

Correspondence

Molecular characterization of chikungunya virus from Andhra Pradesh, India

Sir,

Naresh Kumar *et al*¹ reported an unprecedented outbreak in the Indian Ocean and Reunion Islands in March 2005 and its sporadic fall-out in February 2006²⁻⁴. In India, chikungunya outbreaks occurred in the southern States including Andhra Pradesh after a gap of three decades almost during the period of Indian Ocean Islands episode. Surprisingly, the outbreak was localized mostly in the three districts of Andhra Pradesh, those other urban areas of the State also reported the outbreak of CHIK V⁵. However, the authors did not point out the incidences of CHIK V in those areas during the same period of study. The epidemiological profile showed that the number of confirmed cases reported by the National Institute of Virology, Pune, and National Institute of Communicable Diseases, Delhi, were 1162 out of 11,17,691 suspected cases in the year 2006. Maximum cases reported from the State of Maharashtra were 804 followed by Karnataka with 298 cases, Andhra Pradesh with 248, Gujarat 225, Tamil Nadu 116, Madhya Pradesh 106, Government National Capital Territory (GNCT) of Delhi 67, Kerala 43, Orissa 34, West Bengal 21 and Uttar Pradesh with 4 cases (Personal communication: National Vector Borne Disease Control Programme, Delhi). The data clearly revealed that the outbreak of chikungunya was not confined to Andhra Pradesh but spread to central and south India. The authors have reported the role of *Aedes aegypti* and *A. albopictus* as the main vectors of CHIK V but in India, the role of *Ae. albopictus*, Asian tiger mosquito^{6,7} considered as a sylvatic vector, has not been confirmed. In Indian Ocean Reunion Islands, the role of *Ae. albopictus* as one of the implicated vectors of chikungunya outbreak has already been established⁸ whereas *Ae. aegypti* was absent there but it is the main vector in Asia including India^{9,10}.

While studying the phylogenetic analysis of chikungunya virus with the sequenced APRA-CTR

isolate at nucleotide level with ONN (O'nyong-nyong viruses) as an outgroup, inclusion of other representative members of Semliki Forest (DAK Arbs 6878) and Sindbis-like (Ar Mg 812 and B 448) virus was essential because they were quite distinct from the prototype strain and from all other isolates examined¹¹. In the present context, antigenic relatedness of viruses in the CHIK V and other representative virus clades was desirable because several CHIK V virus strains used to immunize hamster and mice were mostly (80%) Central/East African and West African genotype while 20 per cent genotypes were of Asian ones¹². It has been reported earlier that three CHIK virus (Strain 1455/75, 37997 and Ross) produced specific antibodies while ONN virus-infected mice and hamster did not produce detectable antibodies. More importantly, Asian CHIK V strain (1455/75) produced very little homologous antibodies to be useful in neutralization assays, whereas CHIK V strain 37997 of West Africa and CHIK V strain Ross of Central/East Africa produced adequate antibody titre and were used in 80 per cent plaque reduction neutralization tests (PRNT) indicating distinct antigenic subtypes which have >4-fold difference. Studies shown that no antibody against ONN was produced eliminating the possibility of 2-way cross-neutralization test between CHIK and ONN viruses^{11,12}. Similarly, ONN virus lineage has been considered a distinct group of viruses within the antigenic complex.

Since, genetic conservation of CHIK V is being maintained only between human and peridomestic mosquitoes, a high degree of sequence conservation is observed within several other group of alphaviruses, *e.g.*, North American Eastern equine encephalitis virus^{13,14}, Highlands J virus from North America¹⁵, Western equine encephalitis virus¹⁶ and Sindbis virus¹⁷. North American eastern equine encephalitis viruses

being maintained by an avian reservoir host helps increase virus population size that decreases founder effects and genetic drifts which may explain their sequence conservation¹⁶⁻¹⁹. One does not know the migratory patterns of both passerines and shorebirds to encompass much of Southeast Asia ranging from Yellow Sea and South China sea across Philippines and Indonesia to Australia. Relevance of such avian transmission is very much a likely phenomenon to the districts of Andhra Pradesh because State as a whole is situated in south-eastern part of India adjoining the sea of the Bay of Bengal encompassed by the shorebirds. This has been documented by the migratory routes from India across Indian Ocean to East Africa. Serological testing of passerines and shore-birds in Southeast Asia could reveal the plausible means of viruses dispersal. Similarly, the travel of Human could account for the dispersal of viruses in areas as distant as Indonesia and the Philippines to Basri in central India and the introduction of viruses into Asia from Africa¹¹. Thus, the knowledge of the history of movement of migratory labours, origin of travellers, movement of tourists to CHIK V endemic areas, their return to base countries is an essential prerequisite while correlating to the origin and involvement of peri-urban, urban as well as sylvatic vectors in such an active transmission cycle.

Certain studies²⁰ on CHIK V isolates during the beginning of outbreak was very much related to East-African strain which subsequently developed into distinct variants nucleotide sequence of all Indian Ocean Island viruses share certain areas where they are different from the previously determined sequences of virus protein. Some of these changes occur at the beginning of the outbreak termed as "A version" while others appeared half-way through it but very fast considered as "V version" which constitutes 90 per cent of the sequences²¹.

So, on the basis of such report, the phylogenetic analysis needs to be studied on the distinctive variants of epidemic times to disclose the extent of genetic conservation as also the sequence conservation¹⁰ in this part of South-East Asia.

P. K. Kar*, **B.N. Nagpal****, **V. K. Dua*#**
S.K. Ghosh⁺, **K. Raghavendra****, **R. M. Bhatt⁺⁺**

Anup Anvikar & Aparup Das

*#National Institute of Malaria Research
Field Unit, BHEL, Ranipur

Hardwar 249 403, Uttarakhand

**National Institute of Malaria Research

22 Sham Nath Marg, Delhi 110 054

⁺National Institute of Malaria Research
Field Unit, Bangalore 560 038, Karnataka

⁺⁺National Institute of Malaria Research
Field Unit, RLTRI Campus

Raipur 492 015, Chhattisgarh, India

[#]For correspondence:

vkdua51@gmail.com

References

1. Naresh Kumar CVM, Anthony Johnson AM, Sai Gopal DVR. Molecular characterization of chikungunya virus from Andhra Pradesh, India & phylogenetic relationship with Central African isolates. *Indian J Med Res* 2007; 126 : 534-40.
2. Hochedez P, Jaureguiberry S, Debruyne M, Bossi P, Hausfater P, Brucker G, *et al.* Chikungunya infection in travelers. *Emerg Infect Dis* 2006; 12 : 1565-7.
3. Parola P, de Lamballerie X, Jourdan J, Rovey C, Vaillant V, Minodier P, *et al.* Novel chikungunya virus variant in travelers returning from Indian Ocean islands. *Emerg Infect Dis* 2006; 12 : 1493-8.
4. Jossesan L, Paquet C, Zehgnoun A, Caillere N, Le Tertre A, Solet JL, *et al.* Chikungunya disease outbreak, Reunion Islands. *Emerg Infect Dis* 2006; 12 : 1994-5.
5. Ravi V. Re-emergence of chikungunya virus in India. *Indian J Med Microbiol* 2006; 24 : 83-4.
6. Cordel H. Chikungunya outbreak in Reunion: update. *Eurosurveillance* 2006; 11 [Serial in the internet]. 2006 March 2 [Cited 2006 Mar 3]. Available from <http://www.eurosurveillance.org/ew/2006/060302.asp#3>, accessed on October 10, 2008.
7. Institut National de Veille Sanitaire. Chikungunya outbreak on Reunion: update on June 1st [article in French]. 2006 June 2 [cited 2006 June 6]. Available from http://www.invs.sante.fr/presse/2006/le_point_sur/chikungunya-reunion-020606/index.html, accessed on October 10, 2008.
8. Enserink M. Infectious diseases. Massive outbreak draws fresh attention to little known virus. *Science* 2006; 311 : 1085.
9. Yergolkar PN, Tandale BV, Arankalle VA, Sathe PS, Sudeep AB, Gandhe SS, *et al.* Chikungunya outbreaks caused by African genotypes, India. *Emerg Infect Dis* 2006; 12 : 1580-3.
10. Jupp PG, McIntosh BM. Chikungunya virus disease. In: Monath TP, editor. *The arboviruses epidemiology and ecology*. Vol II. Boca Raton, Florida: CRC Press; 1988. p. 137-57.
11. Powers AM, Brault AC, Tesh RB, Weaver SC. Re-emergence of chikungunya and O'nyong-nyong viruses: evidence for distinct geographical lineages and distant evolutionary relationships. *J Gen Virol* 2000; 81 : 471-9.
12. Calisher CH, Shope RE, Brandt W, Casals J, Karabatsos N, Murphy FA, *et al.* Proposed antigenic classification of registered arboviruses I. Togoviridae, Alphavirus. *Intervirology* 1980; 14 : 229-32.
13. Calisher CH, Karabatsos N. Arbovirus serogroups: definition and geographic distribution. In: Monath TP, editor. *The*

arboviruses: epidemiology and ecology. Vol. I. Boca Raton, Florida: CRC Press; 1988. p. 19-57.

14. Weaver SC, Hagenbaugh A, Bellew LA, Gousset L, Mallampalli V, Holland JJ, *et al.* Evolution of alphaviruses in the eastern equine encephalomyelitis complex. *J Virol* 1994; 68 : 158-69.
15. Cilnis MJ, Kang W, Weaver SC. Genetic conversation of Highlands J Viruses. *Virology* 1996; 218 : 343-51.
16. Brault AC, Powers AM, Chavez CL, Lopez RN, Cachon MF, Gutierrez LF, *et al.* Genetic and antigenic diversity among eastern equine encephalitis viruses from North, Central, and South America. *Am J Trop Med Hyg* 1999, 61 : 579-86.
17. Sammels LM, Lindsay MD, Poidinger M, Coelen RJ, Mackenzie JS. Geographic distribution and evolution of Sindbis virus in Australia. *J Gen Virol* 1999; 80 : 739-48.
18. Weaver SC, Kang W, Shirako Y, Rumenapf T, Strauss EG, Strauss JH. Recombinational history and molecular evolution of western equine encephalomyelitis complex alphaviruses. *J Virol* 1997; 71 : 613-23.
19. Weaver SC. Evolution of alpha viruses. In: Gibbs AJ, Calisher CH, Garcia-Arenal F, editors. *Molecular basis of virus evolution*. Cambridge: Cambridge University Press; 1995. p. 501-30.
20. Weaver SC, Rico-Hesse R, Scott TW. Genetic diversity and slow rates of evolution in New World Alphaviruses. *Curr Top Microbiol Immunol* 1992; 176 : 99-117.
21. Schuffenecker I, Itean I, Michault A, Murri S, Frangeul L, Vaney MC, *et al.* Genome microevolution of chikungunya viruses causing the Indian Ocean outbreak. *PLoS Med*, 2006; 3 : 1058-70.

Authors' response

Sir,

With reference to the queries raised by Kar *et al*¹ regarding our article² on molecular characterization of Chikungunya virus, we would like to submit the following:

(i) CHIK epidemics were mainly observed in Chittoor, Nellore and Kadapa districts of Andhra Pradesh during 2006. So for the initial screening and characterization, we collected the samples from the three districts only. In our further studies, we collected and screened CHIKV in blood samples of suspected cases from other districts as well (unpublished data).

(ii) Higher prevalence of *Aedes albopictus* and observation of E1A226V mutation in CHIKV isolates in Kerala³ further supports the Schuffenecker hypothesis⁴, for rapid CHIKV spread in that region. *Ae albopictus* acted as an alternate vector in CHIKV

outbreak in Kerala⁵. Although there are no confirmed reports of CHIKV isolated from *Ae. albopictus* in India, yet due to its wide prevalence the role of this vector in CHIKV transmission cannot be ignored and needs to be further confirmed.

(iii) ONN virus sequence has been introduced as an outgroup for correct rooting of the tree while studying the phylogenetic relationship of CHIK AP Ra CTR with other isolates. However, the point is well taken that inclusion of members of Semiliki forest Virus (DAK ArB16878) and Sindbis like virus (DAK ArMg812 & B448) was indeed essential to make the analysis more informative.

(iv) As pointed out by Kar *et al*¹, possible role of migratory birds as a reservoir/secondary host for CHIKV cannot be ruled out and needs further investigation. Pulikat and Kolleru lakes are the best known bird sanctuaries in Andhra Pradesh favoured by the seasonal migratory birds from other countries. Since Pulikat and Kolleru lakes area is highly restricted, it is very difficult to screen the migratory birds and study their possible role, if any, in the transmission of CHIKV.

**C.V.M. Naresh Kumar &
D.V.R. Sai Gopal***

Department of Virology
Sri Venkateswara University
Tirupati 517 502, India
*For correspondence:
dvrsaigopal@rediffmail.com

References

1. Kar PK, Nagpal BN, Dua VK, Ghosh SK, Raghavendra K, Bhatt RM, *et al.* Molecular characterization of chikungunya virus from Andhra Pradesh, India. *Indian J Med Res* 2009; 129 : 335-7.
2. Naresh Kumar CVM, Anthony Johnson AM, Saigopal DVR. Molecular characterization of chikungunya virus from Andhra Pradesh, India & phylogenetic relationship with Central African Isolates. *Indian J Med Res* 2007; 126 : 534-40.
3. Santhosh SR, Dash PK, Parida MM, Khan M, Tiwari M, Lakshmana Rao PV. Comparative full genome analysis revealed E1: A226V shift in 2007 Indian chikungunya virus isolates. *Virus Res* 2008; 135 : 36-41.
4. Schuffenecker I, Itean I, Michault A, Murri S, Frangeul L, Vaney MC, *et al.* Genome microevolution of chikungunya viruses causing the Indian Ocean outbreak. *PLoS Med* 2006; 3 : 1058-70.
5. Sudeep AB, Parashar D. Chikungunya: an overview. *J Biosci* 2008; 33 : 443-9.