Defence Life Science Journal, Vol. 01, No. 2, September 2016, pp. 184-187, DOI : 10.14429/dlsj.1.10744

Vertical Transmission of Chikungunya virus in *Aedes aegypti* Mosquitoes from Northern India

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ABSTRACT

Chikungunya virus is now recognised as a resurging arbovirus of global public health significance, with their circulation in both new and old world. It is horizontally transmitted among vertebrates by Aedes mosquitoes. So far, the existence of vertical transmission of Chikungunya virus in *Aedes* vector is riddled with conflicting reports. In this study, presence of Chikungunya virus was detected in adult *Aedes aegypti* mosquitoes that emerged from field-collected larvae from Gwalior, northern India during 2010. This was further confirmed through nucleotide sequencing that revealed the presence of novel east central south African (ECSA) genotype of Chikungunya virus. This provides molecular evidence for vertical transmission of Chikungunya virus in mosquitoes in nature, which may have important consequences for viral survival during inter-epidemic period and adverse climatic conditions.

Keywords: Chikungunya, mosquito, vertical transmission, trans-ovarial transmission, RT-PCR, genome sequencing

1. INTRODUCTION

Chikungunya fever is a resurging mosquito borne viral infection of global public health significance. Its re-emergence since 2005 was associated with large scale epidemics with unusual clinical severity in both old and new world[1] Autochthonous transmission was also witnessed in many places of temperate countries including Italy and France[2,3]. Chikungunya fever is classically characterized by fever, headache, nausea, vomiting, rash, myalgia and a severe, often persistent incapacitating polyarthralgia[1]. However, recently it is also associated with neurological complications.

Chikungunya virus (CHIKV), the causative agent is an enveloped icosahedral virus belonging to the genus *Alphavirus* of the family *Togaviridae*. The genome of CHIKV consists of a linear, single stranded, positive sense ribonucleic acid (RNA) of approximately 11.7 kb in length, and codes for 4 non-structural proteins (nsP1 to nsP4), three structural proteins (capsid, E1 and E2) and two small peptides (E3 and 6K) in two open reading frames[1]. Chikungunya is transmitted by day biting peri-domestic *Aedes* mosquitoes, primarily *Aedes aegypti* and *Aedes albopictus* [3]. The virus is primarily transmitted horizontally in Man-Aedes-Man cycle. The very high level of viremia in human during acute phase of illness, allows efficient horizontal transmission of the virus, resulting in massive outbreaks[4]. Apart from Man-Aedes-Man cycle, other mode of transmission of the CHIKV is not clearly understood. Vertical or trans-ovarial transmission (TOT) refers to the transmission of virus from infected female mosquito to its progenies. TOT is well documented for several arboviruses belonging to genus Bunyavirus, Flavivirus and Alphavirus[5-8]. It plays an important role for maintenance of arboviruses, particularly in adverse climatic conditions. However, literature on TOT in CHIKV is scarce with conflicting reports[9,10]. A recent report from our lab confirms the existence of vertical transmission of CHIKV in *Aedes* mosquitoes[11-14].

Received : 06 August 2016, Revised : 28 August 2016
Accepted : 12 September 2016, Online published : 07 October 2016

2. THE STUDY

Gwalior is located in the state of Madhya Pradesh in northern India (26° 13′ N, 78° 10′ E, altitude=196 m). A major Chikungunya outbreak was reported in Gwalior and adjoining area during 2006 during its re-emergence in India[15]. Sporadic cases were also reported during subsequent years. During an entomological surveillance for Chikungunya virus, 48 larval pools were collected from different parts of Gwalior and adjoining areas during October 2010 to September 2012. The field-collected larvae were reared in the laboratory (temperature 28^0C; relative humidity 75–85%) and the emerged adults were screened at around day 5 of emergence. The mosquitoes in a pool size of ≤ 33 were homogenized in 2 mL tubes with Eagle's minimum essential medium and steel bead using Tissuelyzer and the clarified homogenate were subjected to extraction of RNA employing QIAamp viral RNA mini kit (Qiagen, Germany), according to the manufacturer's protocol. It was eluted in 50 µL of nuclease-free water and stored at −70°C. The presence of

CHIKV specific RNA was detected using Enhanced Avian HS RT-PCR kit (Sigma, USA) employing a primers pair targeting the E1 gene (CHIK13: TTACATCACGTGCGAATAC and CHIK14: CTTTGCTCTCAGGCGTGCGACTTT) with the thermal profile, as reported earlier[15]. Double-pass sequencing of the purified amplified products was carried out with a Big dye terminator cycle sequencing ready reaction kit (Applied Biosystems, USA) on an ABI3130 DNA sequencer following the manufacturer's protocol. The nucleotide sequences thus obtained were analysed using Lasergene 5 software package (DNASTAR Inc, USA). Neighbor-Joining tree was constructed employing the best model of K2+G using the MEGA v5.03 software employing 22 other globally diverse isolates (Table 1)[16]. The tree topologies were evaluated using 10000 replicates of the data set.

The RT-PCR revealed presence of Chikungunya specific amplicon of 500 bp in two RNA samples extracted from adult *Aedes aegypti* mosquitoes, emerged from field collected larvae. Rest of the RNA samples were found negative for Chikungunya virus. RT-PCR is one of the most sensitive methods for detection of virus even in a single mosquito[17]. Further, both the CHIKV specific amplicons were subjected to nucleotide sequencing and the sequences were submitted to GenBank under accession numbers KU747171 and KU747172. Both these sequences revealed 98.2 per cent identity with the prototype CHIKV (S27), isolated from Tanzania in 1953. The phylogenetic analysis classified all these viruses into three genotypic groups as reported earlier[18]. Both the CHIKV sequences deciphered in this study were classified into novel Indian Ocean clade of East-Central-South African (ECSA) genotype (Fig. 1). A large number of viruses isolated from La Reunion, India, Sri Lanka, Singapore, Thailand, Malaysia, France and Italy were also clustered within this group. The close clustering of sequences in this study with recent Indian CHIKV revealed the indigenous local origin of the viruses in mosquitoes.

3. CONCLUSIONS

In conclusion, this study revealed the presence of CHIKV in adult *Aedes aegypti* mosquitoes emerged from larvae in nature, which was confirmed using molecular techniques. The vertical transmission through trans-ovarial route might play a crucial role for maintenance of CHIKV in *Aedes aegypti* in nature, particularly during inter-epidemic period. Aedes eggs are capable of surviving desiccation in extreme environment under adverse conditions for several months[19]. The evidence of TOT of novel ECSA genotype of Chikungunya virus in *Aedes aegypti* mosquitoes in nature shifts the focus of vector control strategy towards simultaneous control of both larvae and adults.

Table 1. Details of the genome sequences of Chikungunya virus isolates investigated in this study

Virus ID. No	Year of isolation	Country of origin	Genotype	Gen Bank Accession No.
16/RMRC/Kendrapara	2010	India	ECS African	JN711133
2010-1909	2010	France	ECS African	FR846305
RGCB356/KL08	2008	India	ECS African	GQ428215
SGEHICHD13508	2008	Singapore	ECS African	FJ445511
India10_DRDE_V1	**2010**	**India**	**ECS African**	**KU747171**
Thudumaladinne-02	2008	India	ECS African	FJ705371
India: Valayam	2009	India	ECS African	HQ599560
LK(EH)CH20108	2008	SriLanka	ECS African	FJ513679
India10_DRDE_V2	**2010**	**India**	**ECS African**	**KU747172**
Delhi-23	2010	India	ECS African	JN048825
05-115	2005	Reunion	ECS African	AM258990
DRDE-06;	2006	India	ECS African	EF210157
IND-09-WB	2009	India	ECS African	JF264892
CU-Chik683	2009	Thailand	ECS African	GU301781
0810bTw	2008	Malaysia	ECS African	FJ807899
ITA07-RA1	2007	Italy	ECS African	EU244823
H2123	1976	South Africa	ECS African	AF192904
S27	1953	Tanzania	ECS African	AF369024
653496	1965	India	Asian	AY424803
SV045196	1996	Thailand	Asian	AF192900
RSU1	1985	Indonesia	Asian	AF192894
IbH35	1964	Nigeria	West African	AF192893
PM2951	1966	Senegal	West African	AF192891
37997	1983	Senegal	West African	AF192892

* Sequences in bold font refer to the sequences deciphered in this study.

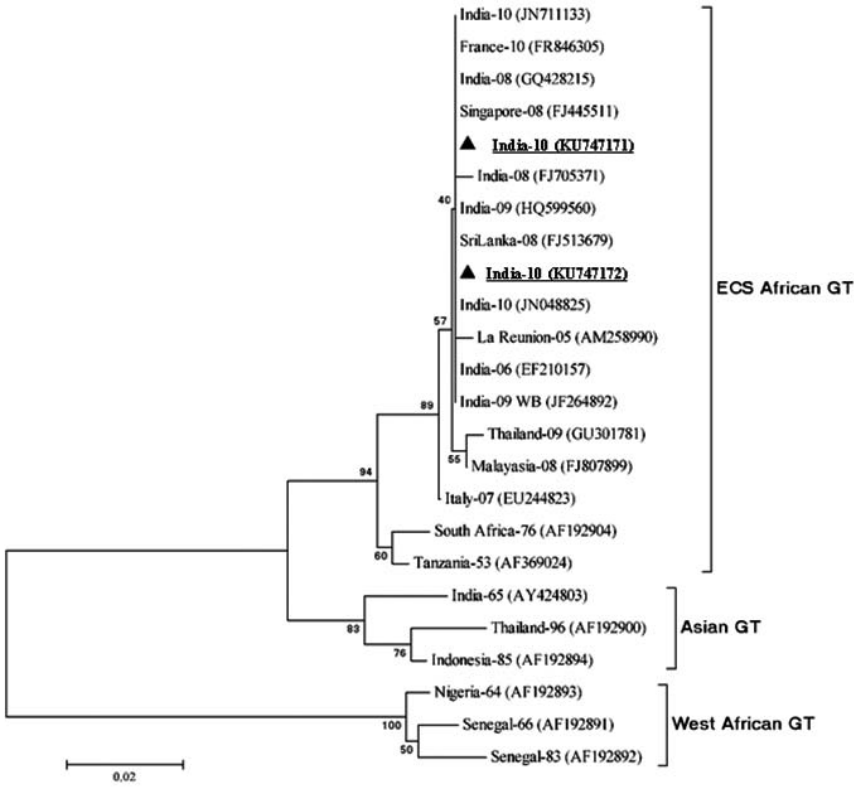

Figure 1. Phylogenetic tree among CHIK viruses generated by Neighbor-Joining method based on the partial nucleotide sequence of E1 gene. Each strain is abbreviated with the country of origin and last two digits of the year of isolation, followed by GenBank accession number in parenthesis. The CHIKV sequenced in this study are underlined and written in Bold fold and underlined. Bootstrap values are indicated at the major branch points.

ACKNOWLEDGMENTS

We thank Director, DRDE, Gwalior for providing necessary facilities for this study. This study was funded by the Defence Research and Development Organization, Ministry of Defence, India.

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