Abstract: Viruses of Flavivirus genus are the causative agents of many common and devastating diseases, including yellow fever, dengue fever etc. so for proper development of efficient anti viral pharmaceutical strategies there is a need for proper classification of viruses of this group. To generate the most diverse phylogenetic datasets for the Flaviviruses to date, we analyzed the whole genomic sequences and phylogenetic relationships of 44 Flaviviruses by using various bioinformatics tools (MEGA, Clustal W, and PHYLIP). We analyze these data for understanding the evolutionary relationship between classified and unclassified viruses and to propose for the reclassification of unclassified viruses which shows sequence similarity and also similar mode of transmission with classified viruses.

Key words: Viruses, Flavivirus, Phylogenetic.

1. INTRODUCTION

Viruses of the Flaviviridae family infect vertebrates and they are primarily transmitted through arthropod vectors (mainly ticks and mosquitoes) [1]. The Flaviviruses constitute a fascinating group of diverse arboviruses that exhibit uniquely clear correlations between phylogenetic relationships and virus-vector-host interactions [2]. Flaviviridae are enveloped, positive single-stranded RNA viruses [3]. The Flaviviridae family includes four genera: Flaviviruses (Yellow fever virus, West Nile virus, Dengue virus, Tick-borne encephalitis viruses), Hepacivirus (Hepatitis C virus), Pestivirus (Classical swine fever virus, Bovine viral diarrhea virus) and Unclassified Flaviviridae (Hepatitis GB virus, GB viruses) [4]. The genus Flavivirus of the family Flaviviridae comprises over 70 viruses, many of which, such as the dengue (DEN) viruses, Japanese encephalitis (JE) virus, St. Louis encephalitis (SLE) virus, and yellow fever (YF) virus are important human pathogens [5]. Flaviviruses may be (i) arthropod-borne, infecting a range of vertebrate hosts through mosquito or tick bites, (ii) presumed to be limited to vertebrates alone or (iii) apparently limited to insects alone. The last group, referred to as the ‘insect-specific’ Flaviviruses, contains tentative members of the genus Flavivirus that appear to replicate only in mosquito cells and not in mammalian cells figure (1) [6].
Figure 1: Life cycle of Flaviviridae virus. The figure was made with tools from www.proteinlounge.com

Pathogenic mosquito- and/or tick-borne Flaviviruses cause a variety of clinical diseases in a wide range of vertebrate species. These disease syndromes include mild/severe febrile illness, “flu-like” syndromes with a rash, or in other cases severe encephalitis or haemorrhagic disease [7]. Flaviviridae have monopartite, linear, single-stranded, positive sense RNA genomes [8]. Ranging from 10 to 12.5 kilo bases (kb) in length [9]. Virions of the Flaviviridae family are enveloped and slightly pleomorphic during their life cycle. They are spherical in shape and usually 40–60 nm in diameter. Their nucleocapsids are isometric and sometimes penetrated by stain. The usual size of the nucleocapsids is 25–30 nm in diameter and they have polyhedral symmetry [10]. The concept that the phylogenetic relationships of the tick-borne Flaviviruses (TBFV) may correlate with their epidemiology, disease association and biogeography was first proposed in 1996 with the publication of the clinical evolution concept of the tick-borne encephalitic Flaviviruses [11]

II MATERIALS AND METHODS

Data collection is an important aspect of any type of research study. Accurate data collection is essential to maintain the integrity of research. So for the purpose of comparative genome analysis of viruses of Flavivirus data needs to be collected which contains the reference sequences. The sequences of 44 viruses of Flavivirus genus are retrieved in this study whose reference sequences had been already available on NCBI (National Center of Biotechnology Information). These sequences include 44 sequences from the Flavivirus. The multiple sequence alignment programs Clustal W offline [12] was used to obtain an optimal nucleotide or amino acid sequence alignment file. Phylograms for the entire sequence were obtained either by MEGA version 5.10 [13] or by PHYLIP version 3.6 [14] based on aligned nucleotide or amino acid sequences.

For tree building various genetic distance matrices were used for the neighbor joining method which calculated bootstrap confidence intervals of 500. We also tested a character state tree building algorithm which consisted of a sequential programs in the PHYLIP package. A strict consensus bootstrap tree was obtained by using the following programs:

(1) SEQBOOT to generate reiterated replicas
(2) DNAPARS to acquire the most parsimony tree of each reiterated data
(3) CONSENSE to build a strict consensus bootstrap tree
(4) DRAWGRAM to draw the phylogenetic tree.
Table 1: Flaviviruses used in phylogenetic studies

<table>
<thead>
<tr>
<th>Flaviviruses</th>
<th>Distribution</th>
<th>Reference no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Murray Valley encephalitis</td>
<td>Australia, Papua New Guinea</td>
<td>NC_000943.1</td>
</tr>
<tr>
<td>Usutu virus</td>
<td>Africa</td>
<td>NC_006551.1</td>
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<tr>
<td>Japanese encephalitis virus</td>
<td>Asia and parts of the pacific</td>
<td>NC_001437.1</td>
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<tr>
<td>West Nile virus</td>
<td>Africa, Asia, Europe</td>
<td>NC_009942.1</td>
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<tr>
<td>West Nile virus</td>
<td>Africa, Asia, Europe</td>
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<tr>
<td>St. Louis encephalitis virus</td>
<td>Americas</td>
<td>NC_007580.2</td>
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<td>Ilheus virus</td>
<td>Central and South America, Trinidad</td>
<td>NC_009028.2</td>
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<td>Tembusu virus</td>
<td>Southeast Asia</td>
<td>NC_015843.2</td>
</tr>
<tr>
<td>Bagaza virus</td>
<td>Central African Republic</td>
<td>NC_012534.1</td>
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<tr>
<td>Ntaya virus</td>
<td>Central and South Africa</td>
<td>NC_018705.3</td>
</tr>
<tr>
<td>Bussuquara virus</td>
<td>South America</td>
<td>NC_009026.2</td>
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<td>Kokobera virus</td>
<td>Australia</td>
<td>NC_009029.2</td>
</tr>
<tr>
<td>Kedougou virus</td>
<td>Senegal</td>
<td>NC_012533.1</td>
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<tr>
<td>Zika virus</td>
<td>Africa, Asia</td>
<td>NC_012532.1</td>
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<tr>
<td>Dengue type 4 virus</td>
<td>Tropical and semitropical areas</td>
<td>NC_002640.1</td>
</tr>
<tr>
<td>Dengue type 2 virus</td>
<td>Thailand</td>
<td>NC_001474</td>
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<td>Dengue type 1 virus</td>
<td>Tropical and semitropical areas</td>
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<td>Dengue type 3 virus</td>
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<td>Chaoyang virus</td>
<td>Republic of korea</td>
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<td>Donggang virus</td>
<td>CHINA</td>
<td>NC_016997.1</td>
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<td>Yokose virus</td>
<td>Japan</td>
<td>NC_005039.1</td>
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<td>Entebbe bat virus</td>
<td>Uganda</td>
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<td>Yellow fever</td>
<td>Tropical areas</td>
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<td>Rio Bravo virus</td>
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<td>Modoc virus</td>
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<td>Apoi virus</td>
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<td>Tyuleniy virus</td>
<td>Russia, Oregon</td>
<td>NC_023424.1</td>
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<td>Kama virus</td>
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<td>NC_023439.1</td>
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<td>Karshi virus</td>
<td>Kazakhstan</td>
<td>NC_006947.1</td>
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<td>Powassan virus</td>
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<td>Alkhumra virus</td>
<td>Saudi Arabia</td>
<td>NC_004355.1</td>
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<td>Langat virus</td>
<td>Southeast Asia, Russia</td>
<td>NC_003690.1</td>
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<td>Omks hemorrhagic fever virus</td>
<td>Russia, Siberia</td>
<td>NC_005062.1</td>
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<tr>
<td>Louping ill virus</td>
<td>Siberia and the UK</td>
<td>NC_001809.1</td>
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<td>Tick-borne encephalitis virus</td>
<td>Europe</td>
<td>NC_001672.1</td>
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<td>Aedes Flavivirus</td>
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<td>Kamiti River virus</td>
<td>USA</td>
<td>NC_005064.1</td>
</tr>
<tr>
<td>Cell fusing agent virus</td>
<td>Unknown</td>
<td>NC_001564.1</td>
</tr>
<tr>
<td>Culex Flavivirus</td>
<td>Japan</td>
<td>NC_008604.2</td>
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<td>Mosquito Flavivirus</td>
<td>China</td>
<td>NC_021069.1</td>
</tr>
<tr>
<td>Quang Binh virus</td>
<td>Vietnam</td>
<td>NC_012671</td>
</tr>
</tbody>
</table>
III RESULTS AND DISCUSSION

The phylogenetic tree figure (2) was constructed by the neighbor-joining method of MEGA for nucleotide sequences. Each number at nodes is the percentage of 1000 bootstrap replicate support. According to phylogenetic tree the whole genus is divided into two groups vector borne and non vector borne. The phylogram reveals that non-vector and vector-borne clusters emerged first from the putative origin of the genus Flavivirus. The latter further branched off to form tick-borne and mosquito-borne virus clusters [15]. These three clusters are well supported by 99% of bootstrap replicates. The non-vector cluster further branched into Montana myotis leukoencephalitis, Riobravo virus, Modoc, Apoi. Mosquito borne viruses can be further divided into mosquito borne culex, Mosquito borne ades. Yellow fever virus group comes under Mosquito borne ades. The viruses belongs to arthropod specific group has mosquito as their vector these all viruses belongs to unclassified Flaviviruses. The Tick-borne encephalitis virus groups include total number of nine viruses Tyuleni, Kama, Karshi, Powassan, Alkhunra, Langat, Omsk Hemorrhagic fever, Louping ill. The mode of transmission of Tick borne encephalitis viruses is through tick. Murray valley encephalitis(MVE), Usutu, Japanese encephalitis, Westnile, St. Louis encephalitis, Ilehus, Tembusu, Bagaza, Nataya, Bussuquara, kokobera these viruses are more closely related with high bootstrap value and they have same mode of transmission by a Culex Mosquito. Dengue 1-4, zika, Kedoungo, chaoyang, donggang these all viruses are Mosquito borne ades viruses and they also have some mode of transmission. This group contains two unclassified Flavivirus chaoyang, donggang and these both viruses share sequence similarity with other viruses of this group. Since some viruses belonging to the unclassified Flavivirus group show similarity and phylogenetic relationship with classified viruses group and their mode of transmission is also same. Aedes Flavivirus, Cell fusing agent virus, chaoyang virus, Culex Flavivirus, donggang virus, Kamiti River virus, Mosquito Flavivirus, Quang Binh virus show similarity with mosquito borne viruses and kama virus with tick borne viruses. So on the basis of phylogenetic relationship shown by unclassified viruses with other grouped viruses and also on the basis of same mode of transmission we can propose for the reclassification of unclassified flaviviruses.
Figure 2: Phylogenetic tree of Flavivirus using amino acid sequence constructed by MEGA.
REFERENCES


