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Low intensity Chikungunya outbreak in rural Western India indicates potential for similar outbreaks in other regions

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ABSTRACT

Objective: To investigate the Chikungunya outbreak in rural Talegaon Dhamdhere Town of Pune City in Western Maharashtra in October–November 2012.

Methods: Samples were collected from people who experienced fever and joint pains since a month ago. Clinical information was collected and entomological investigations were carried out in affected areas. Blood samples collected from hospital and clinics during the outbreak were tested for CHIKV using IgM ELISA (NIV kit), nested RT-PCR and real time RT-PCR. Age specific attack rates were calculated for the epidemic.

Results: The overall attack rate for this outbreak was 4.64/1000 population. The Breteau Index of the area was 12.0 and Adult House Index was 6.2%. A total of 63.6% samples were positive for CHIKV by either ELISA or PCR. Sequence analysis of the partial E1 gene in 4 representative samples found an alanine residue at position 226 of the E1 protein, showing that the CHIKV strain belonged to the ECSA genotype.

Conclusions: The epidemic with low transmission intensity was experienced by this area from the western region of the state. This indicates probability of such outbreaks in areas that have witnessed larger Chikungunya outbreaks in the past. Sero-prevalence studies and surveillance for Chikungunya in wide areas of such regions will help formulate well directed policies.

1. Introduction

Chikungunya (CHIK) is a disease of growing public health concern in India. It is caused by the Chikungunya virus (CHIKV) of the genus *Alphavirus* from the *Togaviridae* family. Since the 1952 Tanzania outbreak, CHIKV has caused outbreaks in various parts of Africa. Major CHIK outbreaks were reported from India in 1963–1964. Since its first isolation in Kolkata in 1963, there had been reports from different parts of India *viz.* Vellore, Chennai, Nagpur[1]. In the western part of the country, major outbreaks with high morbidity were reported from Barsi, Solapur District, Maharashtra in 1973[2]. Subsequently, in the absence of either active or passive surveillance, it seemed that the virus had disappeared from the country until the end of 2005. However,

large scale outbreaks of fever caused by this virus in several states of India including Maharashtra in 2005–2006 have confirmed its reemergence. In the western part of the country, major outbreak with high morbidity was reported in 2005–2006[1,3-5]. Thereafter, sporadic cases continue to be recorded in Maharashtra State[6]. The present study was conducted to investigate the CHIK outbreak in rural Talegaon Dhamdhere Town of Pune City in Western Maharashtra in October–November 2012.

2. Materials and methods

2.1. Community settings and subjects

Talegaon Dhamdhere is a small historic town close to the metropolitan industrial city of Pune in the western part of the country (Figure 1). It is well connected to the city of Pune and hosts weekly market for around 100 000 people residing in villages. Perennial water scarcity in this area leads to a tendency towards storing water in large containers. Cases were reported from the town as well as adjoining hamlets. Majority of the

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reported cases were adults.

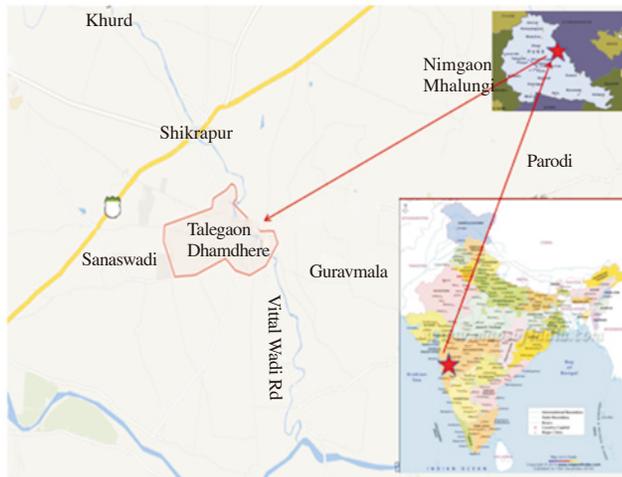


Figure 1. Map of Talegaon Dhamdhare Town in Shirur block of Pune City, Maharashtra.

2.2. Sample collection

A total of 43 blood samples (1 mL each) from patients affected with fever and poly-arthralgia in Talegaon Town of Pune City during October 2012 were referred to National Institute of Virology (NIV), Pune. The post-onset day (POD) for these referred patients was not mentioned. After observing 48.8% IgM positivity (21/43) for CHIKV, epidemiological and entomological investigations were performed by the NIV team. The team collected 67 serum samples from Talegaon Dhamdhare Town. All three major hospitals and all the clinics in the small town were visited to understand areas from which the cases were reported. The team also visited villages and schools around the town that reported CHIK suspect cases. Areas in the town from where maximum numbers of cases were reported were visited for further investigations. Information on suspected CHIK cases reported from other areas of the town and affected villages was collected from the medical officer at the Primary Health Center (PHC) in the town. Demographic information regarding the town was collected from the PHC.

2.3. Mosquito collection

Survey of *Aedes aegypti* was conducted in and around the affected areas. The teams visited patient's households and surveyed for the presence of the vector. Potable as well as non-potable containers were examined. In a locality, around 20 houses were searched at random based on risk factors such as crowded accommodations, water scarcity, proximity to road, communication network, etc. Information was collected with reference to knowledge, attitude and practices (KAP) of the population about mosquito breeding. Larvae and adults were identified using mosquito taxonomy keys. The fed mosquitoes were held for blood meal digestion and head-squashes were prepared by pressing and squashing mosquito head in two glass slides. The head-squashes were tested using indirect immunofluorescence test using anti-CHIKV polyclonal serum adsorbed with mosquito tissue suspension and liver powder respectively. The positive mosquito body was passed by

mosquito inoculation technique for virus isolation.

Samples were screened for CHIKV using IgM ELISA (NIV kit), nested RT-PCR and real time RT-PCR. The age of the patients from whom the samples were collected ranged from 5–70 years. CHIKV IgM test was carried out as per manufacturer's instructions.

2.4. RNA extraction and nested RT-PCR

For nested RT-PCR and real time RT-PCR, RNA was isolated using QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) as per manufacturer's instructions. Nested RT-PCR targeting the *E1* and *NSP 3* genes was carried out as described earlier[1]. Standard CHIKV from NIV repository (African genotype, strain No. 061573; Andhra Pradesh 2006; Accession Number EF027134) was used as positive control and phosphate buffer saline (PBS) as negative control to compare the results.

2.5. Nucleotide sequencing

Nucleotide sequencing was performed for the partial *E1* gene of 4 clinical samples. In brief, the PCR products were purified by using QIAquickPCR Purification Kit (Qiagen) and sequenced using Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) in an automatic sequencer (ABI PRISM 3100 Genetic Analyzer, Applied Biosystems). Multiple sequence alignment of the nucleotide sequences of the *E1* gene was performed using Clustal X, version 1.83. As described earlier samples were processed for virus isolation[1] but virus could not be isolated.

2.6. Real time RT-PCR

Viral load in the serum samples was determined by real time RT-PCR targeting the E3 structural protein region using the standard curve method[7-9]. One-step real time RT-PCR was performed in 25 μ L reaction mixture containing 5 μ L RNA, 12.5 μ L TaqMan One-step RT-PCR 2 \times master mix, 1 μ L 40 \times (RT + RNasin) (Applied Biosystems) each 1 μ L sense (μ mol/L), 1 μ L anti-sense (μ mol/L) primer and 1 μ L TaqMan probe. Real-time one-step RT-PCR was performed in a 96-well format using 7300 real time RT-PCR system and SDS software V 1.0.2 (Applied Biosystems). The amplification program included: reverse transcription at 48 $^{\circ}$ C for 30 min, initial denaturation at 95 $^{\circ}$ C for 10 min, and 50 cycles of denaturation (95 $^{\circ}$ C for 15 s) and annealing and extension (60 $^{\circ}$ C for 1 min)[7-9].

3. Results

A total of 70 out of 110 (63.6%) samples were positive for CHIKV by either ELISA or PCR. Positivity was found higher in males; 48 out of 67 males and 22 out of 43 females were positive (OR = 2.65; 95% CI: 1.10–6.39). Attack rates for different age groups are given in Table 1. The overall attack rate for this outbreak was 4.64/1000 population. Attack rate was highest in the

19–48 age group (6.13/1 000) followed by people aged above 48 years old (5.86/1 000); it was lowest for the population less than 6 years old.

Table 1

Age specific attack rates of chikungunya during an outbreak in a village in rural Pune.

Age group (years)	No. of attacked population			Population	Attack rate/1 000
	Female	Male	Total		
0–6	0	1	1	1561	0.64
7–11	1	1	2	1369	1.46
12–18	1	3	4	1606	2.49
19–48	12	30	42	6848	6.13
48+	7	11	18	3070	5.86
Total	21	46	67	14454	4.64

A total of 49 (73.1%) samples of the 67 samples were found to be positive by serological and molecular methods of which 40/67 samples (59.7%) were found to be positive for IgM antibodies. All IgM positive samples were from 4–30 POD. PCR was done for 31 early POD (≤ 10 POD) samples and 9 samples were found to be positive by nested RT-PCR and real time RT-PCR. A total of 8 samples (< 5 POD) were found to be positive by nested RT-PCR (25.8%) (Figure 2) and 7 samples (≤ 8 POD) were positive by real time RT-PCR (22.5%). In nested RT-PCR positive samples, only one sample is IgM positive for CHIKV antibodies (4 POD). One IgM positive sample which was negative by nested RT-PCR was found to be positive by real time RT-PCR and 2 samples which were negative by IgM and nested RT-PCR were found to be positive by real time RT-PCR. Out of 8 nested RT-PCR positive samples only 50% were found to be positive by real time RT-PCR. RNA load for positive samples was in the range of 10^2 – 10^5 copies/mL. A total of 17 samples were found to be negative for IgM antibodies, nested RT-PCR and real time RT-PCR. Sequence analysis of the partial *E1* gene in 4 representative samples found an alanine residue at position 226 of the *E1* protein (E1:226A), showing the CHIKV strain belonging to the ECSA genotype.

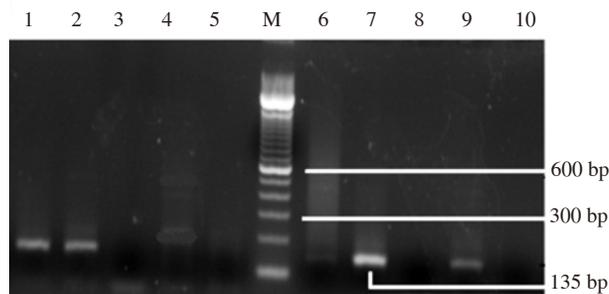


Figure 2. Nested RT-PCR of samples targeting *NSP 3* gene region.

Lane M: Molecular weight marker; Lanes 1, 2, 6, 9: Chikungunya positive samples; Lanes 3–5, 8: Negative samples; Lane 7: Positive control; Lane 10: Negative control.

A total of 80 households were visited for entomological investigations in four localities under Talegaon Dhamdhare PHC. Of these two localities were positive for presence of *Aedes aegypti* larvae and adults. The Breteau Index of the area was 12.0 and Adult House Index was 6.2%. Metal drums (250–300 L) kept indoors were found to be the main breeding sites. A total of 13 *Aedes aegypti* females were collected but the virus could not be isolated.

4. Discussion

There are a very few major reports on CHIK outbreaks from the western part of India after 2006–2007. Since then, sporadic cases were reported in this part of the country. This study was done in the affected Talegaon area of Pune City in Western Maharashtra. The investigation found 21 IgM and PCR positive cases out of the 43 referred samples (48.8% positivity) and 49 out of the 67 (73.1%) collected samples processed. All the patients had acute CHIK-like symptoms such as high fever and polyarthralgia resulting in restricted movements. The IgM positivity in the serum samples demonstrated recent CHIKV infection in the area. It is known that molecular assays like nested RT-PCR and real time RT-PCR have been used for detection of viral RNA in the early phase of infection (≤ 7 days). Hence only 31 early POD samples were processed for molecular testing because rate of detection decreases for late POD samples. A total of 9 (29.0%) additional samples were found to be positive by molecular tests. In our previous reports it was found that both the molecular tests are highly sensitive and have a detection limit of 10–100 virus particles[7,8]. In the present study samples of $\text{POD} \leq 7$ had a significantly high positivity for IgM. These findings are consistent with those of a study conducted to assess sensitivity and specificity of these tests in randomly collected samples[7].

In representative 4 samples sequence analysis of the partial *E1* gene showed that the CHIKV strain belonged to the ECSA genotype and had an alanine residue at position 226 of the *E1* protein (E1:226A). Notably none of the CHIKV isolates from Maharashtra had A226V mutation that has been reported to provide a fitness advantage to *Ae. albopictus* mosquitoes[10].

In the present study, we found that more males were affected than females. Similar observations were reported by other studies[11,12]. However, in another study conducted in an urban field practice area of a private medical college of Chennai and in an outbreak at Vellore, South India, more females were affected than males[13,14]. The maximum number of cases was found in the age group of 19–48 years. This finding was similar to that in the outbreak in rural Maharashtra reported by Ahmad[11]. In a study during the outbreak in Madras City in 1964, it was found that the population in the age group of 5–9 years had the highest morbidity (23.2%)[12]. Another available data showed that the disease presents in more severe form among children[15], but in this outbreak we found that the young adults and adults were the major age groups attacked and children did not experience infections or severe disease during this outbreak.

The attack rates during the 2005–2006 epidemics had reached 45% in some areas[16]. In Bangladesh, the attack rates reported in outbreak among naïve population have been high (29% overall, 38% among adults and 25% among children)[17]. The population was naïve for the African strain during that period. Seroprevalence estimates for CHIK are not available for this part of the country. In a sero-epidemiological study among patients with pyrexia and arthralgia in the northern region of Karnataka State, the investigators reported low prevalence (6.6%) during a period of 2011–2014[18]. Genome analysis has revealed that there is no change in the antigenicity of the virus. The low attack rates (maximum 6.4/1 000) indicate that immunity levels against CHIK

in this population are higher at present compared to the period of 2006–2007. Other limitation of our study was the inability to isolate the CHIKV from serum samples and need for full *E1* gene sequencing rather than partial gene sequencing along with sequencing of the structural genes. There were no reports of any gingivitis, epistaxis or any other forms of frank bleeding reported in earlier outbreaks[10]. There are many areas that were not affected during the 2006–2007 epidemics and remain vulnerable to outbreaks. Surveillance systems need to be established in order to keep a watch on this activity. Population in India is huge and population density is high in many areas. The losses caused by such outbreaks are substantial considering discomfort and loss of productivity over long periods of time. The kind of population and geographical settings observed in this study are not uncommon in the middle, western and southern parts of India especially around the industrial metropolitan cities. Findings from this study may be well generalized to these parts of the country.

The concerned town is close to a major highway which means that there are a lot of communications with the urban and surrounding rural areas. The town has also experienced a decadal growth in the recent past due to industrialization and urbanization in surrounding areas. The weekly market attracts traders and buyers from a large area around the town. Profuse breeding of *Aedes aegypti* was seen in the surrounding villages/towns during earlier surveys. In some other parts of the country like the north eastern state of Meghalaya, *Aedes albopictus* was found to be the predominant vector[19]. This investigation was carried out in the late stages of the outbreak after the doctors requested. Entomological investigations were conducted after two rounds of fogging by the PHC officials. The local health authorities need to keep a close watch and ensure that the vector was maintained under threshold levels.

The outbreak of CHIK fever may be due to a variety of social, environmental, behavioral, and biological changes. The best strategy for control of such outbreak is raising awareness of the community through mass education by public health officials. Vector control measures like spraying insecticides, clearing stored water and personal protective measures are also a key element in control of such outbreak.

Conflict of interest statement

We declare that we have no conflict of interest.

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