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Chikungunya is Declining in West Bengal, India- A Retrospective study (2010-2014)

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Abstract

Re-emergence of Chikungunya virus in West Bengal was detected almost after 40 years when an outbreak of fever occurred in Baduria village (West Bengal, India) in October 2006. After its re-emergence, Chikungunya virus infection/Chikungunya fever unexpectedly spread in the form of devastating epidemics all over the West Bengal which was associated with high fever, crippling joint pain and debilitating arthritic by joint pain that may last for a long time after resolution of infection. Blood samples from clinically Chikungunya suspected cases are routinely referred to the ICMR Virus Unit Kolkata for the diagnosis of Chikungunya infection as it is an Apex Referral Laboratory of National Vector Borne Disease Control Programme {NVBDCP}, Delhi for the detection of Chikungunya in the Eastern part of India. Here we report the activity of Chikungunya from the year 2010-2014. During this period a total of 3573 samples were received from different medical colleges and hospitals of West Bengal. Based on serological study, only 934 (26.14%) samples were positive to Chikungunya IgM antibody by ELISA method. Rest of the 2639 samples were screened and only 755 Chikungunya IgM negative acute samples having ≤ 4days of illness were subjected to RNA extraction, RT-PCR test followed by gel electrophoresis for molecular detection. Only 83 samples (10.71%) produced prominent band at par with the control strain, i.e; 354bp. In the years 2014, RT-PCR test could not detect the Chikungunya specific RNA. Slightly higher percentile positivity was observed amongst the females than males. All most all the age groups were affected. Age group specific attack rate was variable. Even then, highest attack rate was observed in the adult age group, mainly 31-40 followed by age group of 21-30, 41-50, 51+, 11-20 and 0-10 in the every year. It was observed that incidence of Chikungunya infection were much less than it was observed since its re emergence. This study established that the activity of Chikungunya virus in West Bengal is in a declining phase.

Keywords: Chikungunya, Decline, West Bengal.

Introduction

Chikungunya virus (CHIKV) is an arthropod-borne virus (arbovirus) belonging to the genus *Alpha virus* of the *Togaviridae* family[1]. CHIKV was first isolated in Tanzania in 1953[2]. Chikungunya fever (CHIKF) derives its name from Makonde, a language spoken in south Tanzania, and means "that which bends up", referring to the posture of patients afflicted with severe joint pain/ arthritic symptoms characterizing this infection[3]. *Aedes aegypti* and *Aedes albopictus* are the main vectors of CHIKV in Asia and in the Indian Ocean Region[4]. Two distinct transmission cycles have been well documented for CHIKV: an enzootic sylvatic cycle and an endemic/epidemic urban cycle[5]. Human beings serve as reservoir hosts for the virus during epidemic periods whereas during inter-epidemic periods, several other reservoir hosts have been incriminated such as monkeys, rodents and birds[6]. Two stages of the disease are now described: Symptomatic acute illness and the late stage of illness, with persistent arthropathy[7].

After infection with CHIKV, there is a silent incubation period, lasting about 2-4 days (might be1-12 days). Symptomatic patients generally report an abrupt onset of disease, characterized by high fever, poly arthralgia (most characteristic symptom), backache, joint pain in peripheral and large joints (polyarticular, bilateral, symmetrical); headache, fatigue, myalgia predominantly in the arms, thighs and calves without myositis, maculopapular rash predominating on the thorax, facial oedema, bullous rash (children) with pronounced sloughing, localized petechiae and gingivorrhagia are observed[7]. These clinical symptoms appear within 4–7 days from the date of infection. The incidence of CHIKV infection amongst atypical cases, severe cases and hospitalization rate increased with age[7]. In pediatric populations, newborns have a high risk of severe disease. Although comorbidity and increase of age are often linked, underlying respiratory diseases, the use of NSAIDs, hypertension, cardiac disorders and alcohol abuse were associated with increased disease severity[7]. Since the first report, CHIKV attracted worldwide attention when it caused a massive outbreak in the Indian Ocean islands and most cases of

CHIKV infection have been described in Africa and India[8]. Major epidemics appear and disappear cyclically, usually with an inter-epidemic period ranging from 7 to 20 years[9]. A distinctive feature of Chikungunya virus is that it causes explosive outbreaks, before apparently disappearing for a period of several years to decades[3]. In India, epidemics of Chikungunya fever were reported during the last millennium *viz*, 1963 in Kolkata, West Bengal; 1965 in Chennai, Tamil Nadu and 1973 in Barsi, Maharashtra[10-13]. Thereafter, sporadic cases continued to be recorded specially in Maharashtra state during 1983 and 2000[3]. During 2005-2006, 1.4 million Chikungunya cases were reported from different states[14]. States affected by the outbreaks included Andhra Pradesh, Karnataka, Maharashtra, Orissa, Kerala, Tamil Nadu, Andaman & Nicobar Islands, Gujarat, Madhya Pradesh and Delhi[14-18]. The highest incidence rates were reported from the bordering southern Indian Federal states of Kerala and Tamil Nadu[19]. The entire isolated virus up to this period belonged to the Asian genotype[20]. In India, the first CHIKV outbreak was recorded during 1963–1965 in Kolkata (formerly Calcutta)[21]. After that, CHIKV totally disappeared from West Bengal and reemerged in the year 2006[22].

No licensed vaccine against Chikungunya is commercially available, but several strategies are under study. Considering the capacity of CHIKV to emerge, re-emerge, and quickly spread in novel areas, heightened surveillance and preparedness seem to be a priority. In particular, travellers act as carriers who inadvertently ferry pathogens between countries. They can thus serve as a sentinel population providing information on the emergence or re-emergence of an infectious pathogen[23].

Indian Council of Medical Research (ICMR) virus unit Kolkata first detected the reemergence of CHIKV in West Bengal in 2006[22]. Since then it is engaged for the systematic investigation for the detection of Chikungunya infection in West Bengal. The activity of CHIKV in West Bengal from 2006-2009 has been published elsewhere[22]. This paper deals with a retrospective study on comprehensive distribution and determination of Chikungunya cases from 2010-2014 in West Bengal.

Materials and Methods

Geographic and Meteorological Data: West Bengal is a state in the eastern part of India and is the nation's fourth-most populous state, with over 91 million inhabitants with an area of 34,267 sq mi (88,750 km^2). Population density is 1,000/km^2 and bordered by the countries like Bangladesh, Nepal and Bhutan. The Indian states of Odisha, Jharkhand, Bihar, Sikkim, and Assam also encircle on this state. Frequent infiltration of citizens from other countries/states takes place crossing the border which may facilitate the emergence of new infection. Approximately 7.5% of the total population of the India lives in this state. Kolkata ($22^0$82' N, 88^0 80'E) is the capital of West Bengal and seventh largest metropolitan city in area and population in the world. Kolkata experienced the first epidemic of Chikungunya in 1963–1965. The city has an international seaport and international airport. In addition two big railway station (Sealdah, Howrah), which are the busiest in the world, serves as the gateways of the city. The River Ganges partitions the state of West Bengal into two regions; the northern region known as North Bengal and the southern region known as South Bengal. The state consists of 20 districts, of which Darjeeling, Jalpaiguri and Cooch Behar are located in the hilly and cold climatic regions. The main seasons are summer, monsoon, autumn, late autumn and winter. The summer lasts from mid March to mid June, with the temperature ranging from 38 ◦C to 45 ◦C. The monsoon arrives by the middle of June and lasts up to September. A high humid temperature prevails in these two seasons which is favorable for viral growth. The breteau index of the vector mosquitoes, *Aedes agypti* and *Aedes albopictus* are moderately high in Kolkata during these two seasons which serve as the principal/potential vector for Chikungunya.

Patient and Clinical Samples: The ICMR Virus Unit Kolkata functions as an Apex Referral Laboratory for the detection of Chikungunya infection in the eastern part of India. For this reason blood samples from clinically suspected Chikungunya cases along with a short history of illness and duly signed consent form of patients are routinely referred to the ICMR Virus Unit from out patient's dept (OPD) and indoor of different medical colleges as well as different hospitals and private practitioners for the detection of Chikungunya infection, if any. The inclusion criteria which were initially considered for the diagnosis of Chikungunya infection are: high fever, headache, back pain, myalgia, fatigue, joint pain, arthralgia pruriginous maculopapular rash, facial oedema, bullous rash (in children), petechiae and gingivorrhagia. In this study, apart from fever, any two of these criteria were considered. The samples after excluding the possibilities of prokaryotic and bacterial infection by investigation at respective hospitals were referred to ICMR virus unit. This study was duly approved by the institutional ethical committee.

Sample Collection: Approximately 4-5ml blood samples from clinically suspected cases were collected by venous puncture by the health workers and medical technicians. During these five years of study period (2010-2014) a total of 3573 samples were thus received along with a short history of illness. All the samples were transported to ICMR Virus Unit Kolkata, maintaining the cold chain for the detection of Chikungunya infection, if any. The sera/serum were separated by centrifugation at 1500 rpm for 10 min at 4°C and kept at -80° C in aliquots until tested.

Methods employed for the diagnosis of Chikungunya virus infection were serological method by detecting Chikungunya specific IgM antibody by enzyme linked immunosorbent assay (ELISA) and molecular methods by RNA extraction, RT-PCR which is very sensitive and confirmative methods followed by 2% agarose gel electrophoresis.

Serology: All the 3573 samples were tested for the detection of Chikungunya IgM antibody by Chikungunya specific IgM

capture ELISA (Mac ELISA) method, using a kit (prepared by National Institute of Virology, Pune, India) following the prescribed protocol OD was measured at 450nm using an ELISA reader (Thermo scientific multi scan EX).

RNA extraction: For this purpose out of 3573 samples, only 775 Chikungunya IgM negative acute serum samples, having ≤4 days of illness were screened. Attempts were made to isolate the viral RNA from negative control (Nuclease free water), positive control (Gene Bank Accession No-EF027140.1,obtained from the National Institute of Virology, Pune, India) and directly from the screened samples using a Qiagen viral isolation kit (Qiagen, GmbH, Hilden, Germany) with a slight modification of manufacture's protocol.

One step RT-PCR: Reverse transcription and amplification were conducted in a single reaction tube by the procedure described elsewhere using a highly conserved primer pair for the NSP1 gene[22].

Agarose Gel Electrophoresis: RT-PCR products were analyzed by running 2% agarose gel stained with ethidium bromide and examined under ultraviolet light using a digital gel documentation system. The expected size of the external RT-PCR products is 354bp.

Results and Discussion

ELISA: During the five years of study period a total of 3573 samples were received, of which highest number of cases were referred in the year 2012 followed by 2014, 2010, 2011 and 2013.In our 5 years study period (2010-2014), amongst the 3573 common febrile cases, CHIKV infections were detected in 934 samples (26.14%) by ELISA method of which 392 male and 542 female were positive.

Statistical analysis by t-test was employed to determine significant activity of Chikungunya infection amongst the 3573 common febrile cases in West Bengal. A P-value of <0.05 was considered significant. Calculations were done by Microsoft excel.

RT-PCR: From year 2010-2014, amongst the 3573 common febrile cases, 775 samples were screened for the detection of CHIKV RNA by RNA extraction, RT-PCR and followed by agarose gel electrophoresis.

These screened samples were Chikungunya IgM negative acute samples and having <4 days of illness of which 83 samples (10.71%) were positive by RT-PCR or producing prominent band at 354bp in 2% agarose gel electrophoresis which corresponded with control strain of CHIKV(Figure-1).

To determine significant activity of Chikungunya infection, normal deviate tests were performed using the t-test. A P-value of <0.05 was considered significant. Calculations were done by Microsoft excel.

Table-1
Year wise distribution of Chikungunya positive cases by ELISA methods.

Year	No. Collected/ referred	Number Tested	Number Positive		Total Positive	Percentage Positivity	t-test
			Male	Female			
2010	796	796	124	190	314	39.45%	p-value= 0.014421
2011	365	365	68	106	174	47.67%	
2012	1278	1278	148	170	318	24.88%	
2013	236	236	15	31	46	19.49%	
2014	898	898	37	45	82	9.13%	
Total	3573	3573	392	542	934	26.14%	

According to the statistical analysis by t-test, 26.14% Chikungunya IgM ELISA positivity is significant (p-value: 0.014421; <0.05) respect to total 3573collected/referred common febrile cases. Null hypothesis rejected

Table-2
Year wise distribution of Chikungunya positive cases by RT-PCR methods.

Year	No. Collected/ referred	Number Tested	Number Positive		Total positive	Percentage Positivity	t-test
			Male	Female			
2010	796	181	27	36	63	34.81	p-value= 0.001481
2011	365	81	06	07	13	16.05%	
2012	1278	171	02	03	05	2.9%	
2013	236	149	01	01	02	1.43%	
2014	898	193	00	00	00	00	
Total	3573	775	36	47	83	10.71%	

The total of 83 samples (10.71%) were Chikungunya positive by RT-PCR is significant (p-value**:** 0.001481; <0.05) respect to total 775 screened samples by t-test. Null hypothesis rejected.

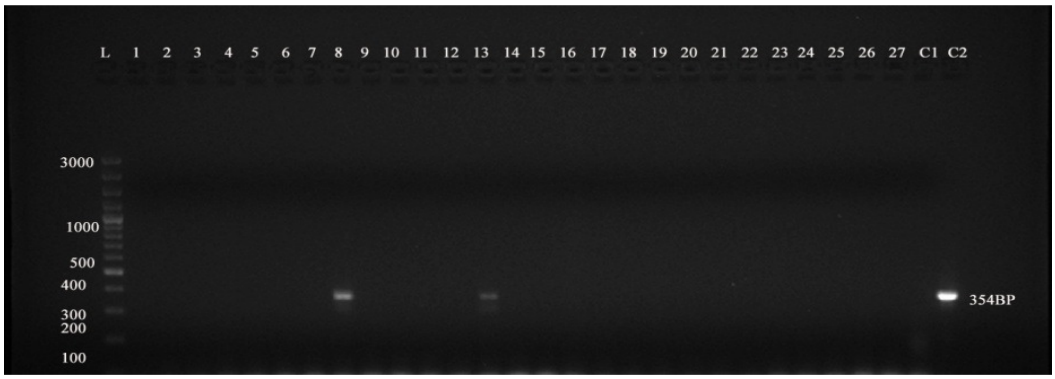

Figure-1
Agarose gel (1.5%) electrophoresis showing band at 354bp, of conserved region ofNSP1of CHIKV. Lane: L-Ladder (GeneRular 100bp Plus DNA Ladder), C1-Negative control, C2-Positive control (634029), Lane 1-27: Virus specific band of patient's samples

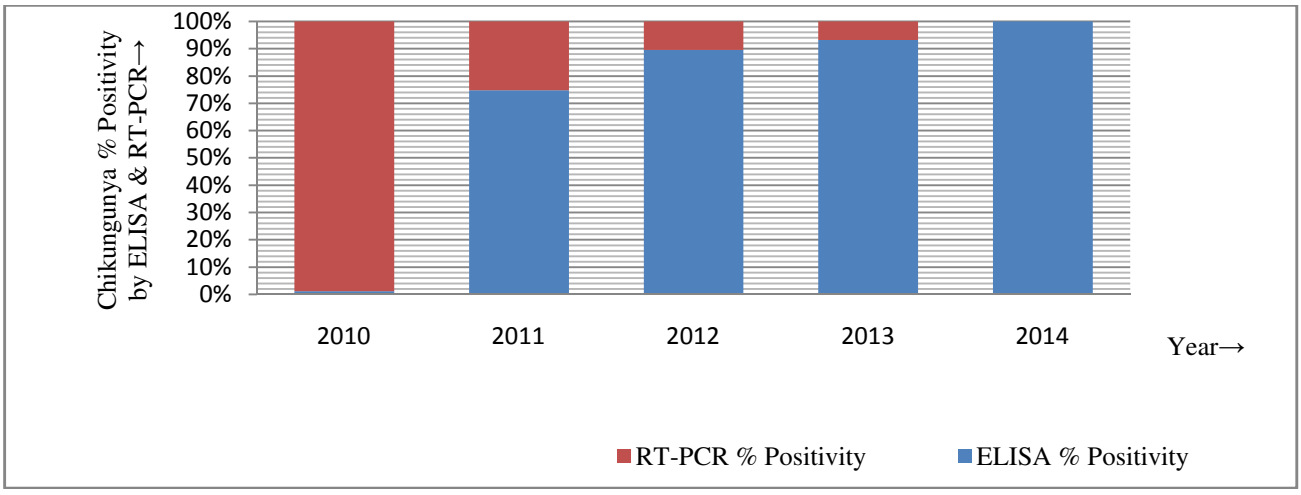

Figure-2
Year wise distribution of Chikungunya percentage positivity by ELISA and RT-PCR method in West Bengal, during study period (2010-2014)

The graphical distribution reveals that, in every year percentage positivity by ELISA was higher than the RT-PCR method. Although IgM antibody was detected every year, but CHIKV RNA could not be detected in the year 2014. It is due to the sharing or cross reactivity of IgM antibody among the other arbo viruses. This result reveals that although CHIKV is still sporadically circulating in West Bengal, but presently it tends to a gradual declining phase.

Table-3
Year and Gender wise distribution of Chikungunya Positive cases in West Bengal during study period (2010-2014)

Year	Male			Female		
	ELISA	RT-PCR	Total	ELISA	RT-PCR	Total
2010	124	27	151	190	36	226
2011	68	06	74	106	07	113
2012	148	02	150	170	03	173
2013	15	01	16	31	01	32
2014	37	00	37	45	00	45

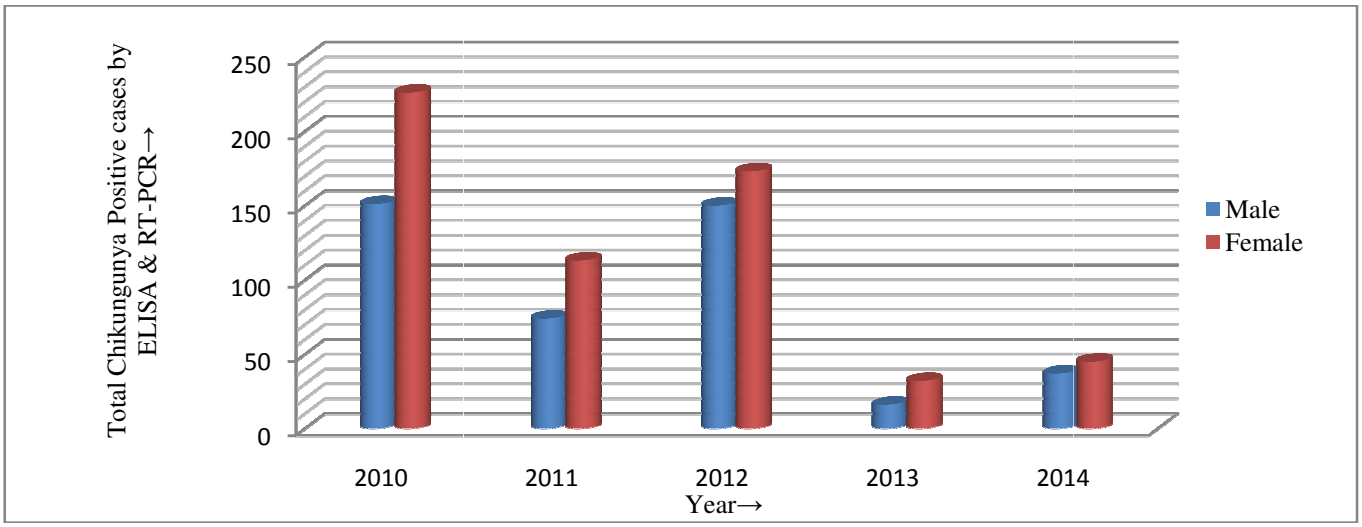

Figure-3
Gender wise distribution of dengue positive cases by ELISA and RT-PCR methods (2010-2014)

Table-4
Year and Age wise distribution of total Chikungunya positive cases (ELISA+ RT-PCR), in West Bengal during study period (2010-2014)

Year	0-10	11-20	21-30	31-40	41-50	51+	Total (ELISA + RT-PCR)
2010	9	29	77	103	76	83	314+63= 377
2011	4	14	38	52	38	41	174+13=187
2012	7	25	65	89	66	71	318+5=323
2013	11	12	8	9	6	2	46+2=48
2014	6	23	20	15	7	11	82+00=82

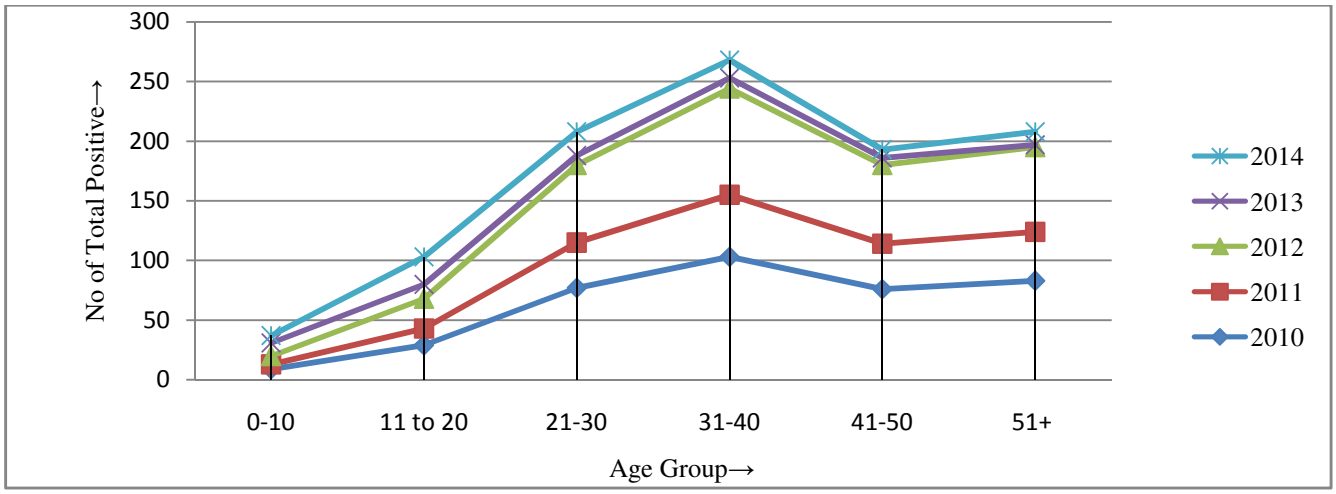

Figure-4
Year and Age wise distribution of total Chikungunya positive cases (ELISA+ RT-PCR), in West Bengal during study period (2010-2014)

During the 5 years study period (2010-2014), it was observed that in every year females were much more affected than the males. It is possibly due to the fact that the vector mosquito *Aedes* is domestic and peri domestic in nature and the females usually reside in the house much longer than the males and thus get exposed largely.

During 5 years study period all most all age groups were affected by Chikungunya infection. The age wise attack rate was variable in different years. Even then, highest attack rate was observed in the adult age group, mainly 31-40 followed by age group of 21-30, 41-50, 51+, 11-20 and 0-10 in the every year.

Discussion: Chikungunya infection is a growing health problem all over the world, as the cyclical incidence of Chikungunya outbreak is increasing. During any outbreak or epidemic, high attack rate is observed. Generally irrespective of age and sex, all are affected by CHIKV infection. In India the first epidemic due to CHIKV was recorded in the city of Kolkata in 1963 which continued up to 1965 [21]. Since then to 2005 no cases of CHIKV were reported from Kolkata or any other places in West Bengal. In 1995 a serological survey conducted on the population of Kolkata revealed the presence of negligible percentage of antibody against CHIKV, that too in the age group of >50 years which amply confirms the infection in the remote past [24]. After a span of more than four decades (44years) the CHIKV re emerged in West Bengal in the year 2006 at Baduria (dist-north 24 parganas), an adjacent village to Bangladesh as well as very close to Kolkata airport[22]. The possibility of re emergence of CHIKV might have been initiated by the travelers. Due to the lack of any herd immunity the disease spread all over the state very rapidly. The state of West Bengal, as a whole is a dengue prone zone and the vector mosquito *Aedes sp* are also abundant here. On the other hand the same vector is also responsible for the spread of CHIKV. Since its reemergence, a striking feature was observed that dengue cases were decreased with the increasing number of Chikungunya cases[22].

During the five years of study period a total of 3573 samples were received, of which highest number of cases were referred in the year 2012 followed by 2014, 2010, 2011 and 2013.In our 5 years study period (2010-2014), amongst the 3573 common febrile cases, CHIKV infections were detected in 934 samples (26.14%) by ELISA method of which 392 male and 542 female were positive. According to the statistical analysis by t-test, 26.14% Chikungunya IgM ELISA positivity is significant (p-value: 0.014421; <0.05) respect to total 3573 collected/referred common febrile cases. Only 83 samples (10.71%) out of 775 screened samples produced prominent band at par with the control strain, i.e; 354bp. The total of 83 samples (10.71%) were Chikungunya positive by RT-PCR is statistically significant (p-value: 0.001481; <0.05) respect to total 775 screened samples by t-test.

The key finding of the present study is that although CHIKV is still circulating in West Bengal but it tends to a gradually declining state. It may be explained by the fact that the populations have acquired the necessary protective immunity against CHIKV. During 5 years study period all most all age groups were affected by Chikungunya infection. The age wise attack rate was variable in different years. Even then, highest attack rate was observed in the adult age group, mainly 31-40 followed by age group of 21-30, 41-50, 51+, 11-20 and 0-10 in the every year. The study also revealed a higher attack rate particularly among females than male which has been corroborated by many other workers.

In the years 2014, RT-PCR test could not detect the Chikungunya specific RNA. This may be possibly due to the unprotected transportation or during thawing of the samples, which might have damaged the viral RNA. This five years investigation only repots referred cases and represent the tip of the ice barge in the population. Total cases are much higher than those dealt with. The clinical features of CHIKV cases almost

mimic dengue infection. In the case of any unknown fever outbreak care should be taken to identify the etiological agent. the cumulative reports for 5 years has revealed that highest number of CHIKV positive cases were detected in the month of November, when the vector density is very high in this state. Similarly observation has been reported from other parts of India. The slow but steady activity of CHIKV needs further in-depth study. Considering the capacity of CHIKV to emerge, reemerge and quick spread all over the country, heightened surveillance and preparedness should be given priority.

Conclusion

The present study reveals that CHIKV is presently at a decline state in West Bengal. The activity of CHIKV over the five years period, reported from West Bengal, is almost same from other part of India. This disappearance or silence of the virus should not be ignored. The re emergence and epidemics are unpredictable phenomena but the impact of such events can be ameliorated by appropriate knowledge and by being in the right state of preparedness. Continuous surveillance on the activity of CHIKV should be taken carefully as when it re-emerges; it takes a devastating and explosive in nature.

Author's Contribution: TK and SC participated in the conception and design of the study. TK standardized and carried out the clinical assessment, immune assays and molecular testing. ASP carried out statistical analysis and interpretation of the data. SC and TK drafted the manuscript. All authors read and approved the final manuscript.SC is guarantor of the paper.

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