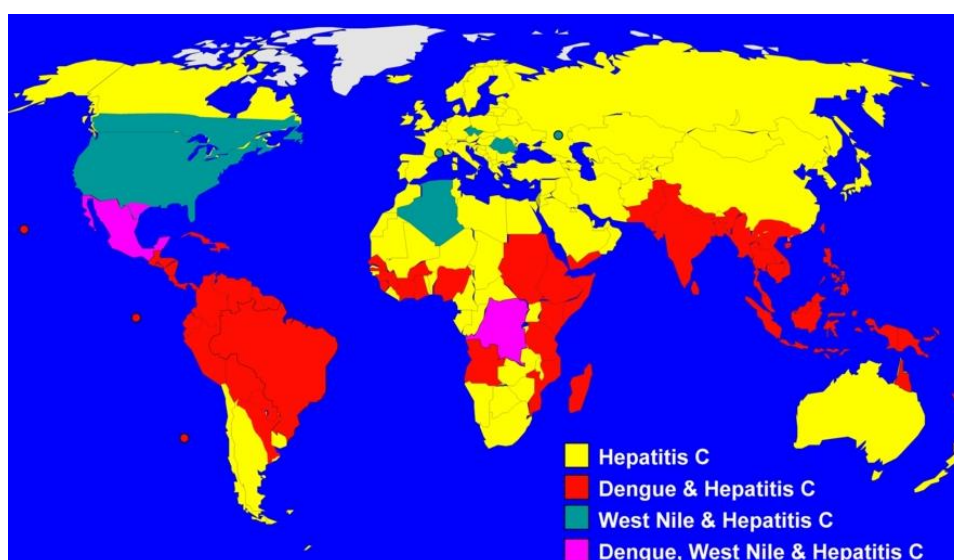


**CHAPTER 1**  
**INTRODUCTION**

## 1.1 BACKGROUND

The arthropod-borne flaviviruses are widespread human pathogens causing serious human diseases globally. Tropical and subtropical regions in the world are highly affected by these viruses causing high mortality rate with no effective treatment.

Flaviviruses are one of the six different taxonomic virus families of arboviruses and their genome is composed of linear, single stranded, infectious, positive sense RNA. These are enveloped viruses. The mode of transmission of flaviviruses infection is through arthropod vectors namely mosquitoes and ticks . Dengue Virus (DEV), West Niles Virus (WNV), Japanese Encephalitis Virus (JEV), Yellow Fever Virus (YFV) and Hepatitis C Virus (HCV) are some of the members of Flaviviruses genus. These viruses are gaining attention because of their re-emergence and incidence throughout the world and additional environmental and demographic consideration suggests that they will continue to emerge in the future.



**Figure 1.1: Distribution of Hepatitis C, Dengue, and West Nile Viruses around the world (<http://www.utmb.edu/>)**

Flaviviruses are able to expand their geographic distribution over years due to the distribution of their vectors, arthropods globally. Amplification of flaviviruses distribution also associated with the changes in viral genetics, host or vector population composition, and environmental structure parallel to the anthropogenic origin. Other than that, global warming, urbanization and increase in population size in tropical regions are some other factors that lead to continuous spreading of flaviviruses into new geographic areas (Yun Young Go et al., 2013). Humans and animals infected by flaviviruses experience symptoms like mild fever, long term fever, hemorrhagic fever and encephalitis often associated with central nervous system. Dengue Viruses are one of the most important flaviviruses, with yearly an estimated 50 – 100 million cases of Dengue and tens-of-thousands of cases of the more severe and sometimes fatal dengue hemorrhagic fever/shock syndrome (DHF/DSS syndromes) (Endy et al., 2010).

There are four significant vertebrate host and vector transmission of flaviviruses comprises of enzootic cycle, epizootic cycle (rural cycle), urban cycle and dead-end hosts or incidental hosts. The first cycle, enzootic cycle is the natural transmission of virus between wild animals (vertebrate hosts) and primary or enzootic insect vectors. This transmission leads to the amplification of the virus in the vector. The vertebrate host is the reservoir host that can nurture a virus indefinitely without any ill effects and can be reinfected several times during their life. Flaviviruses also can be transmitted through epizootic cycle in which the virus is transmitted between domestic animals and the primary insect vectors. Japanese Encephalitis Virus is one of the viruses which transmitted via this cycle in which the virus is transmitted to human through an infected swine. Another way of transmission is urban cycle in which the cycle is between human and insect vectors such as *A.egypti* for Dengue Virus. In this cycle the infection occurs

with every new bite thus making the transmission hyperendemic. Dead- end hosts do not serve for amplification of the virus because they do not develop sufficient viremia to survive the infection. Transmission of West Niles Virus is an example of this type of transmission (Yun Young Go et al., 2013).

Members of flaviviruses share a similar genomic structure and intracellular life cycle but vary in the clinical manifestations. To date there is no antiviral treatment available for these viruses though many studies are being conducted to find cure for diseases caused by these viruses. Therefore, prevention and vaccination is considered as best treatment to fight flaviviruses.

The flaviviruses genome is made up of a single- stranded positive-sense RNA which encodes for polyprotein. The polyprotein precursor consists of three structural protein, capsid (C), membrane (M), and envelope (E), and five nonstructural proteins, NS1-NS5 (Muslum et al., 2013). Correct cleavage of this polypeptide leads to the replication of flaviviruses (Ross et al., 1999). The replication requires both host cell proteases and a virus- encoded two- component protease NS2B-NS3 to interact at the correct binding site (Falgout et al., 1991). The post- translational proteolytic processing of the precursor is performed by NS2B-NS3pro, a trypsin-like serine protease. Catalytic triad (His-51, Asp75- Ser135) is found at the active site of NS2B-NS3pro. The enzymatic properties of the serine protease lead it as a target for drug screening within the drug discovery pipeline. The fact that 80 % of dengue virus infection is decreased with the treatment of cells with a peptide that inhibits NS2B-NS3pro supports this theory (Rothan et al., 2012). Serine protease domain in the N-terminal region requires the

formation of a non-covalent complex with the membrane-bound cofactor NS2B for activity, and has ATP-driven helicase and RNA triphosphatase activities in the C-terminal region (Figure 1.2). The cleavage sites processed by NS2B-NS3pro are indicated by arrows. Partitions of the various functional domains along the primary sequence of NS2B-NS3 are also shown in the partition. The regions of the NS2B proteins expected to associate with membranes are indicated as filled boxes. Evolutionarily conserved residues essential for NS3 enzymatic activities are indicated.

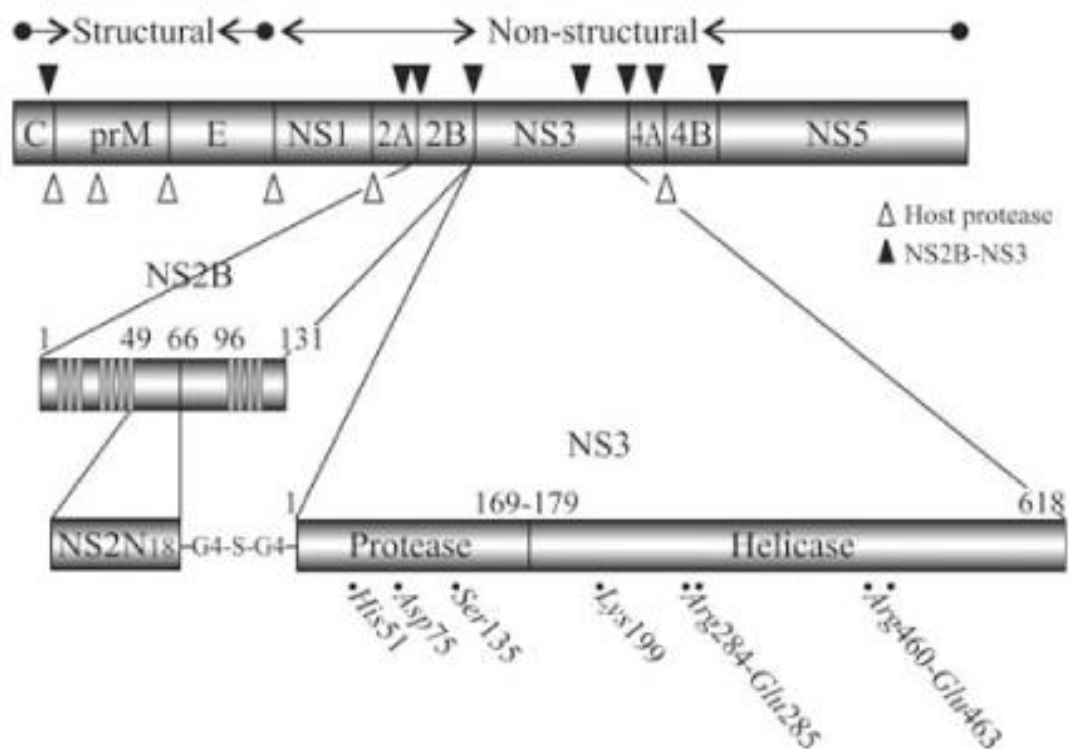


Figure 1.2: Schematic representation of the flaviviral polyprotein by Natarajan, S., (2010).

There are several studies going on to find anti viral therapy for treatment or the prevention of flaviviruses infection. Unfortunately, the findings are still insufficient to find a specific antiviral therapy for the infections with members of the Flaviviridae. Ongoing research has identified possible targets for inhibition, including binding of the virus to the cell, uptake of the virus into the cell, the internal ribosome entry site of hepaciviruses and pestiviruses, the capping mechanism of flaviviruses, the viral proteases, the viral RNA-dependent RNA polymerase, and the viral helicase.

Hence the present study aims to identify the possible binding sites at the serine proteases. This study involves five flaviviruses namely Dengue Virus (DEV), West Niles Virus (WNV), Japanese Encephalitis Virus (JEV), Yellow Fever Virus(YFV) and Hepatitis C Virus (HCV) to identify the binding sites in each protein sequence and extends further to examine if the binding sites involve triads.

## **1.2 OBJECTIVES OF THE RESEARCH**

- 1.2.1 To obtain PDB structures of Yellow Fever Virus(YFV), Japanese Encephalitis Virus(JEV), West Nile Virus(WNV), Hepatitis C Virus (HCV) and Dengue Virus (DENV).
- 1.2.2 To model the serine protease of these viruses
- 1.2.3 To identify the binding sites of inhibitor candidates