Indian J Med Res 151, January 2020, pp 87-92
DOI: 10.4103/ijmr.IJMR_204_19

Quick Response Code:

Laboratory surveillance of chikungunya in Madhya Pradesh, India (2016-2017)

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Received February 4, 2019

***Background & objectives*: Chikungunya (CHIK) is a neglected, re-emerging arboviral disease. Limited information on CHIK-confirmed cases during interepidemic period is available from India. This surveillance study was conducted in Madhya Pradesh (MP), India, during the years 2016-2017, to provide information about CHIK cases.**

***Methods*: Blood samples collected from patients suspected having CHIK were tested by immunoglobulin (Ig) IgM ELISA or real time reverse transcription-polymerase chain reaction (rRT-PCR) for the detection of CHIK virus (CHIKV)-specific IgM antibodies or viral RNA, respectively. Partial envelope-1 gene sequencing was done. Clinical and demographic data were collected and analyzed.**

***Results*: Of the 4019 samples tested, 494 (12.2%) were found positive for CHIKV infection. The positivity was detected in both rural and urban areas. The mean age of CHIK-positive cases was 33.12±18.25 yr. Headache and joint pain were the most prominent symptoms, 34.6 per cent (171/494) of the CHIK cases required hospitalization and six patients with CHIKV infection died. The East/Central/South African genotype of CHIKV was found to be circulating in the study area.**

***Interpretation & conclusions*: Our study recorded a higher CHIK positivity during 2016-2017 in comparison to earlier reports from MP, India. A high proportion of CHIK cases required hospitalization and deaths were also reported, which indicated the severity of the disease in the study area. In-depth molecular analysis of the virus and other risk factors is essential to understand the trends in disease severity.**

Key words Chikungunya - CHIKV - epidemiology - genotype - severity

Chikungunya (CHIK) is a mosquito-borne viral infection, characterized by a sudden onset of fever, muscle and joint pain that is sometimes accompanied by skin rash. The symptoms, such as arthralgia, may last for months to years, especially in adults[1,2]. In 2006, the CHIK virus (CHIKV) re-emerged in India after 32 years, causing the epidemic affecting more than 1.4 million people across the 13 States, and post epidemic, a declining trend was seen till 2011[3,4]. From 2011 to 2015, the National Vector Borne Disease

Control Programme (NVBDCP) reported an average of about 20,000 suspected CHIK cases across the country every year. However, >3-fold rise in suspected CHIK cases was noticed in 2016[5].

The CHIKV has single-stranded positive sense RNA that is about 12 kb long. The virus is classified into three genotypes namely Asian, East/Central/South African (ECSA) and West African, and these genotypes are known to affect the epidemiology of the disease[6]. Prior to 1973, the Asian genotype was circulating in India, which was replaced by the ECSA genotype in 2005-2006[7].

The CHIKV infection gets lower priority in comparison to dengue (DEN) and other infectious diseases, probably because of its non-fatal outcome[8,9]. Further, countrywide limited availability of diagnostic facilities has resulted in underestimating the true burden of this disease. In the recent past, CHIKV has shown increasing severity, and the co-circulation and co-infection of CHIKV along with other infections have increased the severity of the disease[10-12]. The possible long-term impact on health, education and economic growth, per capita income, foreign direct investment and tourism makes CHIK an important clinical entity[13].

There are only a few studies depicting the epidemiology of CHIK during non-outbreak situations[14,15]. We present here the findings of laboratory-based surveillance of CHIK in Madhya Pradesh (MP), India. The finding of this study may serve as the baseline data for future studies and help programme managers, policymakers and clinicians for their endeavours in controlling CHIK in this region.

Material & Methods

The virology laboratory of Indian Council of Medical Research (ICMR)-National Institute of Research in Tribal Health (NIRTH), Jabalpur, is an apex referral laboratory of NVBDCP for DEN and CHIK in MP and Chhattisgarh[5]. Different government hospitals across MP refer blood or serum samples of the admitted and outdoor patients suspected for DEN and CHIK to this laboratory following NVBDCP case definitions[5]. A total of 4019 samples of suspected cases of DEN and CHICK referred during the period of January 2016-December 2017, were included in this study. The study was approved by the Institutional Ethics Committee (NIRTH-201601/03).

Data collection: The clinical and personal information of these cases was collected in the predesigned forms and digitized. The CHIK-positive cases were followed up to three months using telephonic communication. Further, to determine the trends, data regarding population, CHIK cases and climatic conditions were downloaded from reliable sources and analyzed[5,16,17].

Laboratory diagnosis: Samples collected in the acute phase of infection (0-5 days of onset) were tested by the Centers for Disease Control and Prevention (CDC)-developed Trioplex real time reverse transcription-polymerase chain reaction (rRT-PCR) assay that can detect DEN virus (DENV), CHIKV and Zika virus[18]. The samples collected beyond five days post onset of disease were tested for the presence of CHIKV- and DENV-specific immunoglobulin (Ig) IgM antibodies using IgM antibody capture enzyme-linked immunosorbent assay (ICMR-National Institute of Virology, Pune India). All the assays were conducted following respective manufacturer's protocol.

Sequence and phylogenetic analysis: The real-time RT-PCR-positive samples with low cycle threshold (CT) value were randomly selected and subjected to rRT-PCR as described by Naresh Kumar *et al*[19] the resulting *E1* gene amplicon of 330 bases was subsequently sequenced as described earlier[8]. The sequences were curetted using software BioEdit Version 7.0.9.0 (Tom Hall, Ibis Therapeutics, CA, USA) subjected to BLAST (*https://blast.ncbi.nlm.nih.gov/Blast.cgi*) to check the identity; these nucleotide sequences were converted to aminoacid sequences using online ExPASy translate tool (*https://www.expasy.org*) and checked for mutations. The sequences (n=10, 4 from 2016 and 6 from 2017) were submitted to GenBank, National Center for Biotechnology Information (NCBI). The phylogenetic tree of the current sequences along with earlier published CHIKV sequences (n=38) was constructed using the maximum likelihood method based on the Tamura-Nei model in MEGA5 software (*https://mega.software.informer.com/5.0/*) with 1000 bootstrap[8].

Data analysis: The data collected were segregated based on age, sex, region, season, *etc*. The age groups were based on the assumption that children below five years were mostly at home, children aged between 6 and 15 yr were school going, age group of 16-45 yr was of active/earning population of young adults and those 46-60 yr old were considered as old adults, whereas 61 yr and above were senior citizens.

The resulting data were computed using odds ratio and Student's *t* test.

Results & Discussion

During the study, a total of 4,019 samples were tested, of which 494 (12.29%) were positive for CHIKV infection. The positivity was significantly higher [odds ratio (OR): 3.07, 95% confidence interval (CI): 2.18-4.32, $P<0.001$], when compared with positivity with earlier years (2011-2015) when 4.4 per cent (37/848) samples were found positive by this laboratory (unpublished data).

In 2016, a total of 1472 suspected case samples were received from 25 districts, of which 164 (11.1%) samples from 18 districts were found positive. In 2017, a total of 2547 samples were received from 24 districts, of which 330 samples (12.9%) from 20 districts were found positive. The positivity increased during 2017; however, it was not significant. Segregation of the data to determine CHIK cases in rural and urban areas revealed higher positivity in urban (56.07%, n=277) areas than rural (43.92%, n=217) areas.

An increase was observed in suspected and laboratory-confirmed CHIK cases in MP in 2016-2017, and our findings were in concordance with the reported cases by the NVBDCP[5]. Limited epidemiological information during interepidemic periods regarding confirmed CHIK cases was available mainly because of limited diagnostic facilities and non-referral of samples for CHIK by the clinicians. Detection of CHIK cases from both rural and urban areas of more than 20 districts of MP suggested the widespread distribution of this infection in MP.

In 2016, 48 cases were found positive for both DENV and CHIKV IgM antibodies and one case was positive for DENV and CHIKV RNAs. During 2017, 37 cases were detected with DENV and CHIKV IgM antibodies, whereas DENV and CHIKV RNA was detected in 20 samples. This indicated that both these viruses were co-circulating and co-infecting the human population. The mean age of CHIK-positive cases was 33.12±18.25 yr (males: 32.11±18.78; female: 34.26±17.59 yr). Among the positive cases, 57.89 per cent (286/494) belonged to the age group of 16-45 year, while 4.86 per cent (24/494) belonged to the age group of 0-5 yr (Table). Similar findings were recorded during outbreaks from other parts of the country[20,21]. Probably, the age group of 16-45 yr is at higher risk of exposure to the day biting *Aedes aegypti* in home as well as at schools, colleges and their workplaces. Although more males (257/1999, 12.85%) were positive for CHIKV infection than females (237/2018, 11.74%), the difference was not significant (Table). Similar observations have also been reported from other parts of the country[20,22].

Among the total 494 CHIK-positive cases, fever was recorded in 418 (84.6%), followed by headache in 232 (46.9%), chills in 218 (44.1%), arthralgia in 193 (39%), malaise in 183 (37%), rigors in 178 (36%) and body rash in 35 (7%) patients. While 21.3 per cent (35/164) of CHIK-positive patients required hospitalization in 2016, 41 per cent (136/330) were hospitalized in 2017, with the average hospitalization days being 7.9±4.1. One hundred and thirty seven CHIKV-positive cases (adults=117, children=20) could be followed up, of whom six patients died and the rest recovered completely and had no CHIKV-related symptoms; however, joint pain for several weeks was the prominent complaint particularly among the adults (71.7%) than in children (20%). Of the six patients who succumbed to death, two had cancer as an underlying condition; two were tested positive for both CHIKV and DENV by IgM antibodies. No underlying condition was determined in the remaining two patients.

Table. Age- and sex-wise chikungunya positivity in 2016 and 2017

Age group (yr)	Male		Female		Total	
	Tested	Positive (%)	Tested	Positive (%)	Tested	Positive (%)
0-5	97	12 (12.37)	114	12 (10.53)	213	24 (11.27)
6-15	266	20 (7.42)	275	28 (10.18)	541	48 (8.87)
16-45	1223	152 (12.43)	1181	134 (11.34)	2404	286 (11.90)
46-60	257	44 (17.12)	282	39 (13.83)	539	83 (15.40)
61 and above	156	29 (18.59)	166	24 (14.46)	322	53 (16.46)
Total	1999	257 (12.85)	2018	237 (11.74)	4019*	494 (12.29)

*Sex of one suspected case was not available and one was transgender

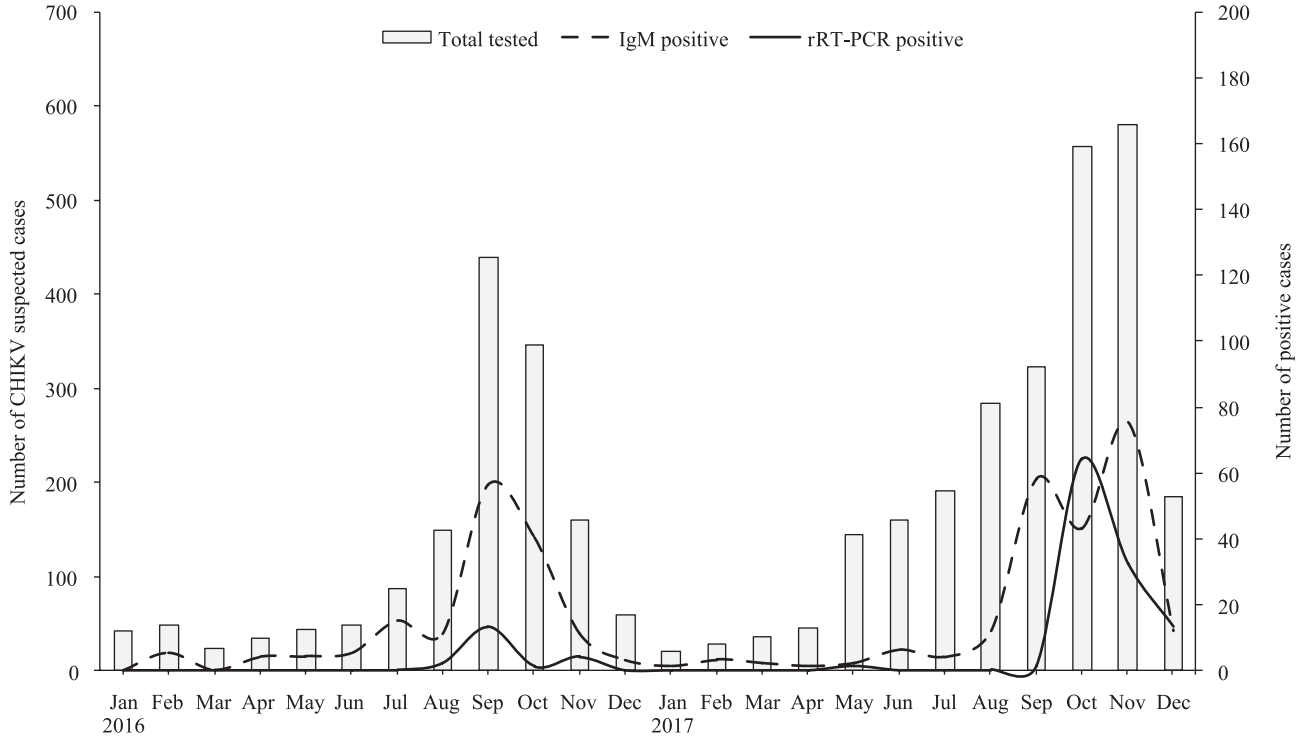

Fig. 1. Graph showing month-wise chikungunya-positive cases; month and year are shown on the X-axis, the number of tested cases are shown on the Y-axis and positive cases are shown on secondary axis.

CHIKV-positive cases were recorded throughout the year, but the cases were significantly higher during the monsoon and post-monsoon period (Fig. 1). Further, rRT-PCR-positive cases were detected only during the monsoon and post-monsoon period in 2016. Interestingly, we detected one and 13 rRT-PCR-positive cases in the months of May and December in 2017, respectively (Fig. 1), IgM positivity was also increased significantly during winter (November-December) of 2017 in comparison to the same period in 2016 (OR: 1.63, 95% CI: 1.28-2.06; *P*=0.001) (Fig. 1). The monsoon and the post-monsoon season are recorded to be favourable for breeding of *Ae. aegypti* mosquitoes in the area[16]. Our observation regarding rRT-PCR positivity in the monsoon and post-monsoon season indicated that campaign to reduce mosquito breeding sites before the monsoon and intensified anti-mosquito measures during the monsoon would be helpful in lowering vector density, which, in turn, would help in reducing disease burden. CHIK rRT-PCR-positive cases were deleted in December 2017, which were not detected in December 2016. Environmental conditions such as rainfall, humidity and temperature are known to affect the occurrence of CHIK cases[23]. However, there was no significant difference in the study area in rainfall, humidity and temperature in 2017 when compared with that of earlier years[16]. The average temperature in May 2017 was 35±1.9°C and in December, it was 19.5±1.8°C, not favouring the mosquitogenic conditions.

Gene sequences from 10 samples (GenBank Accession No. KY315505-KY315508 and MG934546-MG934551) were obtained. Certain synonymous mutations in CHIKV partial *E1* gene were detected in comparison to the virus detected in 2011 (GenBank Accession No. JX911895). The sequencing analysis of partial *E1* gene revealed that the circulating CHIKV belonged to ECSA genotype (Fig. 2). The genetic studies in the past showed that prior to 2000, Asian genotype of CHIKV was circulating in India and it was replaced by ECSA genotype in 2005-2006[24]. The ECSA genotype of CHIKV is shown to produce severe and long-lasting symptoms, even deaths and thus have public health implications[10,12,25]. Since its first detection in 2005, several mutations have been reported in the CHIKV genome[6,24]. It will be worthwhile undertaking full-genome studies to understand polymorphism in CHIKV circulation in Central India.

Our study had a few limitations such as failure to conduct genome-wide sequencing and to test suspected cases, and data were collected only from 24 districts

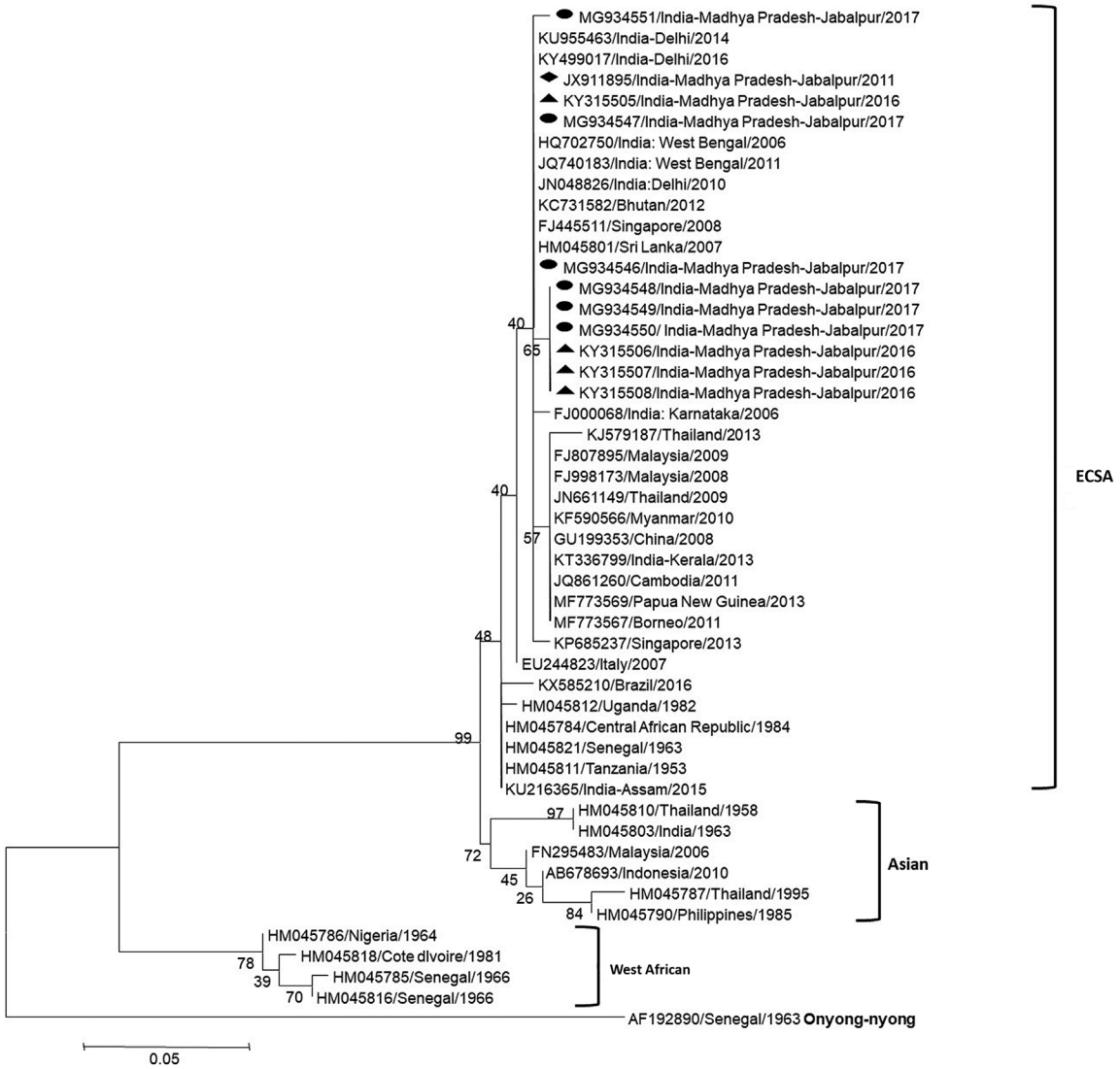

Fig. 2. A phylogenetic tree of chikungunya virus *E1* gene sequences of 2017 (with circle symbol and in bold) was constructed by using the maximum likelihood method based on the Tamura-Nei model in MEGA5 software. The analysis involved four chikungunya virus *E1* gene sequences (with triangle symbol) of 2016 and one (with diamond symbol) of 2011 submitted from Jabalpur, India, and other 38 reference sequences downloaded from National Center for Biotechnology Information database. The strains are represented by their GenBank accession number followed by the country of origin along with State and city followed by the year. Onyong-nyong virus was used as the outgroup.

of MP. On the other hand, depicting CHIK situation in MP, India, by testing more than 4000 samples and documenting year-round data for the period of two years were the strengths of this study.

In conclusion, our study showed that CHIK is an important emerging problem in urban and rural areas of MP, India. Early diagnosis will help in the timely management of patients and quick interventions to control outbreaks. These data would be useful for the preparedness and interventions for the future CHIK outbreaks in the State.

***Acknowledgment*:** Authors thank Servshri Mohan Shukla for his help in sequencing and Santosh Jadhav and Nikilesh Pacharane for assisting in data management. Authors acknowledge the help received from Dr L. Shivlata in molecular analysis, and thank the State Directorate of Health Services, Government of Madhya Pradesh, and doctors and patients of medical colleges and district hospitals.

***Financial support & sponsorship*:** This work was supported by funding from the Centers for Disease Control and Prevention (CDC), USA (cooperative agreement, Grant #6 NU2GGH001903-02). The last author (PVB) acknowledges the Indian Council of Medical Research (ICMR) (Grant No. VIR/43/2011-ECD-I) and National Vector Borne Disease Control Programme (NVBDCP), Directorate of Health Services,

Government of India, New Delhi, for financial support and providing Dengue and Chikungunya IgM kits, respectively. Authors thank the authorities of the CDC, USA and NIV, Pune, India, for providing Trioplex real-time RT-PCR kits and Dr Kayla Laserson for constant support.

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

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