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Identification and genetic characterization of chikungunya virus from *Aedes* mosquito vector collected in the Lucknow district, North India

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ABSTRACT

Chikungunya fever is an emerging mosquito-borne disease caused by the infection with chikungunya virus (CHIKV). The CHIKV has been rarely detected in mosquito vectors from Northern India, since vector surveillance is an effective strategy in controlling and preventing CHIKV transmission. Thus, virological investigation for CHIKV among mosquitoes of *Aedes* (*A.*) species was carried out in the Lucknow district during March 2010 to October 2011. We collected adult mosquitoes from areas with CHIKV positive patients. The adult *Aedes* mosquito samples were pooled, homogenized, clarified and tested for CHIKV by nonstructural protein 1 (nsP1) gene based polymerase chain reaction (PCR). A total 91 mosquito pools comprising of adult *A. aegypti* and *A. albopictus* were tested for CHIKV. The partial envelope protein (E1) gene sequences of mosquito-borne CHIKV strains were analyzed for genotyping. Of 91 pools, 6 pools of *A. aegypti*; and 2 pools of *A. albopictus* mosquitoes were identified positive for CHIKV by PCR. The phylogenetic analysis revealed clustering of CHIKV strains in two sub-lineages within the monophyletic East-Central South African (ECSA) genotype. Novel amino acid changes at the positions 294 (P294L) and 295 (S295F) were observed during analysis of amino acid sequence of the partial E1 gene. This study demonstrates the genetic diversity of circulating CHIKV strains and reports the first detection of CHIKV strains in *Aedes* vector species from the state of Uttar Pradesh. These findings have implication for vector control strategies to mitigate vector population to prevent the likelihood of CHIKV epidemic in the near future.

1. Introduction

Chikungunya virus (CHIKV) is a mosquito-borne *Alphavirus* virus which belongs to family *Togaviridae*, causes an infectious disease chikungunya, a febrile illness accompanied by rashes and typically characterized by debilitating arthralgia (Pialoux et al., 2007). The disease is transmitted to people through bites of CHIKV infected mosquitoes of genus *Aedes,* mainly *A. aegypti* and *A. albopictus* mosquitoes (Brooks et al., 2004). Since the first isolation of virus in Africa in 1952, several CHIKV related epedimics Brooks et al. (2004) have occurred in Africa, South East Asia and India (Arankalle et al., 2007; Volk et al., 2010). In India, the CHIKV outbreak was first recognized in Kolkata in 1963, followed by several epidemics between 1964 and 1973 in South India (Arankalle et al., 2007; Sarkar et al., 1964). No outbreaks were reported from India after 1973 until 2005. However, sporadic cases continued to be recorded in Maharashtra during 1983 and 2000 (Mohan, 2006). In early 2005, the outbreak of chikungunya fever started in many Indian Ocean island nation, and spread through adjoining islands into peninsular India in late 2005 (Lahariya and Pradhan, 2006). The recent resurgence of chikungunya virus in the form of explosive outbreaks after a gap of 32 years in India during 2005–2008 has affected more than 1.3 million people in 13 states (Arankalle et al., 2007; Sudeep et al., 2011). Since then CHIKV related epidemics spread worldwide with unprecedented magnitude has raised a major public health concern.

In Africa, the virus is maintained in a sylvatic cycle among non-human primates and transmitted by forest-dwelling *Aedes* species mosquitoes (Volk et al., 2010). While in Asia, the disease is maintained in an urban cycle and transmitted by *A. aegypti* and *A. albopictus* mosquitoes (Kumar et al., 2008). In 2005–2006 epidemic, *A. albopictus* was the main vector in La Reunion islands in the Indian Ocean, whereas, *A. aegypti* was the main vector in Asia, including India (Reiter et al., 2006; Yergolkar et al., 2006). However, in subsequent years *A. albopictus* enacted the role of prominent vector in outbreaks reported from India in regions with abundance

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of *A. albopictus* mosquitoes (Das et al., 2012; Niyas et al., 2010; Santhosh et al., 2009, 2008; Sumathy and Ella, 2012). The CHIKV strain transmission by *A. albopictus* was attributed to A226V amino acid change in the E1 protein of CHIKV which showed better adaptation and increased fitness in this new vector (Schuffenecker et al., 2006; Tsetsarkin et al., 2007; Vazeille et al., 2007). Later on, it was shown that epistatic mutations in the E2 glycoprotein of CHIKV had an impact on enhanced transmissibility by *A. albopictus* (Sumathy and Ella, 2012; Tsetsarkin et al., 2009). Whereas, in some of the same epidemics and sporadic cases reported from Northern India and other regions of the country, CHIKV strains with E226A sub lineage persisted in areas with predominance of *A. aegypti* population (Sudeep et al., 2011).

CHIKV has been classified into three distinct genotypes: Asian, East/Central/South African (ECSA) and West African (Powers et al., 2000). In India, the CHIKV isolates identified between 1963 and 1973 belonged to Asian genotype, whereas the recent Indian Ocean and Indian subcontinent epidemics were caused by virus strains of the India Ocean lineage (IOL) which have emerged from the ECSA genotype (Arankalle et al., 2007; Volk et al., 2010). Recently, micro-evolutionary changes in the CHIKV genome have resulted in the appearance of new subgroups of ECSA strains (Shrinet et al., 2012; Sumathy and Ella, 2012).

The waves of chikungunya outbreak in India in 2006 which continued through 2010 has least affected the Northern part of India, however, local epidemics and sporadic cases of chikungunya have been documented from the region (Shrinet et al., 2012; Singh et al., 2012a; Soni et al., 2013). Despite of the fact that the virus has been isolated from human sera, mosquito vectors in the regions are rarely examined for CHIKV presence. As during 2006–2008, chikungunya infection was seen in different districts of Uttar Pradesh state (Singh et al., 2012b) and in subsequent non-outbreak period between 2010 and 2011, the region again reflected the CHIKV activity which prompted us to undertake the present study to investigate the presence of CHIKV strain among *Aedes* mosquitoes and perform their molecular characterization to determine the circulating CHIKV genotypes in Lucknow district of the state of Uttar Pradesh during 2010–2011.

2. Material & methods

2.1. Patient'clinical specimen

During the year 2010–2011, 271 case-patients of febrile illness, visited Out/Inpatient departments at Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS), Lucknow, India, a tertiary care hospital in Lucknow. All the case-patients were from different regions of central Uttar Pradesh state in the northern India. The serum samples of febrile case-patients, which included the symptoms suggestive of CHIKV infection such as fever, arthralgia, myalgia, headache and rashes, were investigated for the presence of CHIKV specific IgM antibodies using chikungunya—IgM capture-ELISA at SGPGIMS as described by Yergolkar et al. (2006).

2.2. Mosquito collection and mosquito processing

Identification of CHIKV case-patients from Lucknow prompted us to collect mosquitoes during March 2010 to October 2011 from different localities of Lucknow district where CHIKV positive patients reside for detection of CHIKV in *Aedes* mosquitoes (Fig. 1). The adult *Aedes* mosquitoes collection was conducted in randomly selected houses in neighboring areas where index case was reported in each localities by using two main methods: pyrethrum spray for Indoor collection and assembled battery operated aspirator for outdoor collection in areas where patient serum proved positive for CHIKV by IgM ELISA. Following the guidelines of WHO, use of pyrethrum spray was done to mosquitoes resting inside the house and afterwards mosquito were collected on white standard hospital bed sheets spread on the floor and other flat surfaces in the house (Ponlawat and Harrington, 2005; Who, 1975) Generally March to October is a peak time for mosquito breeding, therefore, a collection was made from March 2010 to October 2011

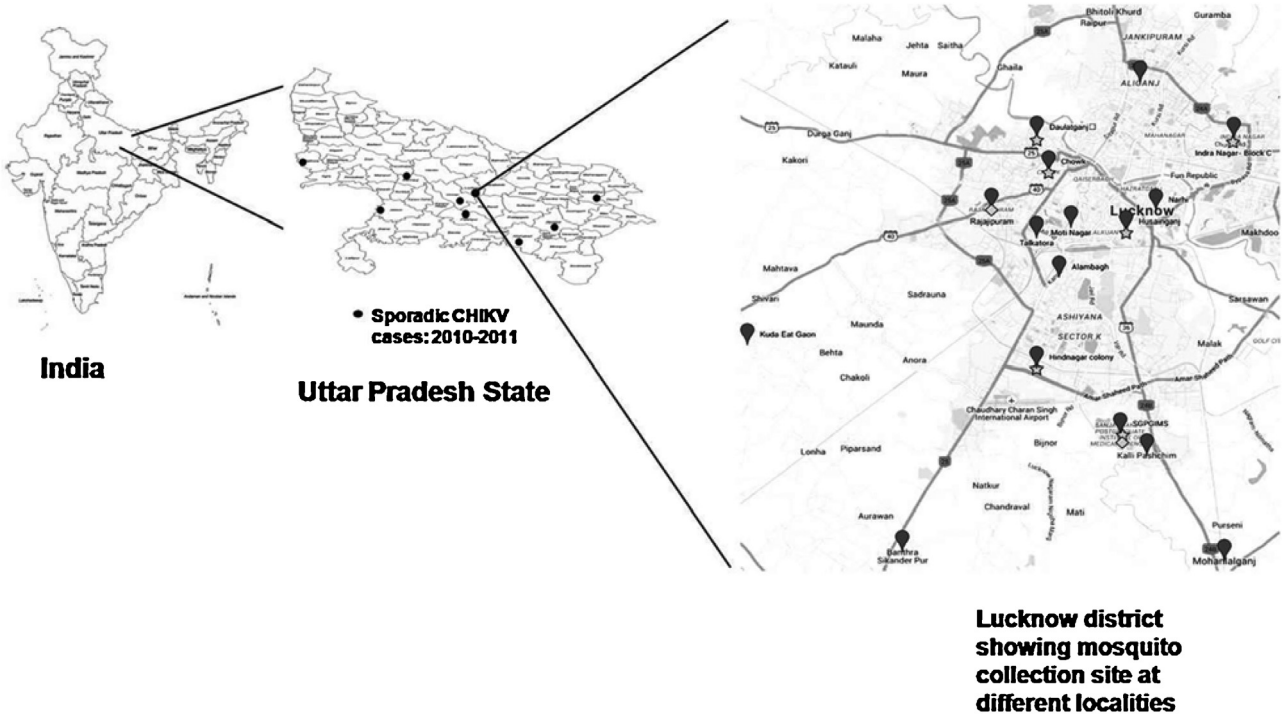

Fig. 1. India Map showing CHIKV sporadic cases in different districts of Uttar Pradesh state during 2010–2011 indicated with black circle and the bubble icon in the Lucknow district map represent the mosquito collection sites; (★) and (♦) indicates urban sites inhabited with CHIKV positive *A. aegypti* and *A. albopictus* mosquito vectors, respectively.