



Revista de Biología Tropical

ISSN: 0034-7744

rbt@cariari.ucr.ac.cr

Universidad de Costa Rica

Costa Rica

Tuan, Le Van; Tuyet Van, Nguyen Thi; Hoang Quan, Nguyen; Duoc, Pham Tho
Phylogeny of Dengue virus type 2 isolated in the Central Highlands, Vietnam

Revista de Biología Tropical, vol. 65, núm. 2, junio, 2017, pp. 819-826

Universidad de Costa Rica

San Pedro de Montes de Oca, Costa Rica

Available in: <http://www.redalyc.org/articulo.oa?id=44950834031>

- How to cite
- Complete issue
- More information about this article
- Journal's homepage in redalyc.org

redalyc.org

Scientific Information System

Network of Scientific Journals from Latin America, the Caribbean, Spain and Portugal

Non-profit academic project, developed under the open access initiative

Phylogeny of Dengue virus type 2 isolated in the Central Highlands, Vietnam

Le Van Tuan, Nguyen Thi Tuyet Van, Nguyen Hoang Quan & Pham Tho Duoc

Department of Virology, Tay Nguyen Institute of Hygiene and Epidemiology, 34 Pham Hung Street, Buon Ma Thuot city, DakLak, Vietnam; levantuan_tihe@yahoo.com, tuyetvan1603@yahoo.com.vn, hoangquandaklak@gmail.com, duocvnn2006@yahoo.com

Received 16-III-2016. Corrected 07-X-2016. Accepted 08-XI-2016.

Abstract: Dengue fever is perhaps the most important viral re-emergent disease especially in tropical and sub-tropical countries, affecting about 50 million people around the world every year. In the Central Highlands regions of Vietnam, dengue fever still remains as a major public health issue. Although four viral serotypes have been currently identified, dengue virus type 2 (DENV-2) was involved in the most important outbreaks during 2010-2012, especially, 2010 when the fatality rate highly increased. Detection of genotype of DENV-2 provided information on origin, distribution and genotype of the virus. In this study, DEN-2 isolated from dengue patients during the 2010-2012 epidemics was amplified and sequenced with E gene. The consensus sequences were aligned with reference E gene sequences of globally available Genbank. Phylogenetic analysis was performed using Neighbor-joining and Kimura 2-parameter model to construct phylogenetic tree. A total of 15 isolates (seven from 2010; one from 2011 and seven from 2012) were obtained from human serum samples. Phylogenetic analysis revealed that Asian genotype 1 is currently circulating locally in Central Highlands region. Isolates of this genotype were closely related to viruses from Thailand, Laos, and Cambodia. It indicated that these epidemics maybe imported into the Central Highlands region from South-East Asia neighbor countries. The study results would help in planning for prevention and control of dengue virus in Vietnam. Continuous monitoring of DENV genotypes is necessary to confirm the current findings and detect possible genotype shifts within the dengue viruses in the future. Rev. Biol. Trop. 65 (2): 819-826. Epub 2017 June 01.

Key words: dengue virus, envelope protein, genotype, phylogenetic analysis, the central highlands.

Dengue fever (DF) is a mosquito-borne viral infection, transmitted by *Aedes aegypti* caused major impact on health and economies in subtropical and tropical countries worldwide. The report of World Health Organization (WHO) indicated that the incidence of dengue has dramatically increased 30-fold since 1955 to 2010, and estimated 50-100 million new infections occurred annually over 100 endemic countries, especially hundreds of thousands of severe cases increased, in Southeast Asian countries (WHO, 2012). Dengue virus (DENV) is a member of the genus *Flavivirus*, family *Flaviviridae*. It is an envelope virus with length 11 kb positive-sense single-stranded RNA genome

(Henchal & Putnak, 1990). There are four antigenically distinct DENV serotypes; DENV-1, DENV-2, DENV-3 and DENV-4 (Henchal & Putnak, 1990) and each serotype shows phylogenetically distinct genotypes (Holmes & Burch, 2000). DENV-2 is classified into six genotypes, including two genotypes confined to the Asian population (Asian 1 and Asian 2), the Cosmopolitan, American/Asian, American, and Sylvatic genotype (Twiddy et al., 2002).

Dengue fever (DF)/Dengue hemorrhagic fever (DHF) is endemic in the Central Highlands region of Vietnam with all four DENV serotypes co-circulating. Multiple serotypes are transmitted during dengue outbreaks

and usually one serotype predominates in outbreak. Major epidemics of DF/DHF in the Central Highlands region were reported in 2010 and 2012 (Dat & Huong, 2010; Duoc, Dat, Trang, & Van, 2014). The dengue epidemic of 2010 with the high incidence estimated around 13 255 infected cases. DENV-2 has been a prominent serotype in many of these outbreaks especially 2010 outbreak (Duoc et al., 2014).

The Central Highlands region has a history of outbreaks of dengue viral infection, however, there is no study reviewing genotype distribution in this region. Therefore, the current study was aimed to determine the circulating genotype in the Central Highlands using isolates collected from outbreaks of DENV-2 (2010 to 2012), and the obtained sequences were compared to other sequences reported from other geographical regions of the world, to deduce a phylogenetic relationship.

MATERIALS AND METHODS

Ethical approval: The study was approved by the institutional review boards of Tay Nguyen Institute of Hygiene and Epidemiology (56/QĐ-VTN/2015).

Data of the period 2008-2012 analyzed in this study, were kindly provided from the epidemiological and virological surveillance system of the Tay Nguyen Institute of Hygiene and Epidemiology, Vietnam Ministry of Health.

Virus strains: The DENV-2 strains used in this study were obtained from patient sera in DF/DHF epidemic in the Central Highlands during 2010 to 2012. All strains were determined as DENV-2 serotype by reverse transcription polymerase chain reaction (RT-PCR) using Promega Access RT-PCR kit (Promega, USA) and previous published DENV type specific primers (Lanciotti, Calisher, Gubler, Chang, & Vorndam, 1992).

Virus stocks were prepared by single passage in C6/36 *Aedes albopictus* cell monolayers in Dulbecco's Modified Eagle's medium (D-MEM) supplemented with 10 % fetal calf serum (FCS). Cells were incubated at 28 °C

for five to seven days and observed for cytopathic effect. The presence of DENV in cell supernatants was confirmed by an immunofluorescence assay (IFA) during which cells were reacted with either DENV group-specific or serotype-specific monoclonal antibodies (MAB)SLE 6B6C-1/FITC conjugate (Sigma-Aldrich, USA), and the serotype-specific MAB: DEN-1 (Hawaii 15F3-1-15 and D2-1F1-3), DEN-2 (NGC 3H5-1-21), DEN-3 (H87 5D4-11-24), DEN- 4 (H241 1H10-6-7) (kindly provided by Pasteur Institute in Ho Chi Minh city, Vietnam).

Preparation of viral RNA, amplification, and sequencing: Viral RNA was extracted from infected cell culture supernatant using QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

Conventional semi-nested PCR was performed using a modified procedure described by Lanciotti and colleagues (Lanciotti et al., 1992). A one-step RT-PCR was performed using the AccessQuick™ RT-PCR System (Promega, Madison, WI, USA) in a 25 µL reaction volume containing 1X AccessQuick™ Master Mix, 5.0 units of AMV Reverse Transcriptase and 0.25 µM (each) of primers D1 and TS2 (Lanciotti et al., 1992) using the following program; reverse transcription at 45 °C for 30 min, inactivation at 94 °C for 3 min, and PCR amplification of 35 cycles under the following conditions: 94 °C for 30 sec, 55 °C for 30 sec, 72 °C for 1 min, and a final extension at 72 °C for 7 min.

RT-PCR and sequencing primers were designed on the basis of published DENV sequences. Four manually designed oligonucleotide primer pairs (Table 1) in order to produce four overlapping fragments covering the complete E gene. E gene of DENV was amplified using one-step RT-PCR amplification (Zhang et al., 2005). Overlapping fragments were amplified using AccessQuick RT-PCR System (Promega, Madison, WI, USA) with four sets of primers covering the entire E gene. Amplified products were purified prior to sequencing using QIAquick PCR purification kit (QIAGEN, Germany) following

TABLE 1
Primers used for amplification and sequencing of the complete DENV-2 envelope gene

Primer name	Sequence (from 5' to 3')	Position
D2E1F	GCAATCCTGGCATAACCAT	850-869
D2E1R	CTGTGATGGAAGCTCTGTGGTG	1421-1450
D2E2F	AGAGGATGGGGAAATGGATG	1231-1250
D2E2R	CCTTTGAGCTGTAGCTTGTC	1803-1823
D2E3F	ATGCACACAGCACTTACAGG	1714-1733
D2E3R	GCTCCAAAGACTTGGTGGA	2243-2261
D2E4F	ATGGCCATTTAGGTGAC	2170-2187
D2E4R	GTTTTCCTGCCTGCATGATT	2707-2726

manufacturer's instructions. Capillary-based Sanger sequencing was used to obtain E gene sequences (1485 nt).

Genotype and phylogenetic analysis of DENV-2: Overlapping nucleic acid sequences obtained from individual sequencing reactions were combined for analysis and edited using the Lasergene package version 8.0 (DNA-STAR Inc., Madison, WI, USA). Contiguous sequences were aligned using ClustalX program (Larkin et al., 2007) and compared with published sequences of DENV isolates in Genbank database. Phylogenetic analysis of complete E gene sequences was performed in MEGA 6.0 program (Tamura et al., 2011) using the neighbor joining (NJ) method.

For genotype classification, we grouped the isolate sequences with the relevant reference sequences based on classification by Twiddy et al. (Twiddy et al., 2002). Phylogenetic trees were constructed from the aligned nucleic acid sequences using algorithms based on distance matrix/neighbor joining (NJ) in MEGA6.0. The reliability of the analysis was evaluated by a bootstrap test with 1 000 replications. DENV-3 (H87, Philippines 1956) strain (Genbank accession numbers FJ850094) was used as out-group to root the tree.

RESULTS

Dengue incidence in the Central Highlands: Dengue fever was endemic in the

Central Highlands region where all four serotypes of dengue virus (DENV) were co-circulated. Epidemiological and virological data has revealed oscillations in disease incidence and serotype prevalence in this region during 2008-2012 (Fig. 1). During 2010-2012, DENV-2 was the most prevalent serotype detected by surveillance and its circulation was associated with increased disease incidence (Fig. 1).

Genotype and nucleotide sequence accession numbers: We determined the complete E gene nucleotide sequences of the 15 DENV-2 strains isolated from 2010-2012 from provinces of the Central Highlands. The sequences of all the strains reported in this paper were deposited in Genbank database (Table 2).

Genotype and phylogenetic tree of DENV-2: A phylogenetic tree was constructed using pair-wise comparison of a 1 485 nt region from the E gene (nt 850-2 726) of virus isolates sequenced in this study. The phylogenetic tree for the genotype was described in figure 2.

The phylogenetic tree demonstrated that all DENV-2 isolates were clustered in Asian genotype 1. Isolates from 2010 epidemic in KonTum province were closely related to the isolates from 2010 epidemic in Laos and Cambodia. However, isolates from 2012 epidemic in DakLak and GiaLai province were closely related to the isolates from 2011 epidemic in Southern Vietnam and 2010 epidemic in Thailand (Fig. 2).

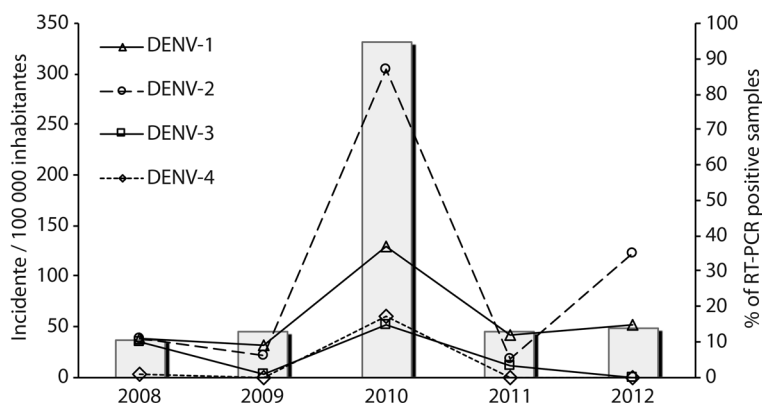


Fig. 1. Dengue incidence and serotype abundance in the Central Highlands region, Vietnam. Changes in the incidence of dengue cases per 100 000 inhabitants (left-hand axis, open bars), and DENV serotype prevalence detected by RT-PCR in children and adults (right hand axis, lines) between 2008 and 2012 in the 4 provinces of the Central Highlands, Vietnam. Data is kindly provided from the epidemiological and virological surveillance system of the Tay Nguyen Institute of Hygiene and Epidemiology, Vietnam Ministry of Health.

TABLE 2
Genotypes of DENV-2 and accession numbers deposited on Genbank

Isolate	Origin	Year	Genotype	Genbank accession no.
43GL10	GiaLai	2010	Asian genotype 1	KP769811
46GL10	GiaLai	2010	Asian genotype 1	KP769812
75GL10	GiaLai	2010	Asian genotype 1	KP769813
07KT10	KonTum	2010	Asian genotype 1	KP706452
14KT10	KonTum	2010	Asian genotype 1	KP706451
23KT10	KonTum	2010	Asian genotype 1	KP706450
32KT10	KonTum	2010	Asian genotype 1	KP706449
30DN11	DakNong	2011	Asian genotype 1	KP769814
07DL12	DakLak	2012	Asian genotype 1	KP671756
129DL12	DakLak	2012	Asian genotype 1	KP706453
134DL12	DakLak	2012	Asian genotype 1	KP706455
142DL12	DakLak	2012	Asian genotype 1	KP706454
16GL12	GiaLai	2012	Asian genotype 1	KP769808
28GL12	GiaLai	2012	Asian genotype 1	KP769809
29GL12	GiaLai	2012	Asian genotype 1	KP769810

DISCUSSION

Dengue fever is one of the world's fastest-growing vector-borne diseases in different geographical regions of the world. It is estimated that over a hundred tropical and subtropical countries with more than 2.5 billion people is at the risk of infection to dengue virus (Huang et al., 2012).

Dengue virus exists as four antigenically distinct viruses designated as serotypes (DENV-1 through DENV-4), belonging to genus *Flavivirus* of family *Flaviviridae*. It has a positive-sense RNA genome that is translated as a single polypeptide and post-translationally cleaved into three structural proteins and seven nonstructural proteins (Henchal & Putnak, 1990). The envelope protein (E) is considered



Fig. 2. Neighbour-joining tree depicting the phylogenetic relationships of dengue serotype 2 viruses based on the envelope gene (1 485 bases). Bootstrap value (in percentage > 65 %) on each node degenerated by using 1 000 replications is shown next to the branches. The Highlands isolates are designated in triangles. Genotypes of DENV-2 are also indicated.

to be the immunodominant protein (Mandl, Guirakhoo, Holzmann, Heinz, & Kunz, 1989). Dengue virus (DENV) also can be divided into different genotypes by the E gene and no particular pattern of genotype distribution can be inferred for DENV-2 as different genotypes spread in diverse locations (Lanciotti, Lewis, Gubler, & Trent, 1994; Wittke et al., 2002).

Central Highlands continues to face challenges with dengue fever outbreak due to problems with mosquitos and the close proximity to regions with high incidences of DENV infection. In order to study the circulating DENV genotypes in the Central Highlands region, genotyping analysis was performed based on complete E gene sequences. Like most molecular studies of DENV, the main focus of the analysis is the E gene, which encodes the major protein component of the virion surface, is the most important antigen with regard to humoral immunity and is associated with other biological activities such as cell attachment, receptor binding and virus assembly (Twiddy et al., 2002). The E gene sequences of the 15 isolates of the Central Highlands region were compared with the sequences of DEN-2 isolates found in Genbank aligned with reference sequences to generate genotype classification.

Some data showed the same serotype of DENV continued years showed that endemic infection of dengue circulating locally may be also the important cause of Dengue epidemic in the Central Highlands region (Duoc et al., 2014). In the study, five isolates (43GL/10, 46GL/10, 75GL/10, 16GL/12 and 29GL/12) were clustered together as Asian genotype 1, suggesting that the isolates of 2012 could be originated from the isolates of 2010. Sequence analysis showed that the DENV-2 strains isolated during the 2012 epidemic were closely related to the strains isolated during the 2010 epidemic of the Central Highlands, suggesting that the 2010 strains had evolved in local and eventually caused the epidemic of 2012.

Phylogenetic tree analysis of 15 DENV-2 isolates also showed that nine strains were closely related to strains in Southern Vietnam, four strains found to be closely related to Laos,

Cambodia and Thailand strains. All these isolates were clustered under the Asian genotype 1. Similar findings observed in previous studies have shown that Asian genotype 1 was the predominant genotype in Vietnam in previous years (Vu et al., 2010). This proved that genotype distribution of DENV-2 remained stable in Vietnam for long time. Asian genotype 1 is also quite common in the region and is widely circulated in India, South East Asia, Africa, the Middle East, and Australia (Fahri et al., 2013).

Previous study showed that the Asian genotype 1 of DENV-2 had displaced the previously dominant American/Asian genotype as the predominant DENV-2 lineage in Southern Vietnam (1995-2009) (Vu et al., 2010). Force of infection has been used widely to understand the intensity of disease transmission within a community (Ferguson, Donnelly, & Anderson, 1999). A large number of susceptible hosts in the population, and an associated increased genotype-specific force of infection of DENV, could help explain the seemingly short period in which genotype replacement have occurred (Twiddy et al., 2002). However, South-East Asian DENV-2 viruses are less susceptible than American lineage viruses to cross-neutralization antibodies elicited by DENV-1 infection (Kochel et al., 2002; Wang et al., 2016). Population wide seroepidemiology, coupled with a better understanding of correlates of immunity, are clearly needed to understand serotype and genotype replacement in all endemic regions.

The displacement of Asian/American lineage viruses by Asian 1 viruses have been also observed in Thailand and Cambodia. In Thailand, the Asian/American genotype of Thai DENV-2 viruses, most likely co-circulated with the Asian genotype 1 for at least a decade prior to 1991, but then were replaced by the Asian 1 lineage from 1992 to 2006 (Lambrechts et al., 2012; Wittke et al., 2002). The same circumstance also occurred in Cambodia as only Asian genotype 1 virus has circulated since 2005 (Vu et al., 2010). This finding showed that Highlands's strains had shared the same genotype of DENV-2 in Laos, Thailand and Cambodia.

All DENV-2 strains isolated in the Central Highlands belonged to Asian genotype 1 that has potentially caused dengue endemic outbreaks in this area. Continuous monitoring of DENV genotype, in combination with a surveillance database, may help improve the local understanding of viral genotype shifts and their relationship with epidemiology.

ACKNOWLEDGMENTS

This work was funded by Tay Nguyen Institute of Hygiene and Epidemiology under Project No VTN/056. The authors wish to thank the Dengue surveillance team at the Tay Nguyen Institute of Hygiene and Epidemiology, for allowing the use of data on dengue incidence, and serotype prevalence for the Central Highlands region, Vietnam. The authors thank Nga Phan by the suggestions made to the manuscript.

RESUMEN

Filogenia del virus del Dengue tipo2 identificado en el Altiplano Central de Vietnam. Posiblemente, la fiebre del dengue es la enfermedad viral recurrente más importante en los países tropicales y subtropicales que afecta cerca de 50 millones de personas cada año en todo el mundo. En las regiones del Altiplano Central de Vietnam, la fiebre del dengue aun se considera como una gran preocupación de salud pública. Aunque los cuatro serotipos virales han sido identificados, el virus del dengue tipo 2 (DENV-2) estuvo involucrado en el brote más importante durante el 2010-2012, especialmente en el 2010 cuando los índices de mortalidad aumentaron considerablemente. El descubrimiento del genotipo DENV-2 proporcionó información del origen, distribución y genotipo del virus. En este estudio, el serotipo DENV-2 identificado de pacientes con dengue durante las epidemias de 2010-2012 se amplificaron y secuenciaron con E gene. Las secuencias consenso se alinearon con secuencias de referencia mundiales de E gene disponibles en GenBank. El análisis filogenético se llevó a cabo utilizando el modelo Neighbor-joining y Kimura 2-parámetros para construir el árbol filogenético. Un total de 15 cepas (siete de 2010, una de 2011 y 7 de 2012) se obtuvieron de las muestras de suero humano. El análisis filogenético reveló que el genotipo asiático 1 circula localmente en la región del Altiplano Central. Las cepas de este genotipo estan muy relacionadas con los virus de Tailandia, Laos y Camboya. El análisis también indicó que estas epidemias pudieron migrar a la región del Altiplano

Central desde los países vecinos del sureste asiático. Los resultados de este estudio pueden ayudar en la planificación de la prevención y el control del virus del dengue en Vietnam. Un monitoreo contante de los genotipos DENV es necesario para confirmar los hallazgos recientes y detectar los posibles cambios del genotipo de los virus del dengue en el futuro.

Palabras clave: virus del dengue, proteína tipo cubierta, genotipo, análisis filogenético, Altiplano Central.

REFERENCES

- Dat, D. T., & Huong, V. T. (2010). Epidemiologic characteristics of Dengue fever in the Central Highlands of Vietnam, 1998-2010. *Journal of Tay Nguyen Preventive Medicine*, 2, 1-3.
- Duoc, P. T., Dat, D. T., Trang, L. T. T., & Van, N. T. H. (2014). Epidemiologic characteristics of Dengue fever in the Central Highlands of Vietnam, 2008-2012. *Journal of Tay Nguyen Preventive Medicine*, 1, 41-47.
- Fahri, S., Yohan, B., Trimarsanto, H., Sayono, S., Hadisaputro, S., Dharmana, E., ... Sasmono, R. T. (2013). Molecular surveillance of dengue in Semarang, Indonesia revealed the circulation of an old genotype of dengue virus serotype-1. *PLOS Neglected Tropical Diseases*, 7(8), e2354. doi: 10.1371/journal.pntd.0002354
- Ferguson, N. M., Donnelly, C. A., & Anderson, R. M. (1999). Transmission dynamics and epidemiology of dengue: insights from age-stratified sero-prevalence surveys. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 354(1384), 757-768. doi: 10.1098/rstb.1999.0428
- Henchal, E. A., & Putnak, J. R. (1990). The dengue viruses. *Clinical Microbiology Reviews*, 3(4), 376-396. doi: 10.12691/ajmr-3-3-4
- Holmes, E. C., & Burch, S. S. (2000). The causes and consequences of genetic variation in dengue virus. *Trends in Microbiology*, 8(2), 74-77. doi: 10.1016/S0966-842X(99)01669-8
- Huang, J. H., Su, C. L., Yang, C. F., Liao, T. L., Hsu, T. C., Chang, S. F., ..., & Shu, P. Y. (2012). Molecular characterization and phylogenetic analysis of dengue viruses imported into Taiwan during 2008-2010. *The American Journal of Tropical Medicine and Hygiene*, 87(2), 349-358. doi: 10.4269/ajtmh.2012.11-0666
- Kochel, T. J., Watts, D. M., Halstead, S. B., Hayes, C. G., Espinoza, A., Felices, V., ..., & Russell, K. L. (2002). Effect of dengue-1 antibodies on American dengue-2 viral infection and dengue haemorrhagic fever. *Lancet*, 360(9329), 310-312. doi: 10.1016/S0140-6736(02)09522-3

- Lambrechts, L., Fansiri, T., Pongsiri, A., Thaisomboonsuk, B., Klungthong, C., Richardson, J. H., ..., & Scott, T. W. (2012). Dengue-1 virus clade replacement in Thailand associated with enhanced mosquito transmission. *Journal of Virology*, 86(3), 1853-1861. doi: 10.1128/jvi.06458-11
- Lanciotti, R. S., Calisher, C. H., Gubler, D. J., Chang, G. J., & Vorndam, A. V. (1992). Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. *Journal of Clinical Microbiology*, 30(3), 545-551. doi: 0095-1137/92/030545-07\$02.00/0
- Lanciotti, R. S., Lewis, J. G., Gubler, D. J., & Trent, D. W. (1994). Molecular evolution and epidemiology of dengue-3 viruses. *Journal of General Virology*, 75(Pt 1), 65-75. doi: 10.1099/0022-1317-75-1-65
- Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., ..., & Higgins, D. G. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics*, 23(21), 2947-2948. doi: 10.1093/bioinformatics/btm404
- Mandl, C. W., Guirakhoo, F., Holzmann, H., Heinz, F. X., & Kunz, C. (1989). Antigenic structure of the flavivirus envelope protein E at the molecular level, using tick-borne encephalitis virus as a model. *Journal of Virology*, 63(2), 564-571. doi: 10.1016/S0092-8674(02)00660-8
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28(10), 2731-2739. doi: 10.1093/molbev/msr121
- Twiddy, S. S., Farrar, J. J., Vinh Chau, N., Wills, B., Gould, E. A., Gritsun, T., ..., & Holmes, E. C. (2002). Phylogenetic relationships and differential selection pressures among genotypes of dengue-2 virus. *Virology*, 298(1), 63-72. doi: 10.1006/viro.2002.1447
- Vu, T. T., Holmes, E. C., Duong, V., Nguyen, T. Q., Tran, T. H., Quail, M., ..., & Simmons, C. P. (2010). Emergence of the Asian 1 genotype of dengue virus serotype 2 in Vietnam: in vivo fitness advantage and lineage replacement in South-East Asia. *PLOS Neglected Tropical Diseases*, 4(7), e757. doi: 10.1371/journal.pntd.0000757
- Wang, C., Katzelnick, L. C., Montoya, M., Hue, K. D., Simmons, C. P., & Harris, E. (2016). Evolutionarily Successful Asian 1 Dengue Virus 2 Lineages Contain One Substitution in Envelope That Increases Sensitivity to Polyclonal Antibody Neutralization. *The Journal of Infectious Diseases*, 213(6), 975-984. doi: 10.1093/infdis/jiv536
- WHO. (2012). Dengue: a global threat - global answers. Global strategy for dengue prevention and control 2012-2020. Geneva: WHO, 43p. ISBN: 9789241504034.
- Wittke, V., Robb, T. E., Thu, H. M., Nisalak, A., Nimmanitya, S., Kalayanrooj, S., ... & Askov, J. G. (2002). Extinction and Rapid Emergence of Strains of Dengue 3 Virus during an Interepidemic Period. *Virology*, 301(1), 148-156. doi: 10.1006/viro.2002.1549
- Zhang, C., Mammen, M. P., Jr., Chinnawirotpisan, P., Klungthong, C., Rodpradit, P., Monkongdee, P., ..., & Holmes, E. C. (2005). Clade replacements in dengue virus serotypes 1 and 3 are associated with changing serotype prevalence. *Journal of Virology*, 79(24), 15123-15130. doi: 10.1128/jvi.79.24.15123-15130.2005