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Outbreaks of dengue in Central India in 2016: Clinical, laboratory & epidemiological study

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***Background & objectives*: Dengue virus (DENV) causes outbreaks and sporadic cases in tropical and subtropical countries. Documenting intricacies of DEN outbreaks is important for future interventions. The objective of this study was to report clinical, laboratory and epidemiological features of DEN outbreaks reported in different districts of Central India in 2016.**

***Methods*: In 2016, outbreaks (n=4) suspected of DEN were investigated by rapid response team. Door-to-door fever and entomological surveys were conducted. Blood samples were collected and tested using NS1 or IgM ELISA; real-time reverse transcription-polymerase chain reaction was done to identify serotypes of DEN virus (DENV). NS1-positive samples were tested for the presence of IgG by ELISA. Clinical and demographic data were collected and analyzed.**

***Results*: Outbreaks occurred in both urban and rural areas in monsoon season and *Aedes aegypti* was identified as the vector. Fever, chills, headache and myalgia were the major symptoms; no fatality was recorded. Of the 268 DEN suspects, 135 (50.4%) were found serologically positive. DEN positivity was higher (n=75; 55.56%) among males and in the age group of 16-45 yr (n=78; 57.8%). DENV 3 followed by DENV 2 were detected as the major responsible serotypes. High attack rates (up to 38/1000) and low cumulative IgG prevalence (14.9%) were recorded in rural areas.**

***Interpretation & conclusions*: Our study showed that DENV 3 was the major serotype responsible for outbreaks that occurred in monsoon. High attack rates and lower number of secondary infections in rural areas indicated that DENV is emerging in rural parts of Central India. Early diagnosis at local level and timely intervention by mosquito control activities are needed to avoid such outbreaks in future.**

Key words Attack rate - dengue outbreak - rural Central India - serotypes

Dengue (DEN) is a mosquito-borne viral disease widespread throughout the tropical and subtropical areas. Dengue virus (DENV) is transmitted by *Aedes* species of mosquitoes[1]. Four distinct serotypes of DENV (DENV-1, DENV-2, DENV-3 and DENV-4) are known to exist, although these are closely related

†Deceased

but infection with one serotype provides only partial and short-lasting immunity; subsequent infection with different serotype in future can lead to severe symptoms[2].

According to a recent global estimate, 390 million people are infected with DENV per year, of whom about 100 million manifest clinically. According to another estimate, people staying in 128 countries are at risk of DENV infection[3]. In recent years, outbreaks from several parts of the world such as USA, China, Japan, Pacific Island countries, Western Pacific regions, Solomon island, the African regions *etc*., have been repeatedly reported[3].

WHO Regions of South East Asia and the western Pacific represent about 75 per cent of the current global burden of DENV infection[4]. In comparison with other Asian countries, DEN was not widespread in India before 1990s although first outbreak of DEN was reported from Kolkata in 1963[4]. Prevalence of DEN has significantly increased since 2001; in addition to an increase in number of cases and severity of illness, its geographical range has also been shifted drastically and is now spreading to rural areas[4].

A recently published meta-analysis showed that more than two dozen DEN outbreaks were reported from India till 2017; most of these were reported from southern States and most occurred after 2000[5]. In 2016, as per National Vector Borne Diseases Control Programme (NVBDCP), India has reported 110,000 cases along with 227 deaths. Madhya Pradesh (MP) has a contribution of 3134 cases and 12 deaths among them[6].

India is known for its hyperendemicity of DEN with the occurrence of both sporadic cases and outbreaks resulting due to infection with DENV belonging to different serotypes, moreover co-infections are also reported[7,8]. Northern India had a major outbreak of DENV 2 in 1996; subsequently, in post-epidemic period, DENV 1 was detected from the same area[7-9]. In 2003, DENV 3 emerged as dominant serotype in Delhi and other parts of north India while recently all four serotypes are reported circulating[7-9]. A nine-year trend study from Mumbai, western India showed dominance of DENV 2, followed by DENV 1 and about three per cent co-infection[10]. Studies done till 2017 showed DENV 2 and DENV 3 as dominant serotypes in the southern region, and DEN 1 and DEN 2 in the eastern India[5].

Circulation of all the four serotypes of DENV has already been reported from Central India, and outbreaks of DENV 1 and 2 have been documented[9,11-14] from tribal and rural areas[11,12]. It has been documented that DENV 2 infection is more severe and is commonly associated with DEN haemorrhagic fever (DHF) and mortality than DENV 1 and DENV 4[11,15]. Hospitalization rates were found to be higher in DENV 3-infected patients than in DENV 1-infected patients[16]. This study was undertaken to present the information on four DEN outbreaks investigated in different districts of Central India (MP) in 2016, elaborating the clinical, laboratory, epidemiological and entomological aspects of DEN disease.

Material & Methods

Outbreak areas and field surveys: In 2016, clusters of cases suspected of DEN were noticed in rural areas of Damoh (population: 14,990), Morena (population: 1236), Sehore districts (population: 2207) and in the urban area of Katni district (population: 15,000)[17] of MP, India. The rapid response team (RRT) of the ICMR-National Institute of Research in Tribal Health, Jabalpur (NIRTH), Jabalpur, comprising clinician, entomologist and epidemiologist along with the support staff visited these areas. Door-to-door fever and entomological surveys were conducted in the affected areas as per NVBDCP guidelines[6]. The case definition given by NVBDCP for DEN was followed. DEN suspects were identified and guided to health camp where they were clinically examined and blood samples (2-3 ml) were collected.

The study was performed after obtaining clearance of the Institutional Ethics Committee (approval no: RMRCT/Ethics committee/2715/2011) and written informed consent was taken from the patients.

Clinical and demographic data were collected in predesigned format. All the blood samples were transported to the Virology laboratory of NIRTH maintaining cold chain. The mosquito control measures were initiated immediately. Collected adults and larvae of mosquitoes were transferred to the laboratory and identified using standard key[18]. The incidence was labelled as outbreak as defined by NVBDCP that is more than five cases reported from a village or a locality with 10-15 thousand population, within a week[6].

Sample processing

ELISA: Serum was separated from blood sample. Samples collected in acute phase of illness (0-5 days, n=137) were tested for the presence of

DENV NS1 antigen by ELISA (Panbio DEN Early ELISA, Standard Diagnostics Inc., Gyeonggi, Republic of Korea). The samples collected after 5th day of onset of illness (n=131) were tested for DENV-specific IgM by monoclonal antibody-based kit manufactured by National Institute of Virology (NIV), Pune, India. Samples collected after five days of illness were also tested for the presence of chikungunya virus (CHIKV)-specific IgM antibodies using kit manufactured by NIV, Pune. All the NS1-positive samples were tested for the presence of IgG for DENV using kit Panbio Dengue IgG Capture ELISA (Standard Diagnostics Inc., Gyeonggi, Republic of Korea), following manufacturer's protocol (Figure).

Serotyping: Acute phase samples found positive by NS1 ELISA (n=59) were subjected to serotyping, using Centers for Disease Control and Prevention (CDC) developed real-time reverse-transcription PCR kit, following manufacturer's protocol[19].

Statistical analysis: Demographic, clinical data and laboratory test result were entered in Microsoft Excel 2007 and analyzed for finding percentages, proportions, odds ratio, Chi-square test; at 95 per cent confidence interval (95% CI). Attack rate during outbreaks was calculated as described by CDC and expressed for per thousand population[20].

Results

In our study, among the investigated DEN outbreaks in four different districts of central India in 2016, three were reported from rural areas and only one from the urban part. Overall positivity was 50.4 per cent as 135 of 268 suspected individuals were tested positive for DEN. Of the 131 samples collected after five days of illness, 30 were positive for CHIKV infection and 13 were positive for both DENV and CHIKV IgM antibodies. Among 135 DEN-positive cases, 75 were tested positive by IgM while 60 by NS1 ELISA (Figure). Serotyping results showed that DENV 3 was the causative agent of outbreaks in rural parts of Damoh, Morena and Sehore districts whereas DENV 2 was responsible for the outbreak in urban areas of Katni district. One sample each was serotyped as DENV 1 and DENV 4 from Damoh and Katni. The IgG studies on NS1-positive samples showed that per cent positivity was higher in urban

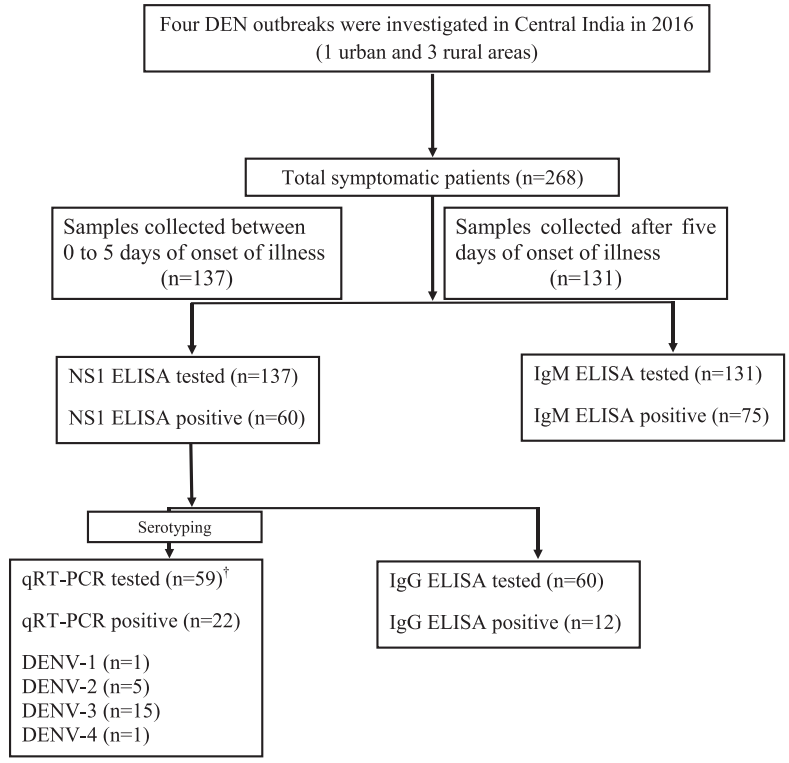

Figure. Flowchart depicting algorithm testing of samples. †One NS1-positive samples could not be tested by real-time reverse transcription-polymerase chain reaction for serotyping due to insufficient sample quantity.

Table I. Cases suspected and found positive for dengue (DEN) during outbreaks by NS1, IgM, IgG ELISA

District	Samples tested	DEN positive			Serotype detected[$]	IgG per cent[$$]	Attack rate/1000
		NS1	IgM	Total (%)			
Morena (R)	76	25	22	47 (61.84)	DENV-3	4 (1/25)	38
Sehore (R)	24	6	7	13 (54.16)	DENV-3	16.6 (1/6)	5.9
Damoh (R)	144	16	40	56 (38.89)	DENV-3	31.2 (5/16)	2.4
Katni (U)	24	13	6	19 (79.16)	DENV-2	38.4 (5/13)	0.5
Total	268	60	75	135 (50.4)			

[$]DENV1 and DENV4 detected from one sample each from Damoh and Katni; [$$]in NS1-positive cases/tested. U, urban; R, rural

areas (38.4%) than in rural areas (14.9%), indicating that secondary infections were more prevalent in urban areas. Further, the calculation of attack rates of DENV revealed that urban areas had attack rates of 0.5 per thousand population, while rural areas had attack rates ranging from 2.4 to 38 per thousand population (Table I).

Door-to-door vector surveillance of affected areas by the RRT revealed that unused containers/ utensils, cement tanks and water stored in the toilets were the main sites for mosquito breeding in rural areas; whereas, water leftover in air coolers harboured mosquito larva in urban areas. Larvae and pupae were collected from the outbreak areas and identified as *Aedes aegypti.*

The age- and gender-wise stratification of data revealed that males (75, 55.56%) were more affected than females (60, 44.4%), though the difference was not significant. Females in urban areas were found affected significantly higher than in rural areas (P<0.05). Female to male ratio was 1:0.9 in urban areas and 1:1.1 in rural areas. Of the 135 DEN-positive cases, maximum [n=78, (57.8%)] belonged to the age group of 16-45 yr (Table II).

Analysis of the symptoms revealed that fever was reported by 116 (86%), chills and rigors by 32 (23.7%), arthralgia by 23 (17%), myalgia by 16 (11.85%), retro-orbital pain was noticed in only two (1.5%) patients and maculopapular rashes and haemorrhage were also the manifestations in two (1.5%) patients each mortality was not reported.

Table II. Age and gender wise dengue positivity distribution of samples collected from DEN outbreak areas

Age group (in yr)	Male		Female		Total	
	Tested	Positive	Tested	Positive	Tested	Positive
0-5	5	1	10	3	15	4
6-15	29	15	20	11	49	26
16-45	86	48	70	30	156	78
46-60	14	8	19	11	33	19
>60	4	3	11	5	15	8
Total	138	75	130	60	268	135

Discussion

In our study, DEN positivity was found to be more than 50 per cent. Similar occurrence of cases during outbreaks has been reported from north, south and western and also from Central India in the past[11,12,21,22]. Co-infection with CHIKV has also been reported earlier[23]. We detected a higher rate of chikungunya monoinfection as compared to the study done in western India[23]. This difference in virus circulation might be due to the difference in the environmental conditions and population's susceptibility and immune response of the region.

It is well known that synergy of temperature, rainfall and relative humidity promotes abundant vector growth, thus affecting the distribution of serologically confirmed DEN cases[24,25]. *Ae. aegypti* was incriminated as the vector during outbreaks. Air coolers and water stored in toilets were the most frequently detected breeding sites, and the toilets were the most often missed breeding site by the field workers. Adults in the age group of 16-45 yr and males were predominantly affected. Most studies in India reported DEN as a disease of young adults where males outnumbered females[7,8,26]. However, Barde *et al*[12] reported that males and females were equally affected in rural MP. The social, behavioural and biological reasons for the specific age group and gender affected need to be evaluated.

Serotype of DENV is known to have a role in determining severity of the disease. DENV 2 is reported to be more severe, responsible for causing

DHF and mortalities[11,15], whereas DENV 3 was found more frequently in hospitalized patients than DENV 1 and DENV 4[16]. DENV 2 was the predominant serotype associated with outbreaks, DEN fever and DHF cases between 1970 and 2000[7,8]. However, DENV 3 is becoming the main serotype in the large outbreaks and sporadic cases of DEN since 2003 in north Indian cities such as Delhi, Gwalior and Lucknow[6,14,22]. DENV 3 has been reported as the aetiological factor for an outbreak in 2003 from Gwalior, an urban area of northern MP[14,26].

IgG antibodies for DEN can be detected for years after first exposure and detection of IgG in early phase of illness can be used to distinguish between primary and secondary infection[11,27]. Our data showed significantly high positivity of DEN IgG from urban area than from the rural areas, indicating that majority of infections in rural areas during outbreaks were primary. Further, high attack rates were observed in the rural areas; similar findings of higher attack rates during DEN outbreaks from villages of the subcontinent were reported earlies[28]. Most of the patients exhibited typical clinical picture of DEN as fever, muscular pain, joint pain, rigors, chills, rash and malaise which were found in consensus with earlier studies[26].

Our study had a limitation of limited number of samples serotyped and IgG tested. However, the important feature of this study was that out of four outbreaks investigated, three were because of DENV 3.

In conclusion, our study described epidemiological and clinical aspects related to DEN outbreaks observed in 2016 in Central India. This study also showed that DEN was no more an urban illness; it was rapidly spreading towards the rural areas of Central India with high attack rates. The continuous shift in DENV serotypes during outbreaks can lead to illness of higher severity, thus updating the relevant related information is necessary.

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***Conflicts of Interest*:** None.

References

1. Centers for Disease Control and Prevention. *Dengue and the Aedes aegypti mosquito*. Available from: *https://www.cdc.gov/dengue/transmission/index.html*, accessed on October 18, 2019.
2. Remy MM. Dengue fever: Theories of immunopathogenesis and challenges for *vaccination. Inflamm Allergy Drug Targets* 2014; *13* : 262-74.
3. World Health Organization. *Dengue and severe dengue*: *Key facts*. Available from: *https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue*, accessed on November 8, 2019.
4. Mutheneni SR, Morse AP, Caminade C, Upadhyayula SM. Dengue burden in India: Recent trends and importance of climatic parameters. *Emerg Microbes Infect* 2017; *6* : e70.
5. Ganeshkumar P, Murhekar MV, Poornima V, Saravanakumar V, Sukumaran K, Anandaselvasankar A, *et al.* Dengue infection in India: A systematic review and meta-analysis. *PLoS Negl Trop Dis* 2018; *12* : e0006618.
6. National Vector Borne Disease Control Programme. Ministry of Health & Family Welfare, Government of India; 2005. Available from: *http://nvbdcp.gov.in/*, accessed on April 12, 2017.
7. Gupta N, Srivastava S, Jain A, Chaturvedi UC. Dengue in India. *Indian J Med Res* 2012; *136* : 373-90.
8. Chakravarti A, Arora R, Luxemburger C. Fifty years of dengue in India. *Trans R Soc Trop Med Hyg* 2012; *106* : 273-82.
9. Dash PK, Parida MM, Saxena P, Abhyankar A, Singh CP, Tewari KN, *et al.* Reemergence of dengue virus type-3 (subtype-III) in India: Implications for increased incidence of DHF & DSS. *Virol J* 2006; *3* : 55.
10. Shastri J, Williamson M, Vaidya N, Agrawal S, Shrivastav O. Nine year trends of dengue virus infection in Mumbai, Western India. *J Lab Physicians* 2017; *9* : 296-302.
11. Barde PV, Shukla MK, Kori BK, Chand G, Jain L, Varun BM, *et al.* Emergence of dengue in tribal villages of Mandla district, Madhya Pradesh, India. *Indian J Med Res* 2015; *141* : 584-90.
12. Barde PV, Kori BK, Shukla MK, Bharti PK, Chand G, Kumar G, *et al.* Maiden outbreaks of dengue virus 1 genotype III in rural central India. *Epidemiol Infect* 2015; *143* : 412-8.
13. Barde PV, Godbole S, Bharti PK, Chand G, Agarwal M, Singh N, *et al.* Detection of dengue virus 4 from central India. *Indian J Med Res* 2012; *136* : 491-4.
14. Dash PK, Saxena P, Abhyankar A, Bhargava R, Jana AM. Emergence of dengue virus type-3 in Northern India. *Southeast Asian J Trop Med Public Health* 2005; *36* : 370-7.
15. Vicente CR, Herbinger KH, Fröschl G, Malta Romano C, de Souza Areias Cabidelle A, Cerutti Junior C. Serotype influences on dengue severity: A cross-sectional study on 485 confirmed dengue cases in Vitória, Brazil. *BMC Infect Dis* 2016; *16* : 320.

16. Singh N, Shukla M, Chand G, Barde PV, Singh MP. Vector-borne diseases in central India, with reference to malaria, filaria, dengue and Chikungunya. *WHO South East Asia J Public Health* 2014; *3* : 28-35.
17. Google Map. Available from: *https://www.google.co.in/maps*, accessed on February 27, 2018.
18. Barraud PJ. *The fauna of British India including Ceylon and Burma. Diptera. family culicidae: Tribes megarhinini and culicini*. Vol. 5. London: Taylor and Francis; 1934. p. 1-452.
19. Centers for Disease Control and Prevention. *DENV-1-4 real-time RT-PCR assay. Division of Vector - Borne diseasees*. Centers for Disease Control and Prevention; 12 July, 2013. Available from: *https://www.cdc.gov/dengue/clinicallab/realtime.html*, accessed on April 12, 2017.
20. Centers for Disease Control and Prevention. Principles of Epidemiology in Public Health Practice. *An introduction to applied epidemiology and biostatistics.* 3rd ed. Atlanta, GA: Centers for Disease Control and Prevention; 2012. Available from: *https://www.cdc.gov/ophss/csels/dsepd/ss1978/lesson3/section2.html*, accessed on April 12, 2017.
21. Prakash O, Singh DD, Mishra G, Prakash S, Singh A, Gupta S, *et al.* Observation on dengue cases from a virus diagnostic laboratory of a tertiary care hospital in North India. *Indian J Med Res* 2015; *142* (Suppl 1): S7-11.
22. Gupta E, Mohan S, Bajpai M, Choudhary A, Singh G. Circulation of dengue virus-1 (DENV-1) serotype in Delhi, during 2010-11 after dengue virus-3 (DENV-3) predominance: A single centre hospital-based study. *J Vector Borne Dis* 2012; *49* : 82-5.
23. Londhey V, Agrawal S, Vaidya N, Kini S, Shastri JS, Sunil S, *et al.* Dengue and chikungunya virus co-infections: The inside story. *J Assoc Physicians India* 2016; *64* : 36-40.
24. Hasan T, Bambrick H. The effects of climate variables on the outbreak of dengue in Queensland 2008-2009. *Southeast Asian J Trop Med Public Health* 2013; *44* : 613-22.
25. Biswas DK, Bhunia R, Basu M. Dengue fever in a rural area of West Bengal, India, 2012: An outbreak investigation. *WHO South East Asia J Public Health* 2014; *3* : 46-50.
26. Sharma S, Dash PK, Agarwal S, Shukla J, Parida MM, Rao PV, *et al.* Comparative complete genome analysis of dengue virus type 3 circulating in India between 2003 and 2008. *J Gen Virol* 2011; *92* : 1595-600.
27. Centers for Disease Control and Prevention. *Laboratory Guidance and Diagnostic Testing: Division of Vector- Borne Diseases*. Centers for Disease Control and Prevention; 3 August, 2017. Available from: *https://www.cdc.gov/dengue/clinicallab/laboratory.html*, accessed on January 21, 2018.
28. Norman G, Theodre A, Joseph A. An insular outbreak of dengue fever in a rural South Indian village. *J Commun Dis* 1991; *23* : 185-90.

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