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

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ABSTRACT

Background & objectives: Delhi, the capital city of India, has so far witnessed several outbreaks of dengue fever since 1967 (last one reported in 2013). Improved virological and entomological surveillance are the only tools that can help in prevention of dengue as well as in the development of dengue control programmes. The aim of the study was to conduct a prospective field study to detect dengue virus in adult *Aedes aegypti* mosquitoes collected from various localities represented by different socioeconomic groups in Delhi.

Methods: The study areas were selected and categorized into high, medium and low income groups on the basis of socioeconomical characteristics of the resident population, where dengue cases were reported during the past three years by MCD. Dengue viral infection was detected in the head squash of each adult mosquito by immunofluorescent assay (IFA) employing monoclonal antibodies against dengue virus (DENV). A total of 2408 females and 1206 males of *Ae. aegypti* were collected and tested by IFA.

Results: Out of 2408 *Ae. aegypti* females, 14 were found positive, with minimum infection rate (MIR) of 5.8 per 1000 mosquitoes. Among the 18 study areas, 11 localities were found positive for dengue virus infection. Low income group (LIG) areas showed highest mosquito infectivity (9.8), followed by medium income group (MIG), *i.e.* 6.2; while least was observed in high income group (HIG), *i.e.* 1.3. No vertical transmission of dengue virus could be detected in 1206 *Ae. aegypti* males collected.

Interpretation & conclusion: The study concludes that there was high MIR in the identified localities of low and medium income groups. Estimation of MIR in a female *Aedes* mosquito in the existing arsenals for dengue surveillance would be an added advantage for early warning of dengue outbreak. The presence of infected mosquitoes in identified localities of Delhi was alarming and require rigorous vector surveillance so that the severe outbreaks can be prevented.

Key words *Aedes aegypti;* dengue virus; immunofluorescent assay; minimum infection rate

INTRODUCTION

Dengue fever is an acute viral disease caused by flavivirus comprising of four different serotypes (DEN-1, DEN-2, DEN-3 and DEN-4). In north India, epidemiology of dengue is changing rapidly and most of the cities have become hyper endemic. Delhi is one of the dengue endemic states in north India[1]. It has so far witnessed several reported outbreaks[2-4] during the past years, *viz.* 1970, 1982, 1988, 1996, 2003, 2006 and 2010[5-11]. There is also a rise in incidence of fatal dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) cases, which are of medical emergencies[12]. In 2013, a severe outbreak occurred in India with a total of 75,454 cases and 167 deaths; wherein Delhi alone registered 5574 dengue cases and six deaths[13].

Over the last few decades, *Aedes aegypti* (Diptera: Culicidae) has replaced *Ae. albopictus*, the earlier principal vector of dengue virus in Asia. It has been previously reported that *Ae. aegypti* has a relatively low receptivity for dengue virus as compared to *Ae. albopictus*[14]. However, it has been acknowledged that *Ae. aegypti* has significantly more receptivity to DEN-2 virus than *Ae. albopictus*[15].

In this study, we report the results of dengue virus detected in *Aedes aegypti* collected during 2013 dengue outbreak of Delhi with the help of immunofluorescent assay (IFA). IFA on individual *Ae. aegypti* has provided important information on vector infection with dengue virus[16-17] that helps in precise estimation of vector infection rate within a particular geographical area[18]. An extensive study on virus detection in field collected *Ae.*

aegypti mosquitoes was hence, undertaken to measure the minimum infection rate and to identify the potential risk areas of dengue infection in Delhi. It will help in developing the appropriate preventive measures and also to estimate the risk of dengue infection.

MATERIAL & METHODS

Study area

The National Capital Territory of Delhi (located at latidute 28°38' N, longitude 77°12' E) covers an area of 1484 km^2. It has a length of 51.9 km and a width of 48.48 km with population of 17.8 million (Census 2011) approximately. The study was conducted in 18 localities of Delhi (Fig. 1) in collaboration with Municipal Corporation of Delhi (MCD), India. The study areas were selected and categorized into high, medium and low income groups on the basis of socioeconomic characteristics of the resident population.

Collection of mosquitoes

A total 2408 female and 1206 male *Ae. aegypti* mosquitoes were collected from human premises from 18 localities of Delhi from June 2013 to May 2014. The study areas were visited once every month during the study period. The mosquitoes were collected from the resting places using aspirators, handnets and torch[19].

Field collected adult mosquitoes were brought to the laboratory of National Institute of Malaria Research, New Delhi. The mosquitoes were sorted out as males and females from each locality and were stored at –80°C. IFA was performed on these mosquitoes to determine the dengue virus minimum infection rate (MIR). MIR is estimated from the numbers of virus-positive female mosquitoes/total number of female mosquitoes tested multiplied by 1000. IFA was also performed with male mosquitoes to investigate the possibility of vertical transmission of dengue viruses.

Virus isolation and detection

The wild caught adult female mosquitoes were subjected to IFA. Individual mosquito was tested for the presence of virus. Head of each mosquito was squashed by pressing it on the glass slide with a cover slip and was processed for detection of dengue virus antigen by IFA. Chitin and other debris were removed carefully from each head spot, fixed with cold acetone. Dengue (DEN) specific monoclonal antibodies (mAbs) (obtained from Na-

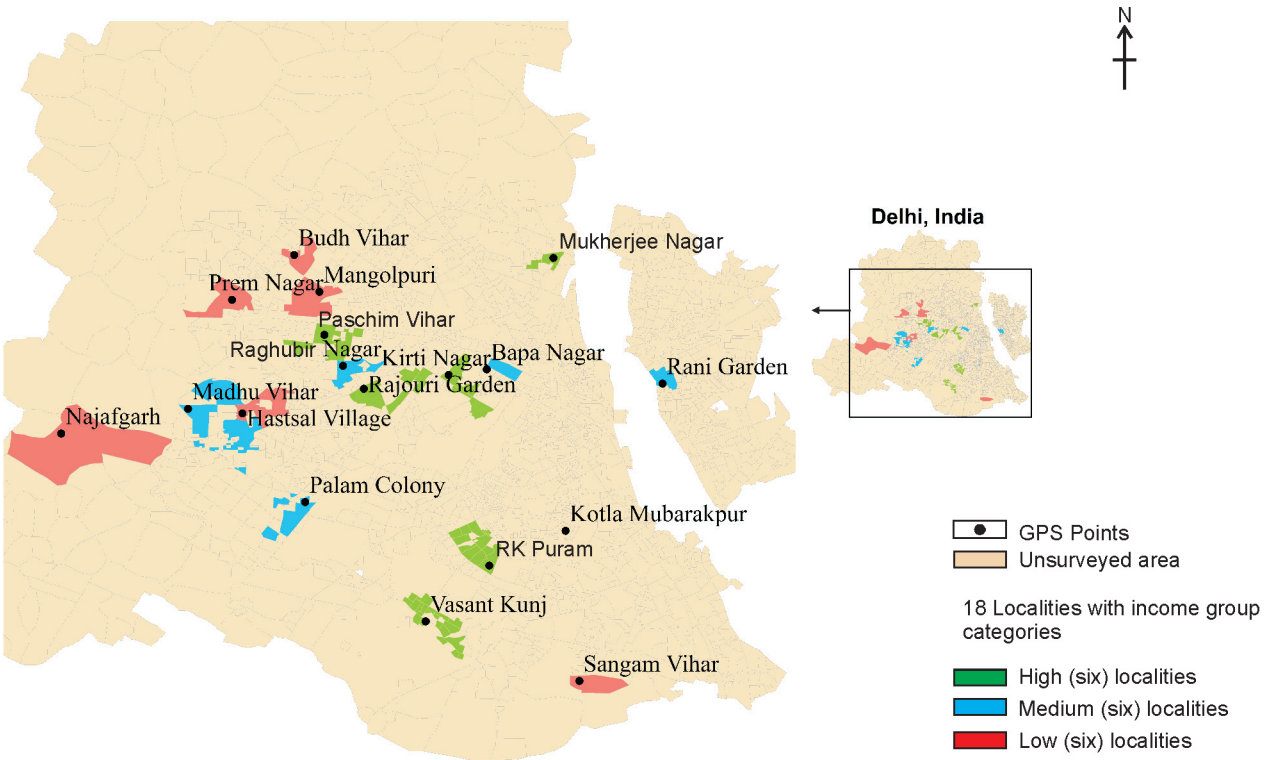

Fig. 1: Map of Delhi showing 18 localities of the study areas.

tional Institute of Virology, Pune) were used on each head spot. After washing these slides with phosphate-buffered saline (PBS) and mounting in glycerol, the bound mAbs were detected by addition of fluorescein isothiocyanate (FITC) conjugated goat anti-mouse IgG (procured from M/s. Sigma, USA) using fluorescence microscope model Axio Scope A1 (Carl Zeiss, Germany). The detection of virus antigen was observed as fluorescence.

Statistical analysis

The data were entered in MS Excel 2007 and statistical analysis was done by SPSS software package (version 20). Pearson's correlation (r) and odds ratio (OR) were calculated to ascertain the relations and relative odds between various income groups and minimum infection rate of *Ae. aegypti*.

RESULTS

In the present study, a total of 2408 adult females *Ae. aegypti* were collected from 18 localities and subjected individually to IFA test employing monoclonal antibodies against DEN virus. Out of these, 14 mosquitoes were found positive for dengue virus with a combined MIR of 5.8. Further, 11 localities out of 18 were found positive for dengue virus infection and infection rate of *Ae. aegypti* mosquitoes were expressed as MIRs. *Ae. aegypti* caught from all study areas had MIRs ranging from 0 to 16.3% (Table 1). Among 18 localities, low income group area showed highest MIR (9.8) followed by medium income group localities (6.2); while least MIR (1.3) was observed in localities with high income group (Table 2). Income groups (high, medium and low) were positively correlated and results were found significant [r (18) = 0.646, p = 0.004] with MIR of *Aedes* mosquitoes collected from the selected localities of Delhi. Odds of getting MIR in *Aedes* mosquitoes was higher in low income group areas as compared to high income group (OR = 8.84, confidence interval (CI) ranging from 1.11 to 69.96) and medium income group (OR = 1.78; CI = 0.59–5.36).

In localities with low income group, five out of six localities were found to have dengue virus infected mosquitoes showing highest MIR (Fig. 2). Budh Vihar was found to have highest MIR in this group, *i.e.* 16.3 followed by Jai Vihar (Najafgarh) 12.3, Mangolpuri 9.2, Prem Nagar (Nangloi) 8.3 and Sangam Vihar 8.1, while Hastsal Village was the only locality from this group where no infected mosquito was found (Table 1). In medium income group localities, highest MIR was observed for Rani Garden (9.1), followed by Raghubir Nagar (7.2),

Table 1. Locality wise distribution of minimum infection rate of *Aedes aegypti* collected in Delhi

Locality	Category (Income group)	No. of female *Ae. aegypti* tested	No. of mosquitoes positive	MIR
Paschim Vihar	High	125	0	0
Rajouri Garden	High	129	1	7.8
R.K. Puram	High	136	0	0
Kirti Nagar	High	103	0	0
Vasant Kunj	High	159	0	0
Mukherjee Nagar	High	139	0	0
Bapanagar	Medium	147	1	6.8
Madhu Vihar	Medium	145	1	6.9
Palam Colony	Medium	123	0	0
Kotala Mubarakpur	Medium	141	1	7.1
Raghubir Nagar	Medium	138	1	7.2
Rani Garden	Medium	110	1	9.1
Mangolpuri	Low	109	1	9.2
Budh Vihar	Low	184	3	16.3
Prem Nagar (Nangloi)	Low	121	1	8.3
Hastsal Village	Low	113	0	0
Jai Vihar (Najafgarh)	Low	162	2	12.3
Sangam Vihar	Low	124	1	8.1
Total		2408	14	5.8

Table 2. Minimum infection rate of *Aedes aegypti* collected from high, medium and low income group categories

Category	No. of female *Ae. aegypti* tested	No. of mosquites positive	MIR
High	791	1	1.3
Medium	804	5	6.2
Low	813	8	9.8

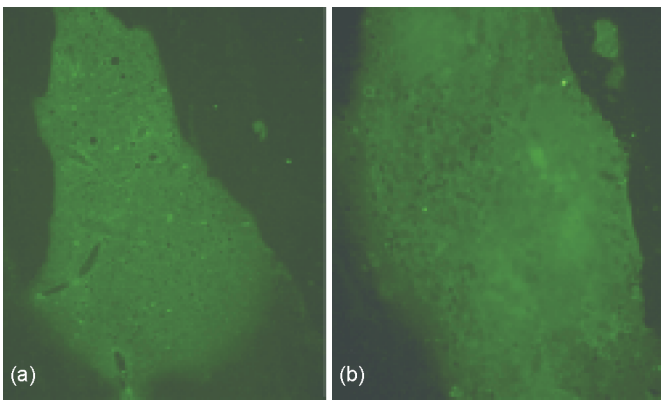

Fig. 2: IFA test performed on the head portion of the virus infected *Ae. aegypti* using monoclonal antibodies specific for dengue showing fluorescence collected from localities with highest MIR (a) Buddh Vihar; and (b) Jai Vihar (Najafgarh).

Kotla Mubarakpur (7.1), Madhu Vihar (6.9) and Bapa Nagar (6.8); whereas no infected vector was detected in Palam Colony. On the other hand, among the six localities with high income group, only one locality, *viz.* Rajouri Garden was found to have *Aedes* mosquitoes infected with dengue virus with MIR 7.8; whereas in rest of the five localities, *viz*. Paschim Vihar, R.K. Puram, Kirti Nagar, Vasant Kunj and Mukherjee Nagar infected mosquitoes were not detected.

None of the field collected male *Ae. aegypti* showed the presence of dengue virus indicating absence of vertical transmission of dengue virus in these localities. Comprehensive studies are further required in this direction to determine if vertical transmission or transovarian transmission of dengue virus is taking place in these localities of Delhi, as mentioned in other studies in India as well as outside India[20-21].

DISCUSSION

As per the results, the overall combined MIR in study localities of all three income groups was found to be 5.8; much lower than the MIR calculated in other study, *i.e*. 90.7 it carried out in Maharashtra state by Ilkal *et al*[16]. Our results demonstrated high MIRs for *Ae. aegypti* in Budh Vihar and Jai Vihar (Najafgarh), *i.e.* 16.3 and 12.3%, respectively which are consistent with the infection rate (18.6) reported in the previous study carried out in Singapore[22]. However, these findings differ from the findings of another study conducted in Singapore by Chan *et al*[19], which reported the MIR of 0.51 for *Ae. aegypti*. The percentage/rate of *Aedes* mosquitoes with dengue virus in different areas indicates risk level, *i.e.* high, medium or low. Higher the MIR, higher will be the risk of dengue transmission. In the present study, localities with low and medium income groups showed higher MIR than the high income group localities, therefore, these areas are more prone to dengue outbreak in Delhi. The risk of acquiring dengue infection is significantly higher in residents of low and medium income group areas of Delhi as compared to high economic group.

The combination of poor, progressively more crowded living conditions, rapidly increasing population density, unstable houses, and water storage practices in low socioeconomic areas of Delhi are the most likely risk factors that not only contribute to the dengue transmission but also lead to the failure of vector control programmes. The presence of infected mosquitoes in these localities is alarming and require careful vector surveillance so that the large outbreaks can be prevented.

In Delhi, there are areas where people with low and medium income have the tendency to store water in various types of containers, *i.e.* plastic, iron, mud pots, *etc.* due to irregular water supply and water shortage. Such water storage practices promote *Aedes* mosquitoes breeding throughout the year[23-24]. The persistence of dengue virus in the community either through transovarian transmission or transvertical transmission is a well known fact. Therefore, areas with persistent *Aedes* breeding can act as foci for the next dengue outbreak and need effective surveillance. Continuous surveillance of dengue infection and *Aedes* breeding is necessary for the prevention and control of dengue in the areas where infected *Aedes* mosquitoes were detected. Such studies of dengue virus detection in mosquitoes indicate that estimation of MIR would be useful monitoring tool for dengue disease transmission in Delhi and other cities.

CONCLUSION

Our results demonstrated that localities with low and medium income groups showing higher MIRs, are at higher risk of dengue transmission than those representing high income group. However, further investigations are needed in order to define critical level of dengue virus infection in the vectors to develop early warning systems. In the absence of dengue vaccine, improved virological and entomological surveillance are the only tools that can help in prevention of dengue as well as in the development of effective dengue control programmes and early warning system for dengue outbreaks.

Conflict of interest: There is no conflict of interest for this study.

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