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Detection of dengue virus in *Aedes* mosquitoes in Delhi, India

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ABSTRACT: Detection of viruses in human sera particularly in endemic areas is cumbersome and laborious. Therefore, an alternative approach, Immuno-fluorescence assay (IFA) was performed to determine dengue virus (DENV) positivity in mosquitoes. A total of 1055 adult *Aedes aegypti* female mosquitoes were tested for IFA test against DENV. Minimum infection rate (MIR) for DENV was found higher during August to November 2016 ranging from 10.75 to 20.83. The average yearly MIR was about 6.64. Higher MIR for *Ae. aegypti* was found in Sarfabad, Noida (12.71) and Khoda Colony, Ghaziabad (11.90). Minimum MIR (4.67) was observed in Sanjay colony (Faridabad). The main contribution of this study resides in the development of a more suitable monitoring system for early detection of viral circulation and to prioritize early intervention in the non-transmission season.

KEYWORDS: *Aedes aegypti*, minimum infection rate, immuno-fluorescence assay, DENV

INTRODUCTION

Dengue fever is a disease of the public health importance caused by arbovirus and transmitted by *Aedes* mosquitoes (Diptera: Culicidae) in both rural and urban areas. The dengue viruses consists of an antigenic sub-group of closely related, yet antigenically distinct virus, serotype DENV 1-4, within the genus Flavivirus, Family Flaviviridae (Defoliart *et al*.,1986). Serotypes produce disease ranging from the relatively mild dengue fever (DF), a self-limiting febrile illness to the severe dengue haemorrhagic fever (DHF) characterized by hemorrhaging with or without fatal shock syndrome (Halstead, 2007).

DF has been the most important arboviral disease in the world, responsible for significant morbidity and mortality, especially in tropical countries (Bhattacharya *et al*., 2013; Khan *et al*., 2013; Restrepo *et al*., 2014). The geographic distribution of dengue has increased over decades. In the 1950s, nine countries reported dengue; today over 100 countries are endemic for DF. Mostly deaths are due to lack of early diagnosis of dengue virus infection caused by four serotypes (Anez and Rios, 2013). DF is the most prevalent arboviral infection worldwide, with up to 40% (2.5–3 billion people) of the world's population living in endemic regions. It is estimated that 50–80 million dengue infections occur each year, with 500,000 cases of DHF, and

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at least 12,000–24,000 deaths, mainly among children under 15 years of age (WHO, 2011).

With the ongoing search for an efficient vaccine and an antiviral drug, prevention is still the best way to control the disease, which is possible through vector control in several forms (Black and Lundkvist, 2013; Resende *et al*., 2013). When adult mosquito density is low, direct entomological monitoring is not a sensitive indicator for outbreak prevention. It is in this particular situation that detection of DENV in vector population becomes a particular element as part of an early alert system which allows to position the vector as the primary element in the transmission cycle during epidemiological evaluations (Chow *et al*., 1998). Surveillance of mosquitoes infected with dengue virus can help to monitor the infection rates within vector mosquito population and provide an early warning signal to predict an impending outbreak of DF (Tewari *et al.,* 2004)

Delhi NCR carries high receptivity and vulnerability to *Ae. aegypti* because of high international traffic as well as from the bordering satellite towns of Noida and Ghaziabad (UP) and Faridabad (Haryana) which require a well-organized and coordinated effort to control the incidence of dengue in Delhi and NCR. A major dengue outbreak with more than 10,000 cases and 425 deaths occurred in Delhi in 1996. In 1967, 1968 and 1969, outbreaks of dengue occurred in Delhi when a number of strains of DENV-2 were isolated from humans. Dengue outbreaks were reported from Delhi in 1970, 1982, 1996, 2003, 2006, 2010, 2013 and 2015 (Kumari Roop *et al*., 2011; Sharma *et al.,* 2014; Chaturvedi and Nagar, 2018). In 2015, the city witnessed 1587 cases, the worst crisis in 20 years with the disease claiming 60 lives. Though Vazeille *et al*. (2003) reported that *Ae*. *aegypti* has a relatively low receptivity for DENV-2 as compared to *Ae*. *albopictus,* Arm strong and Ricco-Hesse (2003) proved that *Ae*. *aegypti* has significantly more receptivity to DENV-2 than *Ae*. *albopictus*. In this study, we report the results of dengue virus detected in *Ae. Aegypti* collected from August 2016 to July2017 from selected localities of NCR, Delhi with the help of IFA. Reliable estimation of natural mosquito infection with arbovirus forms a key element in any surveillance system and is essential for vector incrimination and also for developing appropriate preventive measures.

MATERIALS AND METHODS

The study was conducted in six localities viz., Nithari Sector 30 and Sarfabad (Noida), Khoda colony and Railway Colony (Ghaziabad), Sanjay Colony and Sehatpur (Faridabad), of North Central Railway (NCR). They were selected on the basis of confirmed dengue cases during 2015. NCR has witnessed indiscriminate construction activities causing stagnation of water in containers lying in and around the construction sites. *Aedes* larvae were collected from different localities of study areas by inspecting the water holding containers in domestic and peri-domestic environment. The larvae were bought in the laboratory and reared up to adults. Individual *Aedes* mosquitoes were screened for the dengue virus from each locality. The mosquitoes were sorted out as males and females from each locality. IFA was performed on female *Ae. aegypti* mosquitoes to determine the positivity percentage and dengue virus Minimum Infection Rate (MIR). MIR is estimated from the number of virus-positive female mosquitoes/total number of female mosquitoes tested multiplied by 1000.

Head of each mosquito was squashed by pressing it on the glass slide through 12mm^2 coverslip. The cover slip was then lifted gently and discarded in decontaminating pan. The slides were air-dried and the tissues were fixed with chilled acetone at 4°c for 10 minutes. After washing these slides for 10-15 minutes with PBS (phosphate buffered saline) and mounted in glycerol, the bound were detected by addition of a drop of Florecin isothiocynate (FITC) conjugated goat anti-mouse lgG (procured from M/s sigma, USA). The slides were again incubated for 40 minutes at 37c and counter stained with drop of Evan's blue solution for 5 minutes at room temperature. 5-6 mosquito squash were made in each glass slide for screening. The virus infection rate was expressed as minimum infection rate (MIR) calculated per thousand mosquitoes as described by Gajanana *et al*. (1995).

RESULTS AND DISCUSSION

A total of 1055 adult *Ae. aegypti* females were subjected individually to IFA test against DEN virus. Our results on F1 generation showed 6.64 MIR which seems to be high vertical transmission in generation. Monthly mosquito positivity showed that F1 reared mosquitoes were found positive during the months of August to November 2016. Though in January 2017 more number of mosquitoes were tested but none of the mosquitoes was found positive. The minimum infection rate (MIR) for DENV was found to be higher during the months of August to November 2016 ranging from 10.75 to 20.83 which seem to be very high. The average yearly MIR was 6.64 (Table 1, Fig. 1).

The urban system of Delhi is complex as it is an amalgamation of different socio cultural and socio economic groups as well as populations visiting on daily basis for their day today life processes including jobs, purchase, treatment etc. As per the results of the present study, overall combined MIR of all six localities of NCR was found to be 6.64, which is much lower than the MIR calculated in earlier observations (Ilkal *et al*., 1991). Our results demonstrated high MIR for *Ae. aegypti* in Sarfabad (Noida) and Khoda colony (Ghaziabad) i.e., 12.71 and 11.90 respectively which are consistent with infection rate 9.1 and 9.2 reported in a study carried out in Delhi by Kumar *et al*. (2015). Trasovarial transmission of DENV in *Aedes* mosquitoes is considered an important mechanism for the maintenance of the virus in nature and may be implicated in the occurrence of outbreaks and epidemics of the disease (Arunachalam *et al.,* 2008). Source reduction activities i.e. removal of all water holding and dry containers lying in the urban system of Delhi/NCR during non-transmission season i.e. January to June should be carried out to prevent dengue outbreaks.

The MIR in our study was higher in transmission months (Aug-Nov). It is known in DENV that if MIR reaches 10, suggesting a high risk to the surrounding community. If human herd immunity is high, the probability of transmission will be lower in an area regardless of the magnitude of measures of entomological risk. Conversely, if herd immunity is low, relatively low population densities of *Ae. aegypti* could precipitate an epidemic (Scott and Morrison, 2003). Domestic containers play a crucial role in *Aedes* breeding especially during non-transmission season. Therefore, community should take initiative to clean their own breeding sources otherwise those will act as key containers in transmission season (Nagpal *et al*., 2016a,b)). Such water storage practices promote *Aedes* mosquito breeding throughout the year (Sharma *et al*. 2008; Samuel *et al*., 2019).Such areas with persistent of *Aedes* breeding can act as foci for the next dengue outbreak. Entomological surveillance and vector control measures are essential to prevent devastating disease outbreaks.

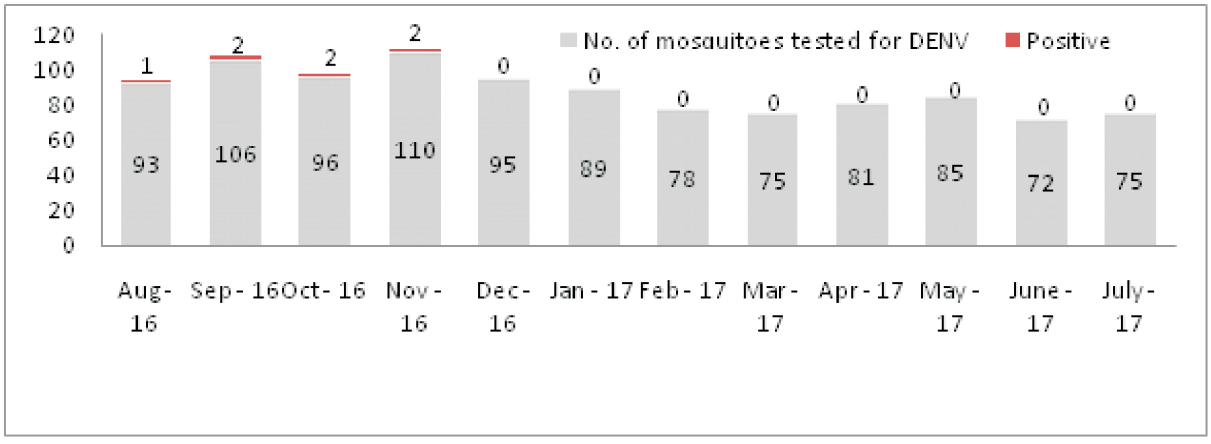

Fig. 1. Number of *Aedes aegypti* mosquitoes tested for DENV in different months (August 2016 to July 2017)

Table 1. Monthly positivity of *Ae. aegypti* for DENV collected from selected localities in NCR, DELHI

Sl. No.	Locality		Aug 16	Sept 16	Oct 16	Nov 16	Dec 16	Jan 17	Feb 17	Mar 17	Apr 17	May 17	Jun 17	Jul 17	Total	MIR
1.	Nithari Sector 30(Noida)	No. of Mosquito	16	17	23	17	14	4	8	13	12	15	14	16	169	0
		Positive	0	0	0	0	0	0	0	0	0	0	0	0	0	
2.	Sarfabad (Noida)	No. of Mosquito	20	25	15	18	28	27	16	14	18	20	18	17	236	12.71
		Positive	1	0	1	1	0	0	0	0	0	0	0	0	3	
3.	Khoda colony (Ghaziabad)	No. of Mosquito	22	26	16	17	25	30	24	18	18	20	19	17	252	11.90
		Positive	0	1	1	1	0	0	0	0	0	0	0	0	3	
4.	Sanjay Colony (Faridabad)	No. of Mosquito	18	20	24	37	15	16	15	18	15	15	8	13	214	4.67
		Positive	0	1	0	0	0	0	0	0	0	0	0	0	1	
5.	Railway Colony (Ghaziabad)	No. of Mosquito	10	12	14	16	8	8	9	8	12	8	7	7	119	0
		Positive	0	0	0	0	0	0	0	0	0	0	0	0	0	
6.	Sehatpur Village (Faridabad)	No. of Mosquito	7	6	4	5	5	4	6	4	6	7	6	5	65	0
		Positive	0	0	0	0	0	0	0	0	0	0	0	0	0	
Total		No. of Mosquito	93	106	96	110	95	89	78	75	81	85	72	75	1055	6.64
		Positive	1	2	2	2	0	0	0	0	0	0	0	0	7	
		Monthly MIR	10.75	18.87	21	18	0	0	0	0	0	0	0	0	6.64	

The study carried out by Kumari Roop *et al*. (2011) in Delhi showed high mosquito positivity in F1 generation mosquitoes. Joshi *et al*. (2002) reported persistence of DENV-3 through transovarial transmission passage in successive generations in *Ae. aegypti* mosquitoes. It is thus important to highlight the implications for future studies on vectorial competence and virus-vector interaction, as well as the mechanism involved in the maintenance of DENV in nature. In this context, it is clear that the main contribution of the study resides in the development of a more suitable monitoring system for the early detection of viral circulation and the risk of epidemics and severe forms of the disease. Our study implies that Noida and Ghaziabad in NCR region having higher MIR and at higher risk of dengue transmission in which better virological and entomological surveillance are required for effective dengue vector control. Such studies should also be integral part of routine surveillance of the city which can provide early evidence of transmission potential area to prioritize early intervention in the non-transmission season.

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