International Journal of Fauna and Biological Studies
Available online at www.faunajournal.com

I J F B S International Journal of Fauna And Biological Studies

ISSN 2347-2677
IJFBS 2016; 3(3): 29-41
Received: 13-02-2016
Accepted: 05-03-2016

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A new dimension in the dengue epidemiology with special reference to the genetic diversity of the virus: A review

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Abstract
The severity of dengue is often ascribed to secondary infection with a virus belonging to a serotype distinct from that of the primary infection. Severe pathogenicity of DENV might be regulated at the genetic level and may be associated with unusual mutational and recombination events which are the two major reasons behind the extensive genetic diversity of DENV. The possible emergence of undesired genetically novel variant DENV in the near future could create further complexity and subsequent complications in the pathogenicity of the disease. This review article is an attempt to understand the significance of the extensive genetic diversity of the dengue virus (DENV) in the development of greater magnitude of the viral pathogenicity as well as in the severity of recurrent outbreaks in the context of changing environment and epidemiology. DENV antigens have been detected from mononuclear cells, lymphocytes, Langerhans cells in the skin, neurons, astrocytes, endothelial cells and hepatocytes, heart and skeletal muscle. This altered tropism of the dengue virus in humans might indicate the fitness strategy of the virus in urban areas since human is the only possible source of viremic vertebrate. Remarkably, the RNA virus has developed the ability to recombine with host dsDNA genomes. The extraordinary abilities of RNA virus like DENV may unlock a new vista in dengue research, which encompasses the relevant proposition of a momentous plausibility of crucial genetic exchange between DENV (+) ssRNA genome and dsDNA of the human host and thereby the possible emergence of genetically novel DENV variants associated with altered pathogenicity.

Keywords: Flavivirus, Dengue, Serotypes, Genetic Diversity, Mutation, Recombination.

1. Introduction
The study of arthropod-borne viruses like Dengue virus (DENV) is relevant in the context of public health because they generally impose an enormous disease burden on humans. The plasticity of the genome of RNA virus together with the extraordinary dispersal potential of arthropod vectors is of a keen interest in the study of arboviral diseases like Dengue [1-3]. Dengue fever was initially mentioned as "water poison" linked with flying insects in a Chinese Medical Encyclopaedia in 992 from Jin Dynasty (265-420 AD). The word "dengue" has been acquired from Swahili phrase Ka-dinga pepo, meaning "cramp-like seizure" [4]. Dengue virus (DENV) is a flavivirus and belongs to the family Flaviviridae. The Dengue virus has four antigenically distinct serotypes (DEN 1, 2, 3, and 4) [1]. The first clinically identified dengue epidemics happened almost simultaneously in Asia, Africa, and North America in the 1780s [4]. Dengue fever has re-emerged as a major public health challenge worldwide; with 2.5 billion people at the risk of infection, more than 100 million cases and 25,000 deaths being reported annually [5].The WHO estimates that almost 2.5 billion people in the world population are at the risk of Dengue infection. This disease is estimated to affect 50-100 million individuals each year in the tropical and subtropical regions. Of these cases, 500,000 develop into severe forms of the disease such as Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). Dengue is gradually becoming a global disease. Compared to nine reporting countries in the 1950s, today the geographic distribution of Dengue includes more than 100 countries worldwide. Dengue/DHF is a significant public health problem in India [1]. Dengue has been considered predominantly an urban disease. Presently, however, rural districts have also been affected by this virus. The greater genetic diversity in any virus population provides considerable opportunity to increase viral fitness in a population. RNA viruses have the potential to adapt rapidly via large population size, shorter replication times, and increased mutation rates [1]. This review article is an attempt to understand the significance of the extensive genetic diversity of the dengue virus (DENV) in the development of greater magnitude of the viral pathogenicity as well as in the severity of recurrent outbreaks in the context of changing environment and epidemiology.

2. Morphology of Dengue Virus

The virion of dengue virus is spherical and 40-50 nm in diameter [6]. It consists of a nucleocapsid which is about 30 nm in diameter enclosed in a lipid envelop. The lipid envelope contains a lipid bilayer and an envelope protein (~51000-59000 Daltons) that facilitates attachment, fusion and penetration. A non-glycosylated internal matrix protein (8500 Daltons) is also present in the lipid envelope [6]. Researches demonstrated that mature dengue virion is characterized by a comparatively smooth surface and 180 copies of envelope protein forming an icosahedral scaffold [7].

3. Genome of Dengue virus

The DENV genome is composed of a single-stranded, positive-sense RNA molecule ~10.7 kb in length. It accommodates a single translated open reading frame (ORF) which encodes a precursor polypeptide (~3390 amino acids) that is further processed catalytically by DENV and host proteases into ten viral proteins: three structural proteins (C, capsid; prM/M, precursor of membrane; E, envelope) which are encoded at the 5' end and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) which are encoded at the 3' end [6] (FIGURE 1) (Table 1). The ORF of dengue virus is flanked at its 5' terminus by an untranslated region (UTR) which is about 100 nucleotides in length and a longer UTR at its 3' terminus which is about 500 nucleotides in length. The 5' terminus of DENV genome contains a type I cap and there is no polyadenylation at 3' terminus [8]. Researches also showed the existence of several multifunctional non-structural proteins [6]. The E protein is the most important among the three structural proteins in regard to virus infectivity. It is glycosylated at two specific sites which are Asn-67 and Asn-153 that is responsible for DENV attachments to receptors on host cells and fusion with host cell membrane [6].

Table 1: List of Non-Structural Proteins of DENV and their functions.

Non Structural Proteins (NS-Proteins)	Functions	References
NS1	Functions in viral RNA replication complex, soluble complement-fixing antigen	6, 200
NS2A	Constituent of RNA replication complex.	
NS2B	NS3 protease cofactor	
NS3	Serine protease, RNA helicase, RTPase/NTPase.	
NS4A	Induces membrane alterations required for virus replication.	
NS4B	Blocks IFN α/β-induced signal transduction.	
NS5	RNA-dependent RNA polymerase (RdRp) and Methyltransferase (MTase)	

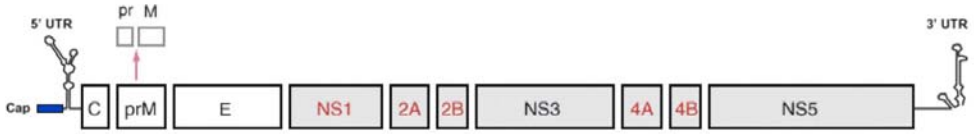

FIGURE 1. Dengue virus RNA genome[200].

4. Significance of 5' and 3' end of DENV RNA genome

The specific structures in 5' and 3'end of DENV genome have a profound role in RNA synthesis [9]. The 5'-UTR of dengue virus are 95-101 nucleotides in length which contain two RNA domains that have a distinct role during viral RNA synthesis [9]. The first domain comprised of ~70 nucleotides is believed to fold into a large stem-loop (SLA) structure. DENV SLA is a Y shaped structure and is thought to function as the promoter for viral RNA directed RNA polymerase (RdRp) [9-14]. In addition, three helical regions (S1, S2, and S3) separated by bulges and highly reactive single stranded regions associated with side stem loop and a top loop are present. The second domain of DENV 5'-UTR is thought to produce a short stem-loop (SLB) which possess important sequence for long range RNA-RNA interaction and genome replication [14]. Inside the coding region downstream of the translation initiation codon, AUG, a stable hairpin (cHP) is also found in DENV genome which is necessary for viral RNA replication [15]. The 3'-UTR of DENV is approximately 450 nucleotides in length and can be divided into three specific domains. Domain I is the most variable region and is located immediately after the stop codon [16]. This domain exhibits substantial size distribution between the viral serotypes which usually ranging from more than 120 nucleotides to less than 50 nucleotides [17-22]. Domain II involves a dumbbell (DB) structure which contains tandemly duplicated nucleotides [17, 20, 22]. These specific domain structures generally function as enhancers of the viral processes [16, 23-26]. Domain III is the most conserved region in the 3'-UTR containing CS1 element which contains a virus cyclization sequence that is complementary to a specific sequence exists in 5' end of the virus genome and involved in long range RNA-RNA interaction between the virus genome [9, 14]. The terminal 3' structure bears a short stem loop (sHP) which is 14 nucleotides in length, followed by a large stem loop that is 79 nucleotides in length. These two adjacent structures containing a total of 93 nucleotides are collectively referred as 3'SL, which is critical for flavivirus replication [9, 27, 28].

5. Different Serotypes and their genotypes of Dengue virus:

The Dengue virus occurs mainly as four antigenically distinct serotypes (DEN 1, 2, 3, and 4) [1, 29]. All the four serotypes are found circulating in India. There is genetic variation within each serotype in the form of phylogenetically distinct subtypes or genotypes. South-east Asia harbors the greatest genetic diversity of dengue virus, suggesting it acts as a viral 'source' population [1, 30]. Recently, a new serotype of dengue virus DEN-5 has been detected from a hospitalized 37 years old farmer in Sarawak state of Malaysia. Researchers demonstrated that this new serotype of dengue virus is genetically similar to other four dengue serotypes, thus thought to be originated from a common predecessor [31]. (Table. 2)

Table 2: Different DENV Serotypes and their distribution.

Dengue Virus Serotypes	Genotypes	Distribution	References
DEN-1	Genotype I	Hawaii in the 1940s (the prototype strains), Japan, China, Southeast Asia and Taiwan.	6, 201
	Genotype II	Thailand in the 1950s and 1960s.	
	Genotype III	Sylvatic origin in Malaysia.	
	Genotype IV	Nauru, Australia, Philippines and Indonesia.	
	Genotype V	The America, Africa, Southeast Asia.	
DEN-2	American	Formerly known as subtype V. Encountered in Latin America, old strains from India (1957), the Caribbean, and the Pacific islands between 1950 and 1970s.	6, 202
	American/Asian	Formerly known as subtype III. Encountered in China, Thailand, Vietnam and Latin America.	
	Asian I	Thailand, Myanmar and Malaysia.	
	Asian II	Formerly known as subtype I and II. Encountered in China, the Philippines, Sri Lanka, Taiwan and Vietnam. It includes the New Guinea C prototype strain.	
	Cosmopolitan	Formerly known as genotype IV. Widely distributed including Australia, the Pacific islands, Southeast Asia, the Indian subcontinent, Indian Ocean islands, Middle East, and both East and West Africa.	
	Sylvatic	Found and isolated from non-human primates in West Africa and Malaysia.	
DEN-3	Genotype I	Indonesia, Malaysia, Thailand, Burma, Vietnam, the Philippines and the South Pacific islands (French Polynesia, Fiji and New Caledonia). Includes the H87 prototype strain.	6, 203
	Genotype II	Thailand, Vietnam and Bangladesh.	
	Genotype III	Singapore, Indonesia, South Pacific islands, Sri Lanka, India, Africa and Samoa.	
	Genotype IV	Puerto Rico and French Polynesia (Tahiti).	
DEN-4	Genotype I	Thailand, Malaysia, the Philippines and Sri Lanka. It includes the H241 prototype strain.	6, 38, 204
	Genotype II	Indonesia, Malaysia, Tahiti, the Caribbean islands (Puerto Rico and Dominica) and the Americas.	
	Genotype III	Thailand (Bangkok, specifically).	
	Sylvatic	Found and isolated from non-human primates in Malaysia.	
DEN-5	Not Reported	Sarawak state of Malaysia	31

6. Major Factors behind DENV Genetic Diversity

A. Mutation

The RNA virus population generally associated with high mutation rates and contain genetic diversity [32]. Studies indicated that mutations are not biased toward a specific nucleotide and the mutation frequency of each nucleotide is usually proportional to its occurrence [33]. Point mutations like transitions (A to G, G to A, C to T or T to C) are found most prevalently in the dengue genome (Table. 3). Mutations are also repeatedly observed in the third codon position. Moreover, it has also been observed that the rare variants like deletions and early termination STOP mutations found at low frequencies in the dengue genome during human infections [33]. Variations in the non-structural proteins have also been found in association with increasing dengue severity [34-39].

Table 3: Types of Mutations majorly found in DENV.

Genomic Components		Types of Mutations	References
Structural Genes	C gene	Nucleotide Substitution	33, 65, 132, 135, 201
	prM gene	Nucleotide substitution	
	E gene	Nucleotide substitution, Deletion	
Nonstructural Genes	NS1	Nucleotide Substitution	
	NS2A	Nucleotide Substitution	
	NS2B	Nucleotide Substitution	
	NS3	Nucleotide Substitution	
	NS4A	Nucleotide Substitution	
	NS4B	Nucleotide Substitution	
	NS5	Nucleotide Substitution, Deletion, Frameshift	
Cis-regulatory region	3'-UTR	Transversion, Transition, Deletion, Insertion	

B. Recombination

Recombination is a momentous impetus behind genomic diversity [40-44]. Recombination events are commonly found in RNA virus, including DENV [45]. Dengue infection with two different strains which is a prerequisite for DENV recombination has been demonstrated in both human and mosquitoes [46-50]. Researchers have demonstrated that recombination can occur in different genetically diverse DENV strains [45, 51-57]. Two major mechanisms have been considered for viral RNA recombination. First one is non-replicative breakage and re-joining and second is replicative template switching [42, 58]. Recombination breakpoint for the (+) ssRNA virus have been found in the non-structural protein and also in the structural protein encoding regions [59, 60]. Researchers reported that three recombination zones in DENV located within the sequences prM-E junction, NS1 and NS3 genes [61]. Interestingly, it has been reported that DENV RNA dependent RNA polymerase (RdRp) exhibits a significant conformational flexibility during the transition from initiation to elongation [62] which might favour the recombination event in virus [61]. Researchers reiterated that RNA recombination events like homologous recombination (at sites with exact sequence match), aberrant homologous recombination (requires sequence homology) and non-homologous

recombination (independent of sequence homology) also occurs in RNA virus [63, 64].

7. Characteristics of Genomic variations leading to the intriguing DENV pathogenesis

Phylogenetic studies indicated that genetic subtypes of the DENV differ (up to 12 %) in the nucleotide sequence of E-gene [65, 66]. Researchers demonstrated that several structural differences are present between two DEN-2 genotypes (such as, the Southeast Asian genotype with DF and DHF and the American genotype with only DF) which results in distinct human clinical presentation [65]. There are six amino acid change differences are encountered, including the pre-membrane protein (prM; amino acid 28 Glu→Lys), the envelope glycoprotein (E; amino acid 390 Asn→Asp), the non-structural protein 4B (NS4B; amino acid 17 Ser→His) and NS5 (amino acid 645 Asn→Asp, 676 Ser→Arg and 800 Lys→Ser). Moreover, the comparative analysis between the American strain and the Thai strains discovered a plausible correlation between the amino acid sequence of the subtypes of DEN-2 strain and the clinical severity of the patients from whom the virus strains were collected [65]. From all DSS cases, three amino acid changes were found in subtype I of DEN-2 strain: D→G change at position 278 in NS1; N→D change at 139 in NS2A; M→I change at 13 in NS3. From all DF cases, five amino acid changes were found in subtype III of DEN-2 strain I→R change at 16 and T→A at 81 in prM; I→M at 136 in NS2A; A→T at 118 in NS3; and T→M at 337 in NS5 [65].

8. Dengue and RNAi

In insects, RNA interference (RNAi) mechanism is considered as a major antiviral defence mechanism [67-73]. In order to transmit into a suitable host, arbovirus like dengue must escape this anti-viral defence [67, 74]. The DENV 3' UTR RNA structures are thought to be linked with the generation of subgenomic flavivirus RNA (sfRNA) [75]. Sf RNA are produced by the incomplete degradation of viral genome by cellular ribonuclease XRN1 which stalled at pseudoknot structures at the 3'-UTR [75-77]. Interestingly, it is shown that sfRNA also inhibit RNAi pathways in mosquitoes [78].

9. Discussion

The increased incidence of dengue has been observed throughout the world since last three decades [1, 79, 80]. Disease severity of dengue is often ascribed to secondary infection with a virus belonging to a serotype other than that of the primary infection. In this context, evolution of the virus is also considered as a major contributing factor behind the enhanced dengue epidemics [81, 82]. Multi-serotype infection and antigenicity of DENV might have profound effect in augmented dengue epidemics [81, 83]. Mutation rates in viruses with RNA genomes can be six orders of magnitude greater than the viruses with DNA genomes as no proof reading enzymes are available for RNA dependent RNA polymerases, thus exhibiting error frequencies nearly equal to 10^{-4} [1, 84]. High mutation rate associated with RNA polymerase introduces approximately one error per genome during each round of viral replication [1, 85]. According to the "Muller's ratchet" theory, a high rate of mutation in RNA virus results the accretion of deleterious non-synonymous mutations and such deleterious mutations are then removed by purifying selection [86. 87]. Researchers reiterated that natural recombinants of dengue virus (DENV) carry multiple recombination events [41]. Analysis of the nucleotide sequences from the DEN-1 and DEN-2 serotypes revealed that a networked evolution has occurred in DENV progeny, indicating the strong probability of occurrence of genetic recombination [88]. The inter-virus genetic recombination occurs successfully which could lead to form new virus variants [1, 89]. Interestingly, intra-serotype homologous recombination has also been identified in the non-structural gene region [90]. Surprisingly, studies demonstrated that the high evolving organisms like virus, bacteria exhibit intra-codon recombination event [91, 92], which is a form of genetic recombination where the nucleotide triplets of the same codon engage in sequence exchange via significant breakpoints within the codon [81]. The different serotypes of DENV also exhibit intra-codon recombination event. This indicates that the genetic recombination event within the codon level has a significant effect to maintain the extensive purifying selection [81]. Studies stated that the viral surface protein of arbovirus is also subjected to strong purifying selection [93].

The DENV subtypes or genotypes differ in both fitness and virulence which have poignant significance in the viral population structure [94]. The abrupt change in genetic composition of one genotype of DENV causes the displacement of another genotype in a specific population [38, 39, 95-97]. This is markedly evident from the phenomenon where replacement of low virulence American genotype of DEN-2 virus by high virulence Southeast Asian genotype of the DEN-2 virus. This has been found in Latin America [98-100]. This replacement might be the result of immune mediated natural selection in which an altered genetic composition in one genotype helped it to evade the cross-immunity generated by the other genotype [101]. The four serotypes of dengue virus have been evolved in non-human primate reservoirs and then leaped over to the humans because of clustering of sylvatic strains with the human strains as a result of increased anthropogenic activity [102] (FIGURE 2). Experimental findings suggest that there is no significant adaptive barrier exists for the emergence of sylvatic DENV in human populations, possibly indicating the DENV might be an "opportunistic virus" and has a significant potentiality to infect different primates [102]. Thus, the co-circulation of different dengue serotypes and increased anthropogenic activity enhance the possibility of genetic changes through recombination and thereby increases a significant plausibility for availability of genetically diverse viral strains. These above apprehensions might be considered as potential reasons behind the emergence of a new variant serotype, DEN-5 [31]. Studies suggested that South-east Asia, possibly harbours DENV "source" population [1, 30]. Thus the finding of a new serotype DEN-5 from Sarawak state of Malaysia of South East Asia [31] further increases the importance of thorough study on the emergence of new virus strains and genotypes or subtypes. In this context, it could also be mentioned that global change of climate might become an important factor behind the sylvatic DENV circulation through the territorial expansion of the dengue vectors [103]. Although, DENV infection is mostly dependent on the considerable interaction of vector mosquito and human host [103] but in near future there is a possibility that the newly emerged serotypes such as DEN-5 could result a severe dengue outbreak in human population with an altered pathogenicity which could further complicate the disease situation in the changing environment.

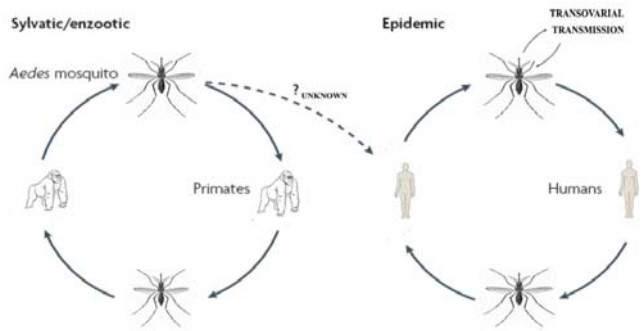

FIGURE 2. Dengue virus Transmission Cycle [6].

Aedes aegypti has been considered to be the principal vector of dengue virus in the urban transmission cycle while *Aedes albopictus* serves as a secondary vector or a maintenance vector [104, 105]. It has been reported that these mosquito vectors has aggressively invaded different ecological areas [106-109]. The global expansion of their distribution and introduction to new continents has been attributed by international trade routes through shipping and increased anthropogenic activities [110-112]. The persistence and the maintenance of the DENV in the nature during inter-epidemic period are results from transovarial transmission of dengue virus by these vector mosquitoes [1]. The outbreak of dengue in India was generally associated with *Aedes aegypti* but interestingly, in Kerala state *Aedes albopictus* has identified as the primary vector for dengue [105]. Thus studying the role of two above mentioned *Aedes* mosquitoes in virus transmission may further elevate our understanding about the vector-virus dynamics in the dengue transmission cycle occurring in natural condition [61]. *Aedes albopictus* has been included within the hundred most dangerous species in the global invasive species database [1, 113]. The genome of this mosquito possesses extensive repetitive sequence and significant groups of transposable elements. It has found that ~68% of genome representing the repetitive sequence [114]. This high repetitive DNA is associated with large genome size and the length of the entire genome is ~40% more than *Aedes aegypti*. This large genome size with extensive repetitive sequence confers a prominent status of "Invasive Species" to *Aedes albopictus* [114]. The RNA sequences related to flavivirus has found integrated in dsDNA genome of these *Aedes* mosquitoes which indicate the possible DNA synthesis from viral RNA by endogenous reverse transcriptase activity [115-117]. This in turn strongly supports the fact that flavivirus has the potential ability to recombine with the mosquito DNA [118, 119]. A relative complex interaction persists between the mosquito's nucleic acid metabolism and DENV replication which might facilitate the generation of recombinant virus [61]. Interestingly, research identified that DENV genome harbours a large number of positions which exhibit a high degree of intra host viral diversity [120]. There are seven positions in DENV genome that shows this high degree of plasticity, which are: two in the E gene, one in the NS1 gene, one in the NS3 gene, one in the 2k peptide at the C terminus of the NS4A gene and two in the NS5 gene. The changes of intra-host genetic diversity during the human infection were encountered more extensively in the NS1, NS2A and E-gene. However, in *Aedes aegypti*, the changes were located in prM, E, NS1, NS3, NS4A (2k peptide) and NS5 genes whereas, in *Aedes albopictus,* the changes were found in E, NS1, NS4A (2k peptide) and NS5 genes. These differences indicate that the DENV which transmitted through an *Aedes aegypti*-human cycle may produce genetically different virus from those transmitted through *Aedes albopictus*-human cycle [120].Thus, it could be fair to mention that the unique genomic properties and the ecological plasticity of both the *Aedes* mosquitoes confer a profound effect on the dengue virus global distribution and maintenance of extensive virus genetic diversity in natural condition. This might be associated with unexpected dengue outbreaks and disease complications.

The other members of the Flaviviridae family like WNV and JEV require replication before transmission. Animal hosts such as pigs and birds play an important role in the maintenance and amplification of the virus. These virus survive by virtue of alternation between vertebrate and invertebrate hosts in a "cycle" where man gets the infection tangentially on accidental intrusion in this pathway [121]. DENV infection requires an invertebrate host (mosquito vector) and a primate host [102, 122]. Interestingly, the flavivirus like WNV, JEV increase their within-host genetic diversity through replication [123]. Albeit, it has been reported that significant purifying selection pressure is also acting on the JEV genome [124, 125] but *in vitro* studies indicated that a single alternate passage in vertebrate cell culture after sequential passage in mosquito cell culture decrease genetic diversity of the flavivirus [123, 126]. Thus, the infection caused by these viruses to human "dead end" host might result in a conspicuous reduction of the chances of the availability of naturally fit genetically variant virus strains. Moreover, the host alteration reduces virus fitness in comparison to single host viruses [127-131]. The essence of dengue virus evolution relies on the fact that, the mutations (deletions and insertions) in the variable regions of 3'-UTR of the DENV genome [54, 132-135] and duplication of a RNA structure at 3'-UTR have a profound role in rapid host specific adaptation without significantly reducing virus fitness [78]. In addition, the NS genes are also thought to have an important role in viral fitness [34]. It is demonstrated that a higher recombination rate per nucleotide probably plays a major role in the more effective purifying selection observed in RNA viruses [93]. Thus, the ability of rapid host adaptation of DENV coupled with genetic recombination events may facilitate the extensive genetic diversity which is then might subjected to purifying selection and thereby may produce naturally fit DENV variant strains. Additionally, the significant role of the cis-regulatory region, 3'-UTR of the DENV RNA genome in facilitating the inhibition of RNA interference pathway in host [78] might enlighten and explain the possible strategy of the virus to bypass or avoid the host developed mechanisms against the virus.

The different serotypes of dengue virus also transmitted between non-human primates and mosquitoes in tropical Asia and West Africa. The presence of this silent zoonotic transmission cycle of DENV could explain about the selection of dengue virus variants with altered host range [136]. Interestingly, a large proportion of asymptomatic dengue cases are found in association with symptomatic dengue cases in a group of affected individuals in a population [137]. It has been also reported that mosquitoes might have the possibility of getting the infection from the individuals who are infected by dengue virus but showed no clinical symptoms [138]. This may add a new dimension in the dengue epidemiology, where some human individuals with asymptomatic dengue infection might

play a potential role as a source in the dissemination of infection [139, 140]. This phenomenon could also facilitate the natural maintenance of dengue virus in the urban areas. However, we are hesitant to refer this human source as a "reservoir host" of dengue virus in urban areas, but may be interpreted as a silent carrier.

Dengue virus shares similarity with the primitive mosquito-borne encephalitis viruses. Interestingly, this virus has significantly evolved a notable biological feature, called "lymphotropism" which segregates them from their more primitive neurotropic ancestors [136]. The virus usually damaged the primate monocyte or macrophage as a principal target cell for replication [141]. Experiments have been revealed that there is a wide range of cellular tropism found in DENV. DENV antigens have been detected from mononuclear cells, lymphocytes, Langerhans cells in the skin, neurons, astrocytes, endothelial cells and hepatocytes, heart and skeletal muscle [142, 143-156]. Researchers reiterated that the C-protein of DENV interacts with the multifunctional host protein Nucleolin (NCL) which facilitates the viral morphogenesis [157]. This altered tropism of dengue virus in humans might indicate the fitness strategy of the virus in urban areas since human is the only possible source of viremic vertebrate. This possibly ensured the sustained transmission of dengue virus in urban situations. The "lymphotropism" phenomenon also provides an opportunity for the survival, maintenance and spread of DENV in urban areas in the absence of any non-human primate reservoir. Moreover, this extraordinary capacity of DENV to replicate in human tissues to high titer level indicates the possibility of emergence of potentially variant strains with altered tropism which could significantly change the disease expression in the host. Therefore, in the context of flavivirus evolution, there is a high possibility that flavivirus like dengue is able to convert itself into a much threatening pathogen by genetic changes which can efficiently capable of causing encephalitis [136], encephalopathy [158] and other neurological complexities. This can be supported by the example which involves the detection of DEN-4 in brain cells and in cerebro-spinal fluid (CSF) of a patient with encephalitis [159, 160]. Interestingly, various neurological manifestations corresponding with DEN-2 and DEN-3 infections have also been found [158]. Thus the possible altered expression of dengue and the burgeoning disease severity might further complicate the existing dengue infection situation in the near future and may create a situation of possible confusion in the disease recognition as well.

Dengue infection with two different strains which is a prerequisite for DENV recombination has been already demonstrated in human [46]. Interestingly, the RNA virus has developed the ability to recombine with the genome of unrelated group of DNA viruses [161]. This further increases the possibility of emergence of entirely new virus strains or subtypes in the environment. Moreover, remarkably researchers reiterated that the RNA virus has also developed the ability to recombine with host dsDNA genomes [162]. Studies indicated that transposon mediated exchanges and the group II intron retro-homing mechanism could facilitate the formation of recombinant virus containing RNA-DNA hybrid genome [161, 163, 164]. The host cell might also use similar related host cell based molecular mechanism to facilitate the formation of recombinant virus. Thus, these extraordinary abilities of RNA virus like DENV may unlock a new vista in dengue research, which encompasses a relevant proposition of a momentous plausibility of crucial genetic exchange between DENV (+) ssRNA genome and dsDNA of the human host. However, any purifying selection within individual hosts may be relatively weak [165], but with the passage of time natural selection might favour the emergence of novel genetically diverse DENV variants, resulted from the crucial genetic exchange between dengue virus genome and the human genome.

Interestingly, it has been demonstrated that the dengue virus can be capable of causing apoptotic cell death in both, *in vitro* and *in vivo* conditions [166]. The crucial induction of programmed cell death by DENV may contribute to severe dengue pathogenesis [142]. Studies demonstrated the presence of apoptotic cells in liver, brain, intestinal and lung tissues which were found in the autopsy examination of fatal DHF/DSS cases [167]. It was also indicated that in the neuroblastoma cells of mice, the notable aggregation of viral proteins in the ER membrane might responsible for the ER stress, which then initiates the apoptotic pathway [168]. The major aspect of DHF/DSS is the induction of apoptosis event in the endothelial cells, which might be caused from DENV-NS1 activating complement system [169, 170]. The apoptosis event in the hepatocytes might be a possible reason of liver damage observed in few dengue infected patients [171, 172]. Studies revealed that megakaryopoiesis could be actively inhibited by DEN-2 virus in *in vitro* condition and is associated with programmed cell death of initial megakaryocyte progenitor subpopulation which may be a significant cause of thrombocytopenia during dengue [173]. Moreover, the apoptosis in the dendritic cells could disable innate immune response or could facilitate the viral escape from the immune surveillance which ultimately results in severe dengue pathogenicity [142]. The severity of dengue infection also results in the undesired destruction of neighboring cells (Bystander effect) and thereby leads to the weakening of the host immune response [174, 175]. Moreover, patient with acute dengue infection has been found with viral myositis, which then leads to the development of rhabdomyolysis in the patient [176]. These instances of severe pathogenicity of DENV might be regulated at the genetic level of the virus and may be associated with unusual mutational and recombination events.

The extensive genetically diverse RNA virus population like DENV thought to consist of a group of closely related non-identical genomes which are referred as viral quasispecies [177-182]. Viral quasispecies might play an important role in the development of the chronic disease [183]. These quasispecies might evolve as a probable result of competition and selection [184]. The accretion of random mutations resulting from the high mutation rate in the RNA genome of DENV might cause the formation of DENV quasispecies [183]. Quasispecies of a flavivirus like the human hepatitis C virus successfully escape from the host immune response when the host immune system has failed to reduce the genetic diversity of the virus [185]. In addition, they could also escape from the pressure of drug treatment [183]. The viral quasispecies contain the genomes with most fitness that is encompassed by a mutant virus spectrum [184]. Moreover, the diverse virus population involves "low-fitness intact viruses" which might show higher fitness in dissimilar environments. The co-infection in association of "high fitness viruses" in disparate environments might be the possible reason in favour of the survival of those "low-fitness intact virus" [183, 186]. Remarkably, studies indicated that RNA

virus like DENV could carry a memory genome in the virus population at the intra-host and inter-host level. The virus population in the form of quasispecies might also carry a molecular memory genome [183]. The memory genome of DENV could result the different cell tropism of different DENV [187]. The co-infection of different DENV strains or genotypes might be considered as the reason behind the formation of viral quasispecies. The DENV population might contain different small minor populations and these populations might be maintained through infections in both mosquitoes and humans. DENV population may be successful to carry the unique genome and genomic combinations which might in turn result in altered cell tropism [183]. Therefore, the uniqueness of DENV genomic constituents and its unconventional interaction with the host genome could further complicate the pathogenicity and severity of the disease.

The evolution of the mosquito borne virus involves the genetic bottleneck and extinction events [188]. The genetic bottleneck causes the emergence of new virus strains and variants from limited members amongst the majority of co-circulating virus in a particular region. The reintroduction of various DENV subtypes in a particular area has been reported [189, 190]. The intricate mutational events in the virus in association of interaction with the host cells may confer an ability of a notable resistance to the positive sense strand RNA virus like dengue against its extinction [184]. Albeit the vertical evolution through random mutation has been considered as the principal factor in the evolution of RNA virus like DENV, but the admixture of genetic constituents between individuals of different strains of the dengue also directs the mode of DENV evolution horizontally by facilitating horizontal evolution [191]. The unique capability of DENV in the maintenance of extensive genetic diversity by high mutation rates, structural modification of cis-regulatory region of its RNA genome and unusual genetic exchange between its different strains, related and unrelated group of viruses and host ds DNA genome in artificial and natural condition is a possible indication of rapid evolutionary adaptation of the DENV in the changing environment.Thus, these unique genetic strategies might indicate the prospective direction of DENV evolution, which is associated with altered disease expression in the changing climate.

Vaccination might produce an ambiance where reasonably low transmission of natural DENV could be found [34]. The World Health Organization (WHO) suggested that the development of tetravalent vaccine is the most effective approach for prevention of dengue [192]. The most important feature of an effective dengue vaccine must include its ability to provide protection against all dengue virus in different dengue endemic geographical regions [193]. The live tetravalent vaccine development has been initiated in Bangkok, Thailand in late 1970 [194]. In addition to this, other significant approaches like recombinant vaccines have also been developed [195]. Inspite of extensive efforts, a safe and effective dengue candidate vaccine was not developed [192]. However, a world renowned multinational pharmaceutical company, Sanofi Pasteur has recently developed the first dengue vaccine Dengvaxia™, which has been approved by Mexico [196]. Studies indicated that recombination events either between strains which are presented in multivalent vaccine or between an attenuated vaccine strain and wild type strain might cause the unwanted emergence of new variant virus with unusual properties [197-199]. Thus, in this changing epidemiological situation, the effectiveness of a vaccine in lowering the transmission of several naturally developing genetically variant DENV in different dengue endemic geographical regions is still a matter of conjecture.

10. Conclusion

The evolutionary arms race between host and virus has always played a pivotal role in shaping the virus evolution. The mutational and unique recombination events might be a principal reason behind their extensive genetic diversity. The mosquito vector provides an environment where changes of viral genotype occur and applies selection pressure on the different viral phenotypes. Hence, vectors have a great chance to influence the evolution of such viruses. An inclusive understanding of host-virus interaction is also needed, to fully assess the factors that regulate the potential for host shifts and geographic expansions. Additionally, rapidly increasing size of the human population and globalization may allow natural selection to play an even more important role in viral evolution, especially, arthropod-borne viruses like dengue virus. Therefore a sustained active surveillance, molecular sequencing studies and phylogenetic analysis of different DENV isolates are required to understand the evolutionary trend of dengue virus with reference to genetic diversity in the changing climate.

11. Acknowledgements

The authors express their sincere thanks to Dr. Dipak Kumar Kar, Principal, Asutosh College for his sustained support for this work. Thanks are also due to Dr. Sibnarayan Datta, Scientist, Defence Research Laboratory, Defence Research and Development Organisation (DRDO), Ministry of Defence (Government of India) for his kind suggestions.

12. Conflict Of Interest

There is no conflict of interest.

13. References

1. Bhattacharya S. Mosquito-Borne Viruses of Public Health Importance: Evolutionary Perspectives and Changing Epidemiology. J Appl Biosci. 2014; 40(1):1-7.
2. Taylor LH, Latham SM, Woolhouse ME. Risk factors for human disease emergence. Philos. Trans R. Soc. Lond. B. Biol. Sc 2001; 356:983-989.
3. Vasilakis N, Deardorff ER, Kenney JL, Rossi SL, Hanley KA, Weaver SC. Mosquitoes Put the Brake on Arbovirus Evolution: Experimental Evolution Reveals Slower Mutation Accumulation in Mosquito Than Vertebrate Cells, Plos Pathogens 2009; (5):e1000467-e1000467.
4. Gupta N, Srivastava S, Jain A, Chaturvedi UC. Dengue in India, Indian J Med Res. 2012; (136):373-390.
5. Mustafa MS, Bansal AS, Rastogi V. Flightless *Aedes* mosquitoes in dengue control. MJAFI. 2011; 67:192-193.
6. Swee Hoe Ong. Molecular Epidemiology of Dengue Viruses from Complete Genome Sequences (Doctoral Dissertation) 2010; University of Basel. Retrieved From: http://edoc.unibas.ch/1155/1/my_thesis_edoc.pdf.
7. Kuhn RJ, Zhang W, Rossman MG, Pletnev SV, Corver J, Lenches E *et al.* Structure of dengue virus: implications for flavivirus organization, maturation, and fusion. Cell 2002; 108:717-725.
8. Chambers TJ, Hahn CS, Galler R, Rice CM. Flavivirus genome organization, expression, and replication. Annu

Rev Microbiol 1990; 44:649-688.
9. Gebhard LG, Filomatori CV, Gamarnik AV. Functional RNA Elements in the Dengue Virus Genome. Viruses 2011; 3:1739-1756.
10. Lodeiro MF, Filomatori CV, Gamarnik AV. Structural and functional studies of the promoter element for dengue virus RNA replication. J Virol. 2009; 83:993-1008.
11. Polacek C, Foley JE, Harris E. Conformational changes in the solution structure of the dengue virus 5' endfig in the presence and absence of the 3' untranslated region. J Virol. 2009; 83:1161-1166.
12. Yu L, Nomaguchi M, Padmanabhan R, Markoff L. Specific requirements for elements of the 5' and 3' terminal regions in flavivirus RNA synthesis and viral replication. Virology 2008; 374:170-185.
13. Filomatori CV, Lodeiro MF, Alvarez DE, Samsa MM, Pietrasanta L, Gamarnik AV. A 5' RNA element promotes dengue virus RNA synthesis on a circular genome. Genes Dev. 2006; 20:2238-2249.
14. Alvarez DE, Lodeiro MF, Luduena SJ, Pietrasanta LI, Gamarnik AV. Long-range RNA-RNA interactions circularize the dengue virus genome. J Virol. 2005; 79:6631-6643.
15. Clyde K, Barrera J, Harris E. The capsid-coding region hairpin element (cHP) is a critical determinant of dengue virus and west nile virus RNA synthesis. Virology 2008; 379:314-323.
16. Alvarez DE, De Lella Ezcurra AL, Fucito S, Gamarnik AV. Role of RNA structures present at the 3'UTR of dengue virus on translation, RNA synthesis, and viral replication. Virology 2005; 339:200-212.
17. Silva RL, de Silva AM, Harris E, MacDonald GH. Genetic analysis of dengue 3 virus subtype III 5' and 3' non-coding regions. Virus Res 2008; 135:320-325.
18. Vasilakis N, Fokam EB, Hanson CT, Weinberg E, Sall AA, Whitehead SS. Genetic and phenotypic characterization of sylvatic dengue virus type 2 strains. Virology 2008; 377:296-307.
19. Roche C, Cassar O, Laille M, Murgue B. Dengue-3 virus genomic differences that correlate with *in vitro* phenotype on a human cell line but not with disease severity. Microbes Infect 2007; 9:63-69.
20. Zhou Y, Mammen MP Jr, Klungthong C, Chinnawirotpisan P, Vaughn DW, Nimmannitya S. *et al*. Comparative analysis reveals no consistent association between the secondary structure of the 3'-untranslated region of dengue viruses and disease syndrome. J Gen Virol. 2006; 87: 2595-2603.
21. Aquino VH, Anatriello E, Goncalves PF, EV DAS, Vasconcelos PF, Vieira DS. *et al.* Molecular epidemiology of dengue type 3 virus in brazil and paraguay, 2002–2004. Am. J Trop Med Hyg. 2006; 75:710-715.
22. Shurtleff AC, Beasley DW, Chen JJ, Ni H, Suderman MT, Wang H. *et al.* Genetic variation in the 3' non-coding region of dengue viruses. Virology 2001; 281:75-87.
23. Bredenbeek PJ, Kooi EA, Lindenbach B, Huijkman N, Rice CM, Spaan WJ. A stable full-length yellow fever virus cDNA clone and the role of conserved RNA elements in flavivirus replication. J Gen Virol. 2003; 84:1261-1268.
24. Lo MK, Tilgner M, Bernard KA, Shi PY. Functional analysis of mosquito-borne flavivirus conserved sequence elements within 3' untranslated region of west nile virus by use of a reporting replicon that differentiates between viral translation and RNA replication. J Virol. 2003; 77:10004-10014.
25. Mandl CW, Holzmann H, Meixner T, Rauscher S, Stadler PF, Allison SL *et al.* Spontaneous and engineered deletions in the 3' noncoding region of tick-borne encephalitis virus: Construction of highly attenuated mutants of a flavivirus. J Virol. 1998; 72:2132-2140.
26. Men R, Bray M, Clark D, Chanock RM, Lai CJ. Dengue type 4 virus mutants containing deletions in the 3' noncoding region of the RNA genome: Analysis of growth restriction in cell culture and altered viremia pattern and immunogenicity in rhesus monkeys. J Virol. 1996; 70:3930-3937.
27. Elghonemy S, Davis WG, Brinton MA. The majority of the nucleotides in the top loop of the genomic 3V terminal stem loop structure are cis-acting in a West Nile virus infectious clone. Virology 2005; 331(2):238-246.
28. Khromykh AA, Kondratieva N, Sgro JY, Palmenberg A. Westaway EG. Significance in replication of the terminal nucleotides of the flavivirus genome. J Virol. 2003; 77(19):10623-10629.
29. Gubler DJ. Dengue and dengue haemorrhagic fever, Clin. Microbiol. Rev. 1998; 11:480-496.
30. Zhang C, Mammen MP Jr, Chinnawirotpisan P, Klungthong C, Rodpradit P, Monkongdee P. Clade replacements in dengue virus serotypes 1 and 3 are associated with changing serotype prevalence. J Virology. 2005; 79:15123-15130.
31. Mustafa Lt Col MS, Rasotgi Col V, Jain Col S, Gupta Lt Col V. Discovery of fifth serotype of dengue virus (DENV-5): A new public health dilemma in dengue control. Medical Journal Armed Forces India. 2015; 71:67-70.
32. Simon-Loriere E, Holmes EC. Why do RNA viruses recombine? Nat. Rev. Microbiol. 2011; 9:617-626.
33. Parameswaran P, Charlebois P, Tellez Y, Nunez A, Ryan EM, Malboeuf CM *et al.* Genome-Wide Patterns of Intrahuman Dengue Virus Diversity Reveal Associations with Viral Phylogenetic Clade and Interhost Diversity. Journal of Virology. 2012; 86(16):8546-8558.
34. Rodriguez-Roche R, Gould EA. Understanding the Dengue Viruses and Progress towards Their Control. BioMed Research International, Article ID 690835, 20 pages, 2013.
35. Tang Y, Rodpradit P, Chinnawirotpisan P, Mammen jr MP, Li T, Lynch JA. Comparative analysis of full-length genomic sequences of 10 dengue serotype 1 viruses associated with different genotypes, epidemics, and disease severity isolated in Thailand over 22 year. The American Journal of Tropical Medicine and Hygiene. 2010; 83(5):1156-1165.
36. Zhao R, Chinnawirotpisan P, Klungthong C, Zhang C, Putnak R. Evidence for inter- and intra-genotypic variations in dengue serotype 4 viruses representing predominant and nonpredominant genotypes co-circulating in Thailand from 1977 to 2001. Virus Genes. 2010; 41(1):5-13.
37. Chen HL, Lin SR, Liu HF, King CC, Hsieh SC, Wang WK. Evolution of dengue virus type 2 during two consecutive outbreaks with an increase in severity in Southern Taiwan in 2001-2002. The American Journal of

Tropical Medicine and Hygiene. 2008; 79(4):495-504.
38. Klungthong C, Zhang C, Mammen Jr MP, Ubol S, Holmes EC. The molecular epidemiology of dengue virus serotype 4 in Bangkok, Thailand. Virology 2004; 329(1)-168-179.
39. Bennett SN, Holmes EC, Chirivella M, Rodriguez DM, Beltran M, Vorndam V *et al.* Selection driven evolution of emergent dengue virus. Molecular Biology and Evolution 2003; 20(10):1650-1658.
40. Awadalla P. The evolutionary genomics of pathogen recombination. Nat. Rev. Genet 2003; 4:50-60.
41. Worobey M, Holmes EC. Evolutionary aspects of recombination in RNA viruses. J Gen Virol. 1999; 80(10):2535-2543.
42. Nagy PD, Simon AE. New insights into the mechanisms of RNA recombination. Virology 1997; 235:1-9.
43. Lai MM. RNA recombination in animal and plant viruses. Microbiol. Rev 1992; 56:61-79.
44. Holland J, Spindler K, Horodyski F, Grabau E, Nichol S, Vande- Pol S. Rapid evolution of RNA genomes. Science 1982; 215:1577-1585.
45. Worobey M, Rambaut A, Holmes EC. Widespread intra-serotype recombination in natural populations of dengue virus. Proc. Natl. Acad. Sci. USA 1999; 96:7352-7357.
46. Bharaj P, Chahar HS, Pandey A, Diddi K, Dar L, Guleria R *et al.* Concurrent infections by all four dengue virus serotypes during an outbreak of dengue in 2006 in Delhi. India Virol. J 2008; 5:1.
47. Loroño-Pino MA, Cropp CB, Farfán JA, Vorndam AV, Rodríguez-Angulo EM, Rosado-Paredes EP *et al.* Common occurrence of concurrent infections by multiple dengue virus serotypes. Am. J Trop Med Hyg. 1999; 61:725-730.
48. Laille M, Deubel V, Sainte-Marie FF. Demonstration of concurrent dengue 1 and dengue 3 infection in six patients by the polymerase chain reaction. J Med Virol. 1991; 34:51-54.
49. Montenegro F, Araújo DC, Maria R, Nogueira R, Maria J, Araújo GD *et al*. Concurrent infection with dengue virus type-2 and DENV-3 in a patient from Ceará. Brazil Mem. Inst. Oswaldo Cruz 2006; 101:925-928.
50. Wang WK, Chao DY, Lin SR, King CC, Chang SC. Concurrent infections by two dengue virus serotypes among dengue patients in Taiwan. J Microbiol Immunol Infect. 2003; 36:89-95.
51. Carvalho SES, Martin DP, Oliveira LM, Ribeiro BM, Nagata T. Comparative analysis of American dengue virus type 1 full-genome sequences. Virus Genes 2010; 40:60-66.
52. Chen SP, Yu M, Jiang T, Deng YQ, Qin CF, Han JF *et al.* Identification of a recombinant dengue virus type 1 with 3 recombination regions in natural populations in Guangdong province. China Arch. Virol 2008; 153:1175-1179.
53. Aaskov J, Buzacott K, Field E, Lowry K, Berlioz-Arthaud A, Holmes EC. Multiple recombinant dengue type 1 viruses in an isolate from a dengue patient. J Gen Virol. 2007; 88:3334-3340.
54. Lavergne A, de Thoisy B, Lacoste V, Pascalis H, Pouliquen JF, Mercier V *et al.* Mayaro virus: complete nucleotide sequence and phylogenetic relationships with other alphaviruses. Virus Res 2006; 117:283-290.
55. Craig S, Thu HHM, Lowry K, Wang XF, Holmes EC, Aaskov J. Diverse dengue type 2 virus populations contain recombinant and both parental viruses in a single mosquito host. J Virol. 2003; 77:4463.
56. AbuBakar S, Wong PF, Chan YF. Emergence of dengue virus type 4 genotype IIA in Malaysia. J Gen Virol. 2002; 83:2437-2442.
57. Uzcategui NY, Camacho D, Comach G, Cuello de Uzcategui R, Holmes EC. Gould EA. Molecular epidemiology of dengue type 2 virus in Venezuela: evidence for in situ virus evolution and recombination. J Gen Virol. 2001; 82:2945-2953.
58. Cooper P. On the nature of poliovirus genetic recombinants. J Gen Virol. 1974; 5:41-49.
59. Santti J, Hyypiä T, Kinnunen L. Evidence of recombination among enteroviruses. J Virol. 1999; 73:8741-8749.
60. Costa-Mattioli M, Ferré V, Casane D, Perez-Bercoff R, Coste-Burel M, Imbert-Marcille BM. *et al*. Evidence of recombination in natural populations of hepatitis A virus. Virology 2003; 311:51-59.
61. Villabona-Arenas CJ, de Brito AF, Zanotto MA. Genomic mosaicism in two strains of dengue virus type 3, Infection. Genetics and Evolution. 2013; 18:202-212.
62. Selisko B, Dutartre H, Guillemot JC, Debarnot C, Benarroch D, Khromykh A *et al.* Comparative mechanistic studies of de novo RNA synthesis by flavivirus RNA-dependent RNA polymerases. Virology. 2006; 351:145-158.
63. Lai MM. RNA recombination in animal and plant viruses. Microbiol. Rev 1992; 56:61-79.
64. Lazzarini RA, Keene JD, Schubert M. The origins of defective interfering particles of the negative-strand RNA viruses. Cell 1981; 26:145-154.
65. Raekiansyah M, Sudiro TM. Genetic variation among Dengue Virus that possibly correlate with pathogenesis. Med J Indones. 2004; 13(3):190-194.
66. Rico-Hesse R. Molecular evolution and distribution of dengue viruses type 1 and 2 in nature. Virology 1990; 174:479-93.
67. Basu P, Bhattacharya S. Perspectives of antiviral RNA interference (RNAi) pathway of insects with special reference to mosquito in the context of dengue infection: a review. International Journal of Mosquito Research. 2014; 1(3):74-81.
68. Campbell CL, Keene KM, Brackney DE, Olson KE, Blair CD. *Aedes aegypti* uses RNA interference in defence against Sindbis virus infection. BMC Microbiol 2008; 8:47.
69. Galiana-Arnoux D, Dostert C, Schneemann A, Hoffmann JA, Imler JL. Essential function *in vivo* for Dicer-2 in host defense against RNA viruses in *Drosophila*. Nat. Immunol 2006; 7:590-597.
70. Keene KM, Foy BD, Sanchez-Vargas I, Beaty BJ, Blair CD, Olson KE *et al*. RNA interference acts as a natural antiviral response to O'nyong-nyong virus (Alphavirus; Togaviridae) infection of *Anopheles gambiae*. Proc Natl Acad Sci USA 2004; 101(49):17240-17245.
71. Vanrij RP, Saleh M-C, Berry B, Foo C, Houk A. The RNA silencing endonuclease Argonaute 2 mediates specific antiviral immunity in *Drosophila melanogaster*. Genes & Development 2006; 20:2985-2995.
72. Wang XH, Aliyari R, Li WX, Li HW, Kim K. RNA interference directs innate immunity against viruses in

adult *Drosophila*. Science 2006; 312:452-454.
73. Zambon RA, Vakharia VN, Wu LP. RNAi is an antiviral immune response against a dsRNA virus in *Drosophila melanogaster*. Cell Microbiol 2006; 8:880-889.
74. Hess AM, Prasad AN, Ptitsyn A, Ebel GD, Olson KE, Barbacioru C *et al*. Small RNA profiling of Dengue virus mosquito interactions implicates the PIWI RNA pathway in anti-viral defence BMC Microbiology 2011; 11:45.
75. Pijlman GP, Funk A, Kondratieva N, Leung J, Torres S. A highly structured, nuclease resistant, noncoding RNA produced by flaviviruses is required for pathogenicity. Cell Host Microbe 2008; 4:579-591.
76. Funk A, Truong K, Nagasaki T, Torres S, Floden N, Balmori Melian E. *et al.* RNA structures required for production of subgenomic flavivirus RNA. J Virol. 2010; 84:11407-11417.
77. Silva PA, Pereira CF, Dalebout TJ, Spaan WJ, Bredenbeek PJ. An RNA pseudoknot is required for production of yellow fever virus subgenomic RNA by the host nuclease XRN1. J Virol. 2010; 84:11395-11406.
78. Villordo SM, Filomatori CV, Sánchez-Vargas I, Blair CD, Gamarnik AV. Dengue Virus RNA Structure Specialization Facilitates Host Adaptation. PLoS Pathog 2015; 11(1):e1004604.
79. Guzman MG, Kouri G. Dengue: An update. Lancet Infect. Dis. 2002; 2:33-42.
80. Gubler DJ. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. Trends Microbiol 2002; 10:100-103.
81. Behura SK, Severson DW. Nucleotide substitutions in dengue virus serotypes from Asian and American countries: insights into intracodon recombination and purifying selection. BMC Microbiology 2013; 13:37.
82. Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ *et al.* Dengue: a continuing global threat. Nat Rev Microbiol. 2010; 8(12):7-16.
83. Gubler DJ, Trent DW. Emergence of epidemic dengue/dengue hemorrhagic fever as a public health problem in the Americas. Infect Agents Dis. 1993; 2:383-393.
84. Steinhauer DA, Domingo E, Holland JJ. Lack of evidence for proof reading mechanisms associated with an RNA virus polymerase. Gene. 1992; 122:281-288.
85. Woelk CH, Holmes C. Reduced Positive Selection in Vector-Borne RNA Viruses. Molecular Biology and Evolution 2002; 19(12):2333-2336.
86. Chao L. Evolution of sex and the molecular clock in RNA viruses. Gene. 1997; 205:301-308.
87. Chao L. Evolution of sex in RNA viruses. J Theor Biol. 1988; 133:99-112.
88. Holmes EC, Worobey M, Rambaut A. Phylogenetic evidence for recombination in dengue virus. Mol. Biol. Evol 1999; 16:405-409.
89. Holmes EC, Twiddy SS. The origin, emergence and evolutionary genetics in Dengue virus. Infect. Genet. Evol 2003; 3:19-28.
90. Su MW, Yuan HS, Chu WC. Recombination in the Nonstructural Gene Region in Type 2 Dengue Viruses. Intervirology 2012; 55:225-230.
91. Arenas M, Posada D. Recodon: coalescent simulation of coding DNA sequences with recombination, migration and demography. BMC Bioinformatics 2007; 8:458.
92. Arenas M, Posada D. Coalescent simulation of intracodon recombination. Genetics 2010; 184:429-437.
93. Hughes AL, Hughes MAK. More Effective Purifying Selection on RNA Viruses than in DNA Viruses. Gene. 2007; 404(1-2):117-125.
94. Holmes EC. RNA virus genomics: a world of possibilities. The Journal of Clinical Investigation. 2009; 119(9):2488-2495.
95. Thu HM, Lowry K, Myint TT, Shwe TN, Han AM, Khin KK *et al.* Myanmar dengue outbreak associated with displacement of serotypes 2, 3, and 4 by dengue 1. Emerg. Infect. Dis 2004; 10:593-597.
96. Wittke V, Robb TE, Thu HM, Nisalak A, Nimmannitya S, Kalayanrooj S *et al.* Extinction and rapid emergence of strains of dengue 3 virus during an interepidemic period. Virology 2002; 301:148-156.
97. Sittisombut N, Sistayanarain A, Cardosa MJ, Salminen M, Damrongdachakul S, Kalayanarooj S *et al.* Possible occurrence of a genetic bottleneck in dengue serotype 2 viruses between the 1980 and 1987 epidemic seasons in Bangkok, Thailand. Am. J Trop Med Hyg. 1997; 57:100-108.
98. Cologna R, Armstrong PM, Rico-Hesse R. Selection for virulent dengue viruses occurs in humans and mosquitoes. J Virol. 2005; 79:853-859.
99. Leitmeyer KC, Vaughn DW, Watts DM, Salas R, Villalobos I, de Chacon *et al.* Dengue virus structural differences that correlate with pathogenesis. J Virol. 1999; 73:4738-4747.
100. Rico-Hesse R, Harrison LM, Salas RA, Tovar D, Nisalak A, Ramos C *et al.* Origins of dengue type 2 viruses associated with increased pathogenicity in the Americas. Virology.1997; 230:244-251.
101. TY Hang VT, Holmes EC, Veasna D, Quy NT, Tinh Hien T, Quail M *et al.* Emergence of the Asian 1 Genotype of Dengue Virus Serotype 2 in Viet Nam: *In Vivo* Fitness Advantage and Lineage Replacement in South-East Asia. PLoS Neglected Tropical Diseases. 2010; 4(7):e757.
102. Vasilakis N, Cardosa J, Hanley KA, Holmes EC, Weaver SC. Fever from the forest: prospects for the continued emergence of sylvatic dengue virus and its impact on public health. Nat Rev Microbiol 2012; 9:532-541.
103. Tabachnick WJ. Challenges in predicting climate and environmental effects on vector-borne disease episystems in a changing world. J Exp Biol 2010; 213:946-954.
104. Gupta M, Bhattacharya S. *Aedes albopictus*: Portrait of a potential vector. IOSR-JPBS. 2015; 10(6):05-09.
105. Bhattacharya S, Srivastava DK, Mandal AK. Studies on The Vectors of Dengue in the Context of Changing Epidemiology. Sci. & Cult 2013; 79(9-10):391-395.
106. Weaver SC, Lecuit M. Chikungunya virus and the global spread of a mosquito-borne disease. The New England Journal of Medicine. 2015; 372:1231-1239.
107. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL *et al.* The global distribution and burden of dengue. Nature. 2013; 496:504-507.
108. Gardner LM, Fajardo D, Waller ST, Wang O, Sarkar S. A predictive spatial model to quantify the risk of airtravel-associated dengue importation into the United States and Europe. Journal of Tropical Medicine. 2012; Article ID 103679, 11 pages.
109. Staples JE, Breiman RF, Powers AM. Chikungunya fever: an epidemiological review of a re-emerging infectious disease. Clinical Infectious Diseases. 2009; 49:942-948.

110. Brown JE, Evans BR, Zheng W, Obas V, Barrera-Martinez L, Egizi A *et al.* Human impacts have shaped historical and recent evolution in *Aedes aegypti*, the dengue and yellow fever mosquito. Evolution 2014; 68:514-525.
111. Gloria-Soria A, Brown JE, Kramer V, Hardstone Yoshimizu M, Powell JR. Origin of the dengue fever mosquito, *Aedes aegypti*, in California. PLOS Neglected Tropical Diseases 2014; 8:e3029.
112. Tatem AJ, Hay SI, Rogers DJ. Global traffic and disease vector dispersal. Proceedings of the National Academy of Sciences of USA. 2006; 103:6242-6247.
113. WHO European Mosquito Control Association: Guidelines for the control of invasive mosquitoes and associated vector borne diseases on the European continent, 2011. (http://www. emca-online.eu/documents/visitors/WHO 2011_revised.pdf)
114. Chen XG, Jiang X, Gu J, Xu M, Wu Y, Deng Y *et al.* Genome sequence of the Asian Tiger mosquito, *Aedes albopictus*, reveals insights into its biology, genetics, and evolution. PNAS 2015; 112(44):E5907-E5915.
115. Rizzo F, Cerutti F, Ballardini M, Mosca A, Vitale N, Radaelli MC *et al*. Molecular characterization of flaviviruses from field-collected mosquitoes in north western Italy, 2011-2012. Parasit Vectors 2014; 7:395.
116. Roiz D, Vázquez A, Seco MP, Tenorio A, Rizzoli A. Detection of novel insect flavivirus sequences integrated in *Aedes albopictus* (Diptera: Culicidae) in Northern Italy. Virol J. 2009; 6:93.
117. Crochu S, Cook S, Attoui H, Charrel RN, De Chesse R, Belhouchet M *et al*. Sequences of flavivirus-related RNA viruses persist in DNA form integrated in the genome of *Aedes* spp. mosquitoes. J Gen Virol. 2004; 85(7):1971-1980.
118. Barrón MG, Fiston-Lavier AS, Petrov DA, González J. Population genomics of transposable elements in *Drosophila*. Annu Rev Genet. 2014; 48:561-581.
119. Geuking MB, Weber J, Dewannieux M, Gorelik E, Heidmann T, Hengartner H *et al.* Recombination of retrotransposon and exogenous RNA virus results in nonretroviral cDNA integration. Science. 2009; 323(5912):393-396.
120. Sessions OM, Wilm A, Kamaraj US, Choy MM, Chow A, Chong Y *et al.* Analysis of Dengue Virus Genetic Diversity during Human and Mosquito Infection Reveals Genetic Constraints. PLoS Negl Trop Dis. 2015; 9(9):e0004044.
121. Bhattacharya S, Basu P. Japanese Encephalitis Virus (JEV) infection in different vertebrates and its epidemiological significance: a Review. International Journal of Fauna and Biological Studies. 2014; 1(6):32-37.
122. Anne Murray NE, Quam MB, Wilder-Smith A. Epidemiology of dengue: past, present and future prospects. Clinical Epidemiology. 2013; 5:299-309.
123. Jerzak GVS, Brown I, Shi PY, Kramer LD, Ebel GD. Genetic diversity and purifying selection in West Nile virus populations are maintained during host switching. Virology 2008; 374(2):256-260.
124. Liu WJ, Zhu M, Pei JJ, Dong XY, Liu W, Zhao MQ *et al*. Molecular phylogenetic and positive selection analysis of Japanese encephalitis virus strains isolated from pigs in China. Virus Res. 2013; 178(2):547-52.
125. Tang WF, Ogawa M, Eshita Y, Aono H, Makino Y. Molecular evolution of Japanese encephalitis virus isolates from swine in Oita, Japan during 1980–2009. Infection, Genetics and Evolution. 2010; 10(2):329-336.
126. Ciota AT, Ngo KA, Lovelace AO, Payne AF, Zhou Y, Shi PY *et al.* Role of the mutant spectrum in adaptation and replication of West Nile virus. J Gen Virol. 2007; 88:865-874.
127. Ciota AT, Kramer LD. Insights into Arbovirus Evolution and Adaptation from Experimental Studies. Viruses. 2010; 2:2594-2617.
128. Vasilakis N, Deardorff ER, Kenney JL, Rossi SL, Hanley KA, Weaver SC. Mosquitoes put the brake on arbovirus evolution: experimental evolution reveals slower mutation accumulation in mosquito than vertebrate cells. PLoS Pathog. 2009; 5:e1000467.
129. Coffey LL, Vasilakis N, Brault AC, Powers AM, Tripet F, Weaver SC. Arbovirus evolution in vivo is constrained by host alternation. Proc Natl Acad Sci USA. 2008; 105:6970-6975.
130. Greene IP, Wang E, Deardorff ER, Milleron R, Domingo E, Weaver SC. Effect of alternating passage on adaptation of sindbis virus to vertebrate and invertebrate cells. J Virol. 2005; 79:14253-14260.
131. Weaver SC, Brault AC, Kang W, Holland JJ. Genetic and fitness changes accompanying adaptation of an arbovirus to vertebrate and invertebrate cells. J Virol. 1999; 73:4316-4326.
132. de Castro MG, de Nogueira FB, Nogueira RM, Lourenco-de-Oliveira R, dos Santos FB. Genetic variation in the 3' untranslated region of dengue virus serotype 3 strains isolated from mosquitoes and humans in Brazil. Virol J. 2013; 10:3.
133. Tumban E, Mitzel DN, Maes NE, Hanson CT, Whitehead SS, Hanley KA. Replacement of the 3' untranslated variable region of mosquito-borne dengue virus with that of tick-borne Langat virus does not alter vector specificity. J Gen Virol. 2011; 92:841-848.
134. Tajima S, Nukui Y, Takasaki T, Kurane I. Characterization of the variable region in the 3' nontranslated region of dengue type 1 virus. J Gen Virol. 2007; 88:2214-2222.
135. Khan E, Hasan R, Mehraj J, Mahmood S. Genetic Diversity of Dengue Virus and Associated Clinical Severity During Periodic Epidemics in South East Asia, In Current Topics in Tropical Medicine, Dr. Alfonso Rodriguez-Morales (Ed.), Intech Publisher, Croatia 2012, 91-108.
136. Monath TP. Dengue: The risk to developed and developing countries. Proc. Nati. Acad. Sci. USA 1994; 91:2395-2400.
137. Vikram K, Nagpal BN, Pande V, Srivastava A, Saxena R, Anvikar A. An epidemiological study of dengue in Delhi, India, Acta Tropica, 2015; 153(2016):21-27.
138. Duonga V, Lambrechts L, Paul RE, Ly S, Laya RS, Long KC. *et al*. Asymptomatic humans transmit dengue virus to mosquitoes, PNAS, 2015; (112)47:14688-14693.
139. Chen WJ, Chen SL, Chien LJ, Chen CC, King CC, Harn MR *et al.* Silent transmission of the dengue virus in southern Taiwan. Am. J Trop Med Hyg. 1996; 55:12-16.
140. Teixeira MG, Barreto ML, Costa MC, Ferreira LD, Vasconcelos PF, Cairncross S. Dynamics of dengue virus

circulation: a Silent epidemic in a complex urban area. Trop. Med. Int. Health 2002; 7:757-762.
141. Monath TP. In The Togaviridae and Flaviviridae, eds. Schlesinger, S. & Schlesinger, M. J. (Plenum, New York). 1985, 375-440.
142. Martins ST, Silveira GF, Alves LR, dos Santos CND, Bordignon J. Dendritic Cell Apoptosis and the Pathogenesis of Dengue. Viruses 2012; 4:2736-2753.
143. Salgado DM, Eltit JM, Mansfield K, Panqueba C, Castro D, Vega MR *et al*. Heart and skeletal muscle are targets of dengue virus infection. Pediatr. Infect. Dis. J 2010; 29:238-242.
144. Balsitis SJ, Coloma J, Castro G, Alava A, Flores D, McKerrow JH *et al*. Tropism of Dengue Virus in Mice and Humans Defined by Viral Nonstructural Protein 3-Specific Immunostaining. Am. J Trop Med Hyg. 2009; 80(3):416-424.
145. Jessie K, Fong MY, Devi S, Lam SK, Wong KT. Localization of dengue virus in naturally infected human tissues, by immunohistochemistry and *in situ* hybridization. J Infect Dis. 2004; 189:1411-1418.
146. Thepparit C, Smith DR. Serotype-specific entry of dengue virus into liver cells: Identification of the 37-kilodalton/67-kilodalton high-affinity lamin receptor as a dengue virus serotype 1 receptor. J Virol. 2004; 78:12647-12656.
147. Wu SJ, Grouard-Vogel G, Mascola JR, Brachtel E, Putvatana R, Louder MK. *et al*. Human skin Langerhans cells are targets of dengue virus infection. Nat. Med. 2000; 6:816-820.
148. Huerre MR, Lan NT, Marianneau P, Hue NB, Khun H, Hung NT *et al.* Liver histopathology and biological correlates in five cases of fatal dengue fever in Vietnamese children. Virchows Arch 2001; 438:107-115.
149. Huang YH, Lei HY, Liu HS, Lin YS, Liu CC, Yeh TM. Dengue virus infects human endothelial cells and induces IL-6 and IL-8 production. Am. J Trop Med Hyg. 2000; 63:71-75.
150. Couvelard A, Marianneau P, Bedel C, Drouet MT, Vachon F, Henin D *et al.* Report of a fatal case of dengue infection with hepatitis: demonstration of dengue antigens in hepatocytes and liver apoptosis. Hum Pathol 1999; 30:1106-1110.
151. King AD, Nisalak A, Kalayanrooj S, Myint KS, Pattanapanyasat K, Nimmannitya S *et al.* B cells are the principal circulating mononuclear cells infected by dengue virus. Southeast Asian J Trop Med Public Health. 1999; 30:718-728.
152. Ramos C, Sanchez G, Pando RH, Baquera J, Hernandez D, Mota J. Dengue virus in the brain of a fatal case of hemorrhagic dengue fever. J Neurovirol. 1998; 4:465-468.
153. Miagostovich MP, Ramos RG, Nicol AF, Nogueira RM, Cuzzi-Maya T, Oliveira AV *et al.* Retrospective study on dengue fatal cases. Clin Neuropathol 1997; 16:204-208.
154. Bhoopat L, Bhamarapravati N, Attasiri C, Yoksarn S, Chaiwun B, Khunamornpong S *et al.*. Immuno histochemical characterization of a new monoclonal antibody reactive with dengue virus-infected cells in frozen tissue using immunoperoxidase technique. Asian Pac J Allergy Immuno. 1996; l14:107-113.
155. Imbert JL, Guevara P, Ramos-Castañeda J, Ramos C, Sotelo J. Dengue virus infects mouse cultured neurons but not astrocytes. J Med Virol. 1994; 42:228-233.
156. Hall WC, Crowell TP, Watts DM, Barros VL, Kruger H, Pinheiro F *et al*. Demonstration of yellow fever and dengue antigens in formalin-fixed paraffin-embedded human liver by immunohistochemical analysis. Am J Trop Med Hyg. 1991; 45:408-417.
157. Balinsky CA, Schmeisser H, Ganesan S, Singh K, Pierson TC, Zoon KC. Nucleolin Interacts with the Dengue Virus Capsid Protein and Plays a Role in Formation of Infectious Virus Particles. J Virol. 2013; 87(24):13094-13106.
158. Jan JT, Chen BH, Ma SH, Liu CI, Tsai HP, Wu HC *et al.* Potential Dengue Virus-Triggered Apoptotic Pathway in Human Neuroblastoma Cells: Arachidonic Acid, Superoxide Anion, and NF-κB Are Sequentially Involved. Journal of Virology. 2000; 74(18):8680-8691.
159. Puccioni-Sohler M, Rosadas C, Cabral-Castro MJ. Neurological complications in dengue infection: a review for clinical practice. Arq Neuropsiquiatr 2013; 71(9-B):667-671.
160. Ramos C, Sánchez G, Pando RH, Baquera J, Hernández D, Mota J *et al*. Dengue virus in the brain of a fatal case of hemorrhagic dengue fever. J Neurovirol. 1998; 4:465-468.
161. Diemer GS, Stedman KM. A novel virus genome discovered in an extreme environment suggests recombination between unrelated groups of RNA and DNA viruses. Biology Direct 2012; 7:13.
162. Stedman KM. Deep Recombination: RNA and ssDNA Virus Genes in DNA Virus and Host Genomes. Annual Review of Virology 2015; 2:203 -217.
163. Zhuang F, Mastroianni M, White TB, Lambowitz AM. Linear group II intron RNAs can retrohome in eukaryotes and may use nonhomologous endjoining for cDNA ligation. Proc Natl Acad Sci USA. 2009; 106:18189-18194.
164. Lambowitz AM, Zimmerly S. Group II Introns: Mobile Ribozymes that Invade DNA. Cold Spring Harb Perspect Biol 2011; 3:a003616.
165. Holmes EC. Patterns of Intra- and Interhost Nonsynonymous Variation Reveal Strong Purifying Selection in Dengue Virus. Journal of Virology. 2003; 77:11296-11298.
166. Marianneau P, Flamand M, Deubel V, Desprès P. Apoptotic cell death in response to dengue virus infection: the pathogenesis of dengue haemorrhagic fever revisited. Clinical and Diagnostic Virology 1998; 10(2-3):113-119.
167. Limonta D, Capó V, Torres G, Pérez AB, Guzmán MG. Apoptosis in tissues from fatal dengue shock syndrome. J Clin Virol. 2007; 40:50-54.
168. Desprès P, Flamand M, Ceccaldi PE, Deubel V. Human isolates of dengue type 1 virus induce apoptosis in mouse neuroblastoma cells. J Virol. 1996; 70:4090-4096.
169. Avirutnan P, Malasit P, Seliger B, Bhakdi S, Husmann M. Dengue virus infection of human endothelial cells leads to chemokine production, complement activation, and apoptosis. J Immunol. 1998; 161:6338-6346.
170. Avirutnan P, Punyadee N, Noisakran S, Komoltri C, Thiemmeca S, Auethavornanan K *et al*. Vascular leakage in severe dengue virus infections: A potential role for the nonstructural viral protein NS1 and complement. J Infect Dis. 2006; 193:1078-1088.
171. Kuo CH, Tai DI, Chang-Chien CS, Lan CK, Chiou SS, Liaw YF. Liver biochemical tests and dengue fever. Am. J Trop Med Hyg. 1992; 47:265-270.

172. Nguyen TL, Nguyen TH, Tieu NT. The impact of dengue haemorrhagic fever on liver function. Res Virol. 1997; 148:273-277.
173. Basu A, Jain P, Gangodkar SV, Shetty S, Ghosh K. Dengue 2 virus inhibits *in vitro* megakaryocytic colony formation and induces apoptosis in thrombopoietin-inducible megakaryocytic differentiation from cord blood CD34+ cells. FEMS Immunology & Medical Microbiology 2008; 53:46-51.
174. Silveira GF, Meyer F, Delfraro A, Mosimann ALP, Coluchi N, Vasquez C *et al.* Dengue virus type-3 isolated from a fatal case with visceral complications induces enhanced pro-inflammatory responses and apoptosis of human dendritic cells. J Virol. 2011; 85:5374-5383.
175. Galluzzi L, Brenner C, Morselli E, Touat Z, Kroemer G. Viral control of mitochondrial apoptosis. PLoS Pathog. 2008; 4:e1000018.
176. Davis JS, Bourke P. Rhabdomyolysis Associated with Dengue Virus Infection. Clinical Infectious Diseases. 2004; 38:e109-11.
177. Domingo E, Biebricher C, Holland JJ, Eigen M. Quasispecies and RNAVirus Evolution: Principles and Consequences (Landes Bioscience, Austin, TX), 2001.
178. Domingo E, Holland JJ, Biebricher C, Eigen M. In Molecular Basisof Virus Evolution, eds. Gibbs A, Calisher C. & Garcı´a-Arenal, F. (Cambridge Univ. Press, Cambridge, U.K.), 1995, 171-180.
179. Holland JJ, de la Torre JC, Clarke DK, Duarte E. Quantitation of relative fitness and great adaptability of clonal populations of RNA viruses. J Virol. 1991; 65(6):2960-2967.
180. Eigen M, Biebricher CK. In RNAGenetics, eds. Domingo, E., Ahlquist, P. & Holland, J. J. (CRC, Boca Raton, FL), 1988; 3:211-245.
181. Eigen M, Schuster P. The Hypercycle: A Principle of Natural Self- Organization (Springer, Berlin), 1979.
182. Domingo E, Sabo D, Taniguchi T, Weissmann C. Nucleotide sequence heterogeneity of an RNA phage population. Cell. 1978; 13(4):735-744.
183. Kurosu T. Quasispecies of dengue virus. Tropical Medicine and Health. 2011; 39(4):29-36.
184. La´zaro E, Escarmı´s C, Pe´rez-Mercader J, Manrubia SC, Domingo E. Resistance of virus to extinction on bottleneck passages: Study of a decaying and fluctuating pattern of fitness loss. PNAS. 2003; 100(19):10830-10835.
185. Von Hahn T, Yoon JC, Alter H, Rice CM, Rehermann B, Balfe P *et al.* Hepatitis C virus continuously escapes from neutralizing antibody and T-cell responses during chronic infection *in vivo*. Gastroenterology. 2007; 132:667-678.
186. Aaskov J, Buzacott K, Thu HM, Lowry K, Holmes EC. Long-term transmission of defective RNA viruses in humans and *Aedes* mosquitoes. Science. 2006; 311:236-238.
187. Kinoshita H, Mathenge EG, Hung NT, Huong VT, Kumatori A, Yu F. *et al*. Isolation and characterization of two phenotypically distinct dengue type-2 virus isolates from the same dengue hemorrhagic Fever patient. Jpn J Infect Dis. 2009; 62:343-350.
188. Scott TW, Weaver SC, Mallampalli VL. Evolution of mosquito-borne viruses. Morse 55, ed. The Evolutionary Biology of Viruses. New York: Raven Press, 1994, 293-324.
189. Nisalak A, Nimmanitya 5, Innis B. Severe dengue infec tions among children in metropolitan Bangkok, 1973-1989: description of epidemics and characterization of virus sero types associated with them. Southeast Asian J Trop Med Hyg Public Health. 1990; 21:678-679.
190. Winter PE, Nantapanich 5, Nisalak A, Udomsakdi 5, Dewey RW, Russell DK. Recurrence of epidemic dengue hem orrhagic fever in an insular setting. Am J Trop Med Hyg. 1969; 18:573-579.
191. Chan JM, Carlsson G, Rabadan R. Topology of viral evolution. Proceedings of the National Academy of Sciences of the United States of America. 2013; 110(46):18566-18571.
192. Thu HM. Virus diversity and the emergence of Dengue (Doctoral Dissertation). Queensland University of Technology, Brisbane, Australia, 2004. Retrieved From: http://eprints.qut.edu.au/16081/1/Hlaing_Myat_Thu_Thesis.pdf
193. Wittke V, Rob TE, Thu HM, Nisalak A, Nimmannitya S, Kalyanrooj S. Extinction and rapid emergence of strains of dengue 3 virus during an interepidemic period. Virology. 2002; 301:148-156.
194. Bhamarapravati N, Yoksan S. Live attenuated tetravalent dengue vaccine. In Dengue and Dengue Haemorrhagic Fever, Edited by Gubler, DJ and Kuno G, CAB International, Oxford. 1997, 367-377.
195. Trent DW, Kinney RM, Huang CY-H. Recombinant DNA vaccines. In Dengue and Dengue Haemorrhagic Fever, Edited by Gubler, DJ and Kuno G, CAB International, Oxford. 1997, 379-403.
196. Dengvaxia®, world's first dengue vaccine, approved in Mexico. Http://www.sanofipasteur.com/en/articles/dengvaxia-world-s-first-dengue-vaccine-approved-in-mexico.aspx. Accessed on: 6thjanuary, 2016.
197. Taucher C, Berger A, Mandl CW. A *trans*-Complementing Recombination Trap Demonstrates a Low Propensity of Flaviviruses for Intermolecular Recombination. Journal of Virology. 2010; 84(1):599-611.
198. Guglani L, Kabra SK. T Cell Immunopathogenesis of Dengue Virus Infection. Dengue Bulletin 2005; 29:58-69.
199. Stephenson JR. Understanding dengue pathogenesis: implications for vaccine design. Bull World Health Organ 2005; 83(4):308-314.
200. Perera R, Kuhn RJ. Structural proteomics of dengue virus. Annu Rev Microbiol 2008; 11:369-377.
201. Goncalvez AP, Escalante AA, Pujol FH, Ludert JE, Tovar D, Salas RA *et al*. Diversity and evolution of the envelope gene of dengue virus type 1. Virology. 2002; 303:110-119.
202. Twiddy SS, Farrar JJ, Nguyen VC, Wills B, Gould, EA, Gritsun T *et al.* Phylogenetic relationships and differential selection pressures among genotypes of dengue-2 virus. Virology 2002; 298:63-72.
203. Lanciotti RS, Lewis JG, Gubler DJ, Trent DW. Molecular evolution and epidemiology of dengue-3 viruses. J Gen Virol. 1994; 75:65-75.
204. Lanciotti RS, Gubler DJ, Trent DW. Molecular evolution and phylogeny of dengue-4 viruses. J Gen Virol. 1997; 78:2279-2286.