

# Chikungunya Outbreak in Nagpur: A Prospective Study during July 2024

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**Running Title:** Chikungunya in Nagpur – India

**Abstract:** Aim: Chikungunya is a mosquito borne disease caused by chikungunya virus, a re-emerging epidemic disease, this study shows the outbreak of chikungunya in Nagpur in India and the utility of RT-PCR for the diagnosis of CHIKV. Materials and Methods: A Prospective study conducted at Mani Microbiological Laboratory Pvt Ltd., Dhantoli, Nagpur. In this study, patients in the age of 1 year old to 67 years old had the symptoms of chikungunya were admitted to hospital in July 2024. EDTA Blood samples were collected and diagnosed with HRM-A RT-PCT (High resolution melt-curve analysis, Real time polymerase chain reaction) technology. The data were analyzed and portrayed in this study. Results: A total of 91 samples screened for Chikungunya out of 91 cases 58 (63.73%) of cases were diagnosed positive for Chikungunya by RT-PCR, out of which 37.93% of individuals were adults above the age of 40. Huge positives were tested in the 4<sup>th</sup> week of July 2024 had 40 positives. And almost all had the symptom of fever, head ache and body pain. Conclusion: An alarming rate of increasing CHIKV in world-wide distribution, it can lead to spread over the new region and cause CHIKF and febrile illness. Every individual need to know the cause of mosquito borne diseases, by draining and washing the containers, tanks; prevent the stagnant water; growing fishes in well, ponds may eat the larvae of mosquito. Knowledge about the dry day has to be practiced in growing population. In this article, we present the outbreak of Chikungunya infection in Nagpur, July 2024, India.

**Keywords:** Chikungunya fever (CHIKF), Chikungunya virus (CHIKV), Polyarthralgia, Epidemiology, Disease resolution

## 1. Introduction

Chikungunya infection, a mosquito borne disease and causes endemic in tropical regions like India. It is a re-emerged disease over past 2 decades and infected over a million of people per year. Chikungunya fever caused by CHIKV pathogen (family: Togaviridae; genus: alphavirus). CHIKV not known before the early 19<sup>th</sup> century, in early 1950s the CHIKV first recognized in East Africa.[1] It has three different genotypes: Asian, West African & East central South African. CHIKV is a positive sense, single stranded RNA virus.[2] Further ECSA divided into sub-lineage Indian Ocean lineage (IOL). First outbreak of CHIKV in 1952 in Tanzanian as a debilitating arthritic disease. Based on the Kimakonde words for "to become contorted. Symptoms include fever and joint pain. These typically occur two to twelve days after exposure. Other symptoms may include headache, muscle pain, joint swelling, and a rash. Symptoms usually improve within a week; however, occasionally the joint pain may last for months or years. Subsequently, symptoms develop after 2–6 days of incubation, including high fever and severe arthralgia. Overlapping symptoms and common vector with dengue and malaria present many challenges for diagnosis and treatment of this disease.[3] Humans and other animals are exposed to arboviruses by getting bitten by haematophagous arthropods. The Bunyaviridae, Togaviridae, Flaviviridae, Reoviridae, and Rhabdoviridae are the five primary Arbovirus families that cause infections in humans and other animals. Dengue virus, Mayaro virus, yellow fever virus, west Nile virus, Rocio virus, Saint Louis encephalitis virus, Venezuelan equine encephalitis virus and Oropouche virus are the other arbovirus species that related to cause disease in Human beings. [4] In 1963, Chikungunya was first time reported in India. After a period of 32 years, In 2005, Chikungunya re-emerged in India. Currently, every part of the country has

become endemic for the disease with outbreaks resulting in huge economic and productivity losses. Several mutations have been identified in circulating strains of the virus resulting in better adaptations or increased fitness in the vector(s), effective transmission, and disease severity.

In India, Maharashtra state is the third most crowded by population and second largest state by land area. A huge population of over 112.4 million (2011 census), it's about 9.28 % with total population. Monsoon: June – October, Winter: October – February and Summer: February – June in that region. [5] Environmental circumstance is suitable for the growth of *Aedes aegypti*. National Health Mission observed lots of outbreaks in India, In January 2024 – 79 cases, February – 72 cases, March – 36 cases, April – 46 cases and 11 cases in May month. CHIKV infection also observed in other parts of India i.e. Karnataka, Tamil Nadu and Andhra Pradesh. During the period 2005 – 2017, CHIKV epidemiology were wide spread over the Indian subcontinent and southeast Asian regions, around 6.11% of suspects to positive. [6] Re-emergence of CHIKV with E1-A226V mutation had huge impact in health care settings. [7] In 2006, ESCA CHIKV strain as causative agent in Delhi for first time. Whole genomes of viruses were sequenced, and between 2005 and 2020, complete genomes stored in the Virus Pathogen Database and Analysis Resource (ViPR) were used to perform genome-scale phylogenetic & evolutionary analyses of CHIKV strains originating from mosquitoes and humans from all over India. [8]

In comparison to the east as well as northeast parts of India, the southern, northern & regions have greater rates of CHIKV transmission. Nonetheless, a greater percentage of CHIKV-susceptible people in the eastern along with northeast regions points to these areas' potential for future outbreaks. [9] The first outbreak of Chikungunya in India

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was in 1963 in Calcutta and the infected patients with atypical symptoms; in 1973 again an outbreak in India, moreover 37.5% was the morbidity rate in Barsi, Maharashtra state. In 1970s Asian genotype was observed in India; later in 2005 and 2008 a re-emerged CHIKV with ECSA lineage; south Indian state of Kerala in 2007, E1: A226V was observed. Presently, there are no licensed vaccines or antivirals available; however, India has initiated several efforts in this direction including traditional medicines. [10-13]

## 2. Methods

Microbiological Laboratories, Nagpur located in the state of Maharashtra, India. EDTA Blood or plasma samples were collected from the co-operative hospitals and labs, patients suspected with tropical fever or vector borne disease. An overall of 91 patient specimens were diagnosed with-in July 2024, samples collected from the symptomatic and suspected patients were processed within 24 hours. A minimum of 1.5 ml of Ethylenediaminetetraacetic acid blood specimen used for processing. Specimens were tested by Real-Time Polymerase chain Reaction (RT-PCR) with High Resolution melt curve analysis (HRMA) technology.

### RT-PCR

#### Extraction of RNA

EDTA blood were subjected for RNA extraction using QIA amp viral RNA kit (Qiagen, Germany) 140 µl of blood or plasma mixed with 560 µl of viral lysis buffer and incubated in room temperature for 10 minutes. Later equal volume of ethanol and wash step were followed with the manufactures protocol. Positive control and negative control were taken from the Chikungunya infected and un-infected patients. Extracted nucleic acid elution were stored in -20°C for later use.

#### CHIKV detection

CHIKV targets were amplified using Quant Studio 5 RT-PCR (Therm Fisher Scientific, USA) using HRMA technology. 10 µl of unknown patient RNA sample, 0.5 µl of reverse transcriptase enzyme, 2 µl of primers and other components of Type-it HRM kit (Qiagen). Amplification started with reverse transcription at 42°C for 20 mins; activation of Taq polymerase and denaturation of reverse transcript nucleic acid at 95°C for 5 mins; and 95°C for 10 secs; 55°C for 30secs and 72°C for 15 secs repeated for 40 cycles. After the cycling HRM initiated at 70°C and gradually raising 0.1°C in 2 secs interval up-to 90°C, run was analysed in Quant Studio 5 software. Chikungunya positive sample shows high melt peak at 80±2°C.

## 3. Results

During the study period there a total of 91 samples screened for Chikungunya. Out of this, 58 (63.73%) patients were found to be positive for Chikungunya infection; of the 91 positive cases 45 patients male and 46 patients female, positivity rate of male 55.17% (32/58) & female 44.83% (26/58).

In the 58 positive blood specimens, 8 (13.79%) of patients were in the age group under 10 years old; 10 (17.24%) of patients in the age range of 11-20 years; 6 (10.34%) of patients in 21-30 years; 12 (20.69%) of patients in mid age people 31-40 years; 22 (37.93%) of patients in 40-67 years old. Collected specimens were from the age group 1-67 years old and there is no infants or children under the age of 1 year were tested. In the first week of July, nil sample were tested for Chikungunya; an outbreak started from the second week had 4 (6.89%) positives of CHIKV; positivity rate of third week was 1 (1.72%) positive; at the end of July month fourth week had huge amount of positive in compare with the previous weeks, 40 (68.96%) patients detected positive for CHIKV in 7 days; and in 29<sup>th</sup> July 13 (22.41%) positives were reported. On 29<sup>th</sup> July 24 samples screened and turns 54.17% (13/24) of the suspects positive.

By real-time PCR analysis positivity level were determined by comparing melt intensity (rate of change of frequency or df/dt) and cyclic threshold, 34 patients were detected high level positive (df/dt in more than 3 and ct in the range upto 19); 18 patients were in medium level positive (df/dt in 1.5≥ 3 and ct in the range 20-29); 6 patients in low level (df/dt in 0.5≥1.5 and ct in the range 30-40).

Clinical features of the positive patients with, almost 91.4 % cases have experienced Chikungunya fever (CHIKF); head ache in 84.5% of the cases; body ache/body pain in 74.1% of the patients; nausea/vomiting leads for tiredness in 70.7% of patients; rashes appeared in 62.1% of the patients; arthralgia – includes wrist pain – 67.2% patients, elbow pain – 74.1% of patients, shoulder pain – 44.8% of patients; arthritis includes ankle pain – 84.5% of patients, knee pain – 77.6% of patients. Polyarthritis – a multiple joint pain observed in 23 (39.65) patients, all are in the age group of middle and senior citizen.

## 4. Discussion

In this study, huge male patients detected positive in compare with female patients, mainly in senior citizen CHIKV positivity rate was higher. According to World Health Organization (WHO) Mosquitoes are the primary carriers of the Chikungunya virus; these mosquitoes, *Aedes aegypti* and *Aedes albopictus* are also capable of spreading the Dengue and Zika viruses. This kind of mosquito bites most often in the daytime. Mosquitoes lay egg in stagnant water. An unaffected mosquito bites on a person whose blood contains CHIKV, the insect has the potential to consume the virus. After that, the virus multiplies within the mosquito over a few days, enters its glands of saliva, and once the mosquito bites a human, it can spread to another person. Once more, the virus multiplies in this freshly infected individual until it reaches elevated levels throughout their blood, at that point it can spread to additional mosquitoes and continue the cycle of transmission.[14]

In India 2023, totally 2,00,064 suspected cases were reported by 35 states (includes union territories), Out of these 11477 samples were tested positive; In 2024, 69,395 suspects and 3066 tested positives for Chikungunya infection. From Maharashtra, 14,495 suspected cases were

tested and 794 cases turns positive. [15] For vector control includes, personal prophylactic measures, biological control, chemical control, elimination of mosquito breeding and Health education, larval control with the concept of “dry day”, frequent cleaning of water tanks, washing and sunlight drying. Polyarthrits, a condition that cause pain in three or more joints without inflammation, fever [102°C] and other complication includes headache, vomiting, loss of appetite, myalgia, arthralgia and maculopapular rash. Guillain-Barré syndrome, myelitis, hemorrhage, cranial nerve palsies, bullous skin lesions, nephritis, hepatitis, retinitis, myocarditis and uveitis. [16] as per WHO record, in India CHIKV appearance have vanished after the Barsi out break in 1973. After a 32 years hiatus, the virus again emerged in 2006 and unleashed a massive outbreak in 13 states includes Karnataka, Andhra Pradesh, Maharashtra, Tamil Nadu, Madhya Pradesh, Kerala and Gujarat were the first states to be impacted. Isolated viruses are different from the previous outbreak in 1963-1973 was Asian genotype, it belongs to African genotype. None of the isolates from India exhibited the A226V mutation in E1 protein were observed in the outbreak spread on Reunion Island. Only the Indian isolate from 2007 was found to carry the A226V mutation. It was discovered that the CHIKV isolate belonged to the E1:226A genotype of Eastern Central Southern Africa. In 2009 and 2010, Maharashtra also reported incidents. [17]

CHIKV evolution has been a significant driver of epidemics in India, hence, the need to focus on proper surveillance, and implementation of prevention and control measure in the country. The first vaccine candidate was an inactivated CHIKV vaccine for Chikungunya in 1970. Today, many vaccines, particularly mRNA vaccines, are undergoing clinical trials; these have shown great promise against COVID-19. mRNA-lipid nanoparticle (mRNA-LNP) vaccines express the CHIKV E2-E1 antigen, which has been shown to induce humoral and cellular responses in mice (C57BL/6). [18] A "Common Protocol for Uniform Evaluation of Public Health Pesticides for use in Vector Control" was put forward in 2023 by the Central Insecticides Board & Registration Committee, the National Centre for Disease Control, the Indian Council of Medical Research, and the National Centre for Vector Borne Diseases Control. [19]

## 5. Conclusion

An alarming rate of increasing CHIKV in world wide distribution, it can lead to spread over the new region and cause CHIKF and febrile illness. It had huge impact in tropical and sub-tropical countries health care settings. Every individual need to know the cause of mosquito borne diseases, by draining and washing the containers, tanks; prevent the stagnant water; growing fishes in well, ponds may eat the larvae of mosquito. Vaccination for CHIKV needs to practiced and prior diagnosis of this disease will decrease the spread and mortality among the population.

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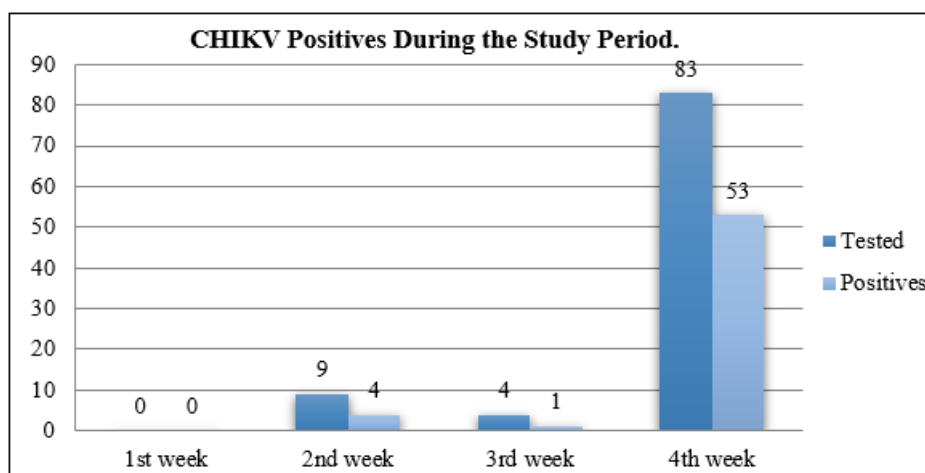
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**Table 1:** Age and Gender wise Chikungunya positives.

Gender	1-10	11-20	21-30	31-40	41-67
Male	5	6	2	5	14
Female	3	4	4	7	8
Total	8 (13.7)	10 (17.2)	6 (10.3)	12 (20.69)	22 (37.93)

**Table 2:** Clinical symptoms of the Chikungunya affected patients

Symptoms	Number (N=58)	%
Fever	53	91.4
Head ache	49	84.5
Body ache	43	74.1
Nausea/Vomiting	41	70.7
Rash	36	62.1
Arthralgia		
Wrist	39	67.2
Elbow	43	74.1
Shoulder	26	44.8
Arthritis		
Ankle	49	84.5
Knee	45	77.6



**Figure 1:** Chikungunya Weekly positives during the study period