

Distribution of dengue virus types in *Aedes aegypti* in dengue endemic districts of Rajasthan, India

Bennet Angel & Vinod Joshi

Desert Medicine Research Centre (ICMR), Jodhpur, India

Received January 16, 2008

Background & objectives: Dengue haemorrhagic fever (DHF) is the major cause of sustained morbidity/mortality among human cases of dengue in dengue endemic areas of Rajasthan. Screening of mosquitoes collected from disease endemic settings and typing the virus could provide significant epidemiological information for prospective risk of DHF. We therefore carried out a study on different dengue virus types as occurring in field collected *Aedes aegypti* mosquitoes from four dengue endemic districts of Rajasthan, India.

Methods: Adult *Ae. aegypti* were collected from the human dwellings of urban, peri-urban and rural settings of four dengue endemic districts of Rajasthan, India. The field collected adults were fed on 4 per cent glucose solution and kept in the laboratory for 3-4 days. The adult field collected *Ae. aegypti*, were subjected to indirect fluorescence antibody test (IFAT) following standard procedure. Commercially acquired monoclonal antibodies against DEN types 1, 2, 3 and 4 were used. The remnants of IFA test subjected mosquitoes were made into viral suspension which was inoculated into the cell culture medium and mouse brain to confirm the presence of virus as shown by IFA test.

Results: Of the 498 adult *Ae. aegypti* tested, 78 (15.6%) were positive by IFA test. Among urban areas, desert area (Jodhpur) showed highest (21.6%) mosquito infectivity followed by 7.1 per cent in forest and river area (Kota) and least (3.2%) in semi-arid area (Jaipur). Among rural settings also, desert area showed maximum (25.0%) natural infection in mosquitoes followed by rural setting-1 of semi-arid area (24.1%). Among urban setting of desert area, all the four dengue types *viz.*, DEN-1, 2, 3 and 4 were detected. In semi-arid area, urban settings showed presence of DEN-3, whereas among rural settings, rural-1 showed all the four DEN types, rural-2 showed DEN-1 and DEN-3, rural-4 showed DEN-3 and DEN-4, and rural-3 showed no mosquito infections. In forest and river area, among urban settings only, three DEN types, 1, 2 and 4 were observed.

Interpretation & conclusion: In desert and semi-arid areas of Rajasthan, where people possess tendency of over- and sustained storage of domestic water, present observations on occurrence of all four dengue virus types may have important bearing on the epidemiology of DHF in the area.

Key words *Aedes aegypti* - dengue haemorrhagic fever - dengue virus types - desert area - IFA test

Rajasthan, is one of the dengue endemic States in India¹⁻⁴. Along with dengue fever (DF), its more severe form, dengue haemorrhagic fever (DHF) is the major

cause of sustained morbidity/mortality among human cases of dengue in this area (Hospital data, unpublished reports). To estimate the risk of DHF and to develop

appropriate preventive measures there is a need to identify the foci of occurrence of multiple strains of dengue circulating in an endemic setting. Screening of mosquitoes of a setting and typing the virus they carry could be a tool to attain the above.

The present study was undertaken in the districts with arid, semi-arid ecology, forest and river characteristics and in non arid districts of Rajasthan, India⁵. Owing to water scarcity and irregular water supply in most of the districts of this arid State, people have tendency to store the domestic water⁶. This practice ensures the availability of domestic breeding dengue vectors *Ae. aegypti* throughout the year and hence persistence of dengue virus. We have observed that persistence of virus through vertical route in the mosquitoes could be a retention mechanism of virus during inter epidemic period of disease (unpublished data). Realizing that availability of more than one type of dengue strain in the available vector fauna could be a significant risk factor for the possibility of DHF, we undertook an extensive study on virus typing in field collected mosquito samples of *Ae. aegypti* from urban and rural areas of four dengue endemic districts of Rajasthan.

Material & Methods

Selection of study areas and collection of mosquitoes: Adult *Ae. aegypti* were collected from the human dwellings of urban, peri-urban and rural settings of dengue endemic districts of Rajasthan, India, from November 2006 through June 2007. The study areas were visited thrice, once during each set of months viz., November-January, February-March, and April-June. The study areas were selected on the basis of their socio-ecological characteristics as classified in the literature⁵. Jodhpur district represents desert area, Jaipur district represents semi-arid area, Kota district represents the area of forest and river, and Bharatpur district represents non desert area. The adult mosquitoes were collected from human premises from urban and rural areas and from tree holes of peri-urban settings. The field collected adults were brought to the laboratory of Desert Medicine Research Centre, Jodhpur, and kept in barraud cages at about 25°C ambient temperature and 60-70 per cent relative humidity. The mosquitoes were fed on 4 per cent glucose solution and were kept in the laboratory for 3-4 days after bringing from the field.

Virus isolation and typing: The wild caught adult females were subjected for indirect fluorescence

antibody test (IFAT) after the digestion of blood⁷. Individual mosquitoes (not pools) were tested for the presence of virus. Head of each of mosquitoes tested was teased and divided into four parts. Each part was made into spot squash by pressing it on the slide through cover slip. From each of spot chitin was removed carefully. Dengue (DEN) specific monoclonal antibodies (MABs) of types 1, 2, 3 and 4 (obtained commercially from M/s Fitzgerald, Germany) were used on each of the parts of head squash tested. The part of head squash showing positive IFA test by reacting against a particular type of monoclonal antibody (DEN 1, 2, 3 or 4) was recorded to contain that particular dengue type. Fluorescence iso thio cyanate (FITC), procured from M/s Sigma, USA, was used, and Fluorescence Microscope model BH2 RFL1 PM 10 ADS, (OLYMPUS, Japan) was used to view the detection of virus as fluorescence.

To avoid possible bias in examining IFA test slides, negative controls (mosquitoes from areas other than study areas, showing no fluorescence), positive controls (mosquitoes from areas other than study areas, showing fluorescence) were shown to blinded observer to match the unbiased observations for IFA test positive and IFA test negative slides. Blinded and non blinded observers scored equally in positive and negative slides.

To confirm that IFA +ve mosquitoes contain viruses, remnants of a sample of mosquitoes showing positive IFA test were used for preparing suspension in bovine albumin. About 22 µl of suspension was intracerebrally inoculated into infant albino mice (2 days old). The mice which developed sickness, were sacrificed in their last stage of activity, brains were dissected out and used to prepare virus suspension in phosphate buffer saline (PBS). Some tissues of mouse brain were made into a thin film by pressing on slide through cover slip and slides were again subjected to IFA test to confirm the presence of virus. To further verify the results of IFA test, remnants of subsequent laboratory reared collection of mosquitoes (other than used for present study) being screened regularly in the laboratory and from IFA test positive, were ground, homogenized and made into a filtered suspension which was inoculated in the cell culture medium (Mitsubishi and Maramorosch medium). After 3-4 days samples from cell culture flasks were subjected to IFA test to confirm that IFA +ve mosquitoes contained viral infections. Cell lines C6/36 commercially procured from National Institute

of Cell Sciences, Pune, India, were used. Ethical approval for the study was obtained prior to the work.

Results

A total of 498 blood fed as well as unfed adult female *Ae. aegypti* were subjected individually to IFA test employing monoclonal antibodies against DEN 1, 2, 3 and 4. Of these, 78 (15.6%) were positive by IFA test. Among urban settings of all the four study areas, desert area showed highest (21.6%) mosquito infectivity followed by 7.1 per cent in forest and river area and least (3.2%) in semi-arid area. Among rural settings also, desert area showed maximum (25.0%) natural infection in mosquitoes followed by rural setting-1 of semi-arid area (24.1%). In peri-urban settings, one of 11 mosquitoes tested showed positive IFA test in desert area. No mosquito infectivity was observed in the non desert area (Table).

Among urban setting of desert area, all the four dengue types *viz.*, DEN-1, 2, 3 and 4 were detected.

Maximum presence (37.1%) of DEN -2 and DEN-3 (31.4%) was observed, while DEN-1 and DEN-4 were relatively less prevalent (14.2 and 17.1% respectively). In peri-urban setting of desert area DEN-3 was observed whereas, among rural settings DEN-2 was detected.

In semi-arid area, urban settings showed presence of DEN-3, whereas among rural settings of this area, rural-1 showed all the four DEN types present, rural-2 showed DEN-1 and DEN-3, rural-4 showed DEN-3 and DEN-4 and rural-3 showed no mosquito infections.

In forest and river area, among urban settings, three DEN types, 1, 2 and 4 were observed, whereas, DEN-3 was not observed from these settings. Among rural settings of this area, in rural-1, only DEN-2 was present, in rural-2, DEN-1 and DEN-4 were present.

The data showed that while in desert and semi-arid areas, all the four DEN types were observed in urban settings, in forest and river area, DEN-3 was not observed. In non arid area no mosquito infection was observed. Pooled data suggested that in urban settings

Table. Distribution of dengue types in *Ae. aegypti* of four endemic areas

Urban/ Rural	Total tested	Total positive	% positive	Positive for DEN-1	% positive	Positive for DEN-2	% positive	Positive for Den-3	% positive	Positive for DEN -4	% positive
<i>Desert:</i>											
Urban	162	35	21.6	5	14.2	13	37.1	11	31.4	6	17.1
Peri-urban	11	1	9.0	0	0.0	0	0.0	1	100	0	0.0
Rural	16	4	25.0	0	0.0	4	100	0	0.0	0	0.0
<i>Semi-arid:</i>											
Urban	31	1	3.2	0	0.0	0	0.0	1	100	0	0.0
Rural -I	62	15	24.1	4	26.6	5	33.3	3	20.0	3	20.0
Rural-II	32	4	12.5	2	50.0	0	0.0	2	50.0	0	0.0
Rural-III	4	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Rural-IV	26	3	11.5	0	0.0	0	0.0	2	66.6	1	33.3
<i>Forest & river:</i>											
Urban	70	5	7.1	3	60.0	1	20.0	0	0.0	1	20.0
Rural-I	27	3	11.1	0	0.0	3	100	0	0.0	0	0.0
Rural-II	36	4	11.1	1	25.0	0	0.0	0	0.0	3	75.0
<i>Non arid:</i>											
Urban	21	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
<i>Total:</i>											
Urban	284	44	15.4	11	25.0	14	31.8	12	27.2	7	15.9
Peri-urban	11	1	9.0	0	0.0	0	0.0	1	100	0	0.0
Rural	203	33	16.2	7	21.2	12	36.3	7	21.2	7	21.2
Grand total:	498	78	15.6	18	23.0	26	33.3	20	25.6	14	17.9

per cent positivity for DEN-2 was 31.8 per cent and in rural settings it was 36.3 per cent (Table).

Discussion

In desert and semi-arid areas of Rajasthan, India, where people have a tendency of over- and sustained storage of domestic water, the present observations on occurrence of all four dengue virus (DEN) types in field collected *Ae. aegypti* show important bearing on the possibility of emergence of DHF in the area. It was interesting to observe that in both desert as well semi-arid areas, among urban settings, all the four dengue types were observed, but in forest and river area DEN-3 was not detected. These observations highlighted the specificity of areas for maintaining particular DEN types.

It has been reported that mosquitoes could be used as indices of propagating dengue viruses⁸. In the present study, the mosquito infections detected through positive fluorescence of IFA test and their subsequent confirmation in cell culture medium, and mouse brain indicate this technique, as the possible screening tool to estimate risk of DHF in an area. As study area is outbreak prone, the present approach of virus typing in field collected mosquito samples could yield a powerful surveillance indicator to investigate current scenario of virus types available and develop predictors of the prospective risk of occurrence of DHF.

Acknowledgment

Authors acknowledge the TDR/WHO/UNDP (Project ID A20766) for providing financial support for the present study.

References

1. Ghosh SN, Pavri KM, Singh KR, Sheikh BH, D'lima LV, Mahadev PV, *et al.* Investigations on the outbreak of dengue fever in Ajmer city, Rajasthan state in 1969. Part I: Epidemiological, clinical and virological study of the epidemic. *Indian J Med Res* 1974; 62 : 511-22.
2. Ilkal MA, Dhanda V, Hassan MM, Mangla M, Mahadev PV, Shetty PS, *et al.* Entomological investigations during outbreak of dengue fever in certain villages in Maharashtra State. *Indian J Med Res* 1991; 93 : 174-8.
3. Ghosh SN, Sheikh BH. Investigations on the outbreak of dengue fever in Ajmer city, Rajasthan in 1969. Part II: results of serological tests. *Indian J Med Res* 1974; 62 : 523-33.
4. Chuhan GS, Rodrigues FM, Shaikh BH, Ilkal MA, Khangaro SS, Mathur KN, *et al.* Clinical & virological study of dengue fever outbreak in Jalore city, Rajasthan. *Indian J Med Res* 1985; 91 : 414-8.
5. *Resource Atlas of Rajasthan*. Jodhpur: State Remote Sensing Application Centre, Department of Science and Technology, Government of Rajasthan; 1978.
6. Joshi V, Sharma RC, Sharma Y, Adha S, Sharma K, Singh H, *et al.* Importance of socio-economic status and tree holes in distribution of *Aedes* mosquitoes (Diptera: *Culicidae*) in Jodhpur, Rajasthan, India. *J Med Entomol* 2006; 43 : 330-6.
7. Kuberski TT, Rosen L. A simple technique for the detection of dengue antigen in mosquitoes by immunofluorescence. *Am J Trop Med Hyg* 1977; 26 : 533-7.
8. Rosen L, Gubler D. The use of mosquitoes to detect and propagate dengue viruses. *Am J Trop Med Hyg* 1974; 23 : 1153-60.

Reprint requests: Dr Vinod Joshi, Scientist 'F' & Head, Laboratory of Virology & Molecular Biology, Desert Medicine Research Centre
Jodhpur 342 005, India
e-mail: vinodjoshi@dmrcjodhpur.org