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Phylogenetic and time-scale analysis of dengue virus types 1 and 4 circulating in Puerto Rico and Key West, Florida, during 2010 epidemics

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Background: Dengue is caused by dengue virus (DENV-1 to -4) and transmitted by the mosquito *Aedes aegypti*. Dengue is endemic in Puerto Rico (PR), and in the US has caused sporadic epidemics in Texas and Hawaii. More recently autochtonous transmission has occurred in Key West, Florida (KW). The aim of this study was to perform phylogenetic and time-scale analysis of DENV-1 and -4 circulating in PR and KW in 2010.

Methods: The study included four DENV-1 and two DENV-4 isolates obtained from PR and KW during 2010 dengue epidemics. Phylogenetic analyses were performed using maximum-likelihood and Bayesian methods. Time-scale analysis (TSA) was performed using the Bayesian approach employed by BEAST.

Results: Our phylogenetic and TSA revealed that 2010 PR DENV-1 strains constitute a new lineage within the genotype V. The analyzed DENV-1 KW strain clustered with a strain isolated from mosquito pools collected in KW during 2010, and with a number of Nicaraguan and Mexican strains. DENV-4 isolates obtained from PR belong to genotype II and associated with strains from PR that circulated during 1980s–1990s, and with other Caribbean and Central American strains. The time of the most recent common ancestor (*tMRCA*) for DENV-1 2010 PR strains is between 7-15 years (y) (mean; m=11 y), while for 2010 DENV-1 from KW is between 16-34 y (m=25 y). Clusters containing the 2010 PR and KW isolates separated from older PR isolates between 19-40 y ago (m=29 y). The *tMRCA* for the 2010 DENV-4 clade is between 14-18 y (m=16 y). The mean nucleotide substitution rate for DENV-1 was of 4.95 (95%HPD: 3.26-6.68) x 10-4 substitutions/site/year (s/s/y), and for DENV-4 was of 8.98 (95%HPD: 6.16–12.0) x 10-4 s/s/y.

Conclusion: The PR 2010 DENV-1 strains are associated to Caribbean and South American strains and our analysis revealed the circulation of a new lineage of DENV-1 in the island. On the contrary, 2010 DENV-4 strains closely associated with older PR strains indicating that this old lineage still circulate in PR. This is the first report on the phylogeny and time-scale analysis of DENV circulating in PR and KW during 2010 epidemics.

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Characterization of Coxsackievirus A20 as the donor strain at the nonstructural region of the recombinant type 1 circulating vaccine-derived polioviruses in the Philippines

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Background: The genomic sequences of circulating vaccine-derived polioviruses (cVDPVs) are said to have been mixed-up with genomes of human enterovirus (HEV)-C species. In the Philippines, a year after the country's polio-free declaration in 2000, type 1 cVDPV which caused wild polio-like paralysis was reported. Genomic sequences showed that the capsid region is homologous to Sabin 1 strain, however, the nonstructural sequences downstream at the 2ABC region were derived from an unidentified HEV-C species. In this study, we seek to identify and characterize the recombinant counterpart at the nonstructural region of type 1 cVDPVs in the Philippines.

Methods: Amplification and sequencing the viral protein 1(VP1) and 2ABC regions were performed on 108 HEV-C isolates collected from 1992-2008 among acute flaccid paralysis cases. Thereafter, near full-length genome of the putative recombinant donor strain was performed. Sequence and recombination analyses were done using MEGA 5.0 and SimPlot software respectively.

Results: Phylogenetic analysis showed that the near full-length sequence of sample 24-PHL-2000 is closely related to both CVA20a and PV 1. The polyprotein 1 which encodes for the structural region clearly categorized 24-PHL-2000 under the CVA20 cluster but diverged away and grouped with the type 1 cVDPV in the nonstructural regions. This was supported by prominent nucleotide sequence identities as well as by similarity plot and bootscanning analyses which showed the high genomic interrelatedness of 24-PHL-2000, CVA20 and type 1 cVDPVs in the whole genomes. Furthermore, there is an evidence of a single intertypic recombination event with the Philippine type 1 cVDPVs based on bootscanning analysis, notably at the non-structural coding regions of the 24-PHL-2000 strain and revealed the crossover site at around 3500 bp position.

Conclusion: In conclusion, we found sample 24-PHL-2000, which is a CVA20, to be the donor strain of the Philippine type 1 cVDPVs. With only few remaining countries endemic for polio, the emergence of cVDPV with potential to recombine with circulating HEV-C species as reported in this study, poses a threat to the ongoing global eradication efforts. This study undercores the importance of recombination studies and may allow an understanding of the evolutionary dynamics and genomic transfers of the nonstructural proteins among HEVs.

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