measures, specifically targeting the elimination of pupal containers in human dwellings like earthen pots, plastic drums, etc. were undertaken by the local authorities, which generated public awareness among the people regarding DENV and its vector breeding behaviour. The State Health Department, Odisha also implemented strategic measures, aiming primarily at source reduction of vectors by eliminating the most productive containers in the affected as well as nearby areas to prevent the further spread of dengue. Hence, the study suggests extensive entomological surveys with greater emphasis on intradomecile vector control methods for reducing the transmission of DENV to new areas.

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Correspondence to: Dr Rupenangshu K. Hazra, Scientist 'D', Department of Medical Entomology, Regional Medical Research Centre, Nalco Square, Bhubaneswar-751 023, India
E-mail: rupenkh@yahoo.co.in

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