Seroprevalence and Clinical Characteristics investigation of Dengue Virus Infection in Rural Bangladesh during 2019 Dengue outbreak

By

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A thesis submitted to the Department of Mathematics and Natural Science in partial fulfilment of the requirements for the degree of
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Declaration

It is hereby declared that,

1. The thesis submitted "Seroprevalence and Clinical Characteristics investigation of Dengue Virus Infection in Rural Bangladesh during 2019 Dengue outbreak**"** is my own original work while completing degree at BRAC University.

2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.

3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.

4. I have acknowledged all main sources of help.

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Approval

The thesis titled "Seroprevalence and Clinical Characteristics investigation of Dengue Virus Infection in Rural Bangladesh during 2019 Dengue outbreak" submitted by Afsana Rashed (Student ID: 15326007) has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Science in Microbiology on 27 February 2021.

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Abstract

Dengue virus (DENV), a single-stranded RNA virus belonging to Flaviviridae family member, is transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes. Dengue is a mosquito-borne viral disease that has become more prevalent in the last few decades. DENV infection which may remain asymptomatic or manifest as life threatening dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS) is one of the most emerging viral diseases in the world. As the clinical presentations ranging from asymptomatic to severe illness may lead to death if improperly managed, the information on the incidence of DENV infections along with the clinical manifestations are significant in preventing future outbreaks. In this study, DENV infections were investigated in clinically suspected dengue fever patients from Faridpur Medical College hospital during September 2019 epidemic in rural Bangladesh. The objective of the current study was to explore the circulating dengue serotypes in rural Bangladesh during that season. A total of 66 serum samples were obtained from Faridpur Medical college Hospital during 2019 DENV epidemic in Bangladesh which were tested for dengue virus using nucleic acid extraction, conventional RT-PCR assay for the presence of DENV RNA, agarose gel electrophoresis followed by nucleotide sequencing using DENV type specific primers that targeted the dengue virus serotype 1–4. DENV RNA was detected from 10 cases (15% of 66 patients). The DENV sequencing using type specific primers detected DENV-3 in eight patients, DENV 2 in one patient and one sample was untypeable in the sequencing method. Also, analysis of clinical observations showed that vomiting, headache, lethargy and restlessness were the most prevalent complains among the investigated dengue positive patients. Other common signs and symptoms were conjunctivitis, arthritis, hepatomegaly and skin rash. Isolation of DENV RNA and the updated knowledge of the circulating DENV serotypes and associated clinical manifestations are of utmost significance for understanding and predicting possible future emergence of a different serotype which can help in public health preparedness tremendously.

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List of Acronyms

DENV	Dengue Virus
DF	Dengue Fever
DHF	Dengue Hemorrhagic Fever
DSS	Dengue Shock Syndrome
Cap	Capsid
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
NS	NonStructural
Nt	Nucleotide
Aa	Amino acid
Pol	Polymerase
VP	Viral Protein
UV	Ultraviolet
L	Liter
PrM	Membrane Protein
µL	Microliter
G	Gram
Mg	Microgram
PCR	Polymerase Chain Reaction
RT	Reverse Transcriptase
CDC	Centers for Disease Control and Prevention

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Chapter 1: LITERATURE REVIEW

1.1 General introduction

Dengue is a mosquito borne infection caused mostly by dengue viruses (DENV) that is responsible for the most common arthropod borne viral disease in people with almost 100 million infections per year(Tuiskunen Bäck & Lundkvist, 2013). DENVs consist of four antigenically distinct serotypes (DENV-1, DENV-2, DENV-3, DENV-4) that are members of the *flaviviridae* family under the genus flavivirus. All four DENV serotypes have emerged from sylvatic strains in the forests of South-East Asia. At present, DENV is considered to be the most common cause of tropical arboviral diseases globally that affects more than two thirds of the worlds total population ranking as the most important mosquito borne viral disease around the world. Dengue viruses are also called arthropod-borne viruses, commonly called as arboviruses and represents taxonomically diverse group of viruses. This classification is done according to antigenic relationships, molecular biology of the virus, morphology, and replicative mechanisms and most importantly, uniqueness in their transmission between arthropod vectors and vertebrate hosts (Harapan et al., 2020). Virus families that fall under arboviruses are *Togaviridae*, *Flaviviridae, Bunyaviridae, Rhabdoviridae, Orthomyxoviridae,* and *Reoviridae.* Of all these families, Flavivirus genera represents the most clinically significant symptoms that are characterized by fever, severe headache, muscle and joint pain, nausea and vomiting, eye pain and rash. The virus is transmitted mainly by the *Aedes aegypti and Aedes albopictus* mosquitoes. *Aedes aegypti* mosquito, an anthropophilic domestic breeding sub-species is mainly responsible for the transmission of virus from human to human (Geo F. Brooks, 2007).

Historically, the origin of dengue and where and how this arbovirus first appeared in human polulations is ambiguous. The initial description of dengue was established by a physician in Philadelphia named Benjamin Rush, where he reported a febrile outbreak in 1780 (B.W.J. Mahy, 2008). It is thought that dengue like syndrome might have occurred in China several times in the first millennium AD(Tuiskunen Bäck & Lundkvist, 2013). In fact, the primary DENV infection was common in North America, the Caribbean, Asia, Australia, presumably due to the widespread ecology of mosquito vectors during the 18th

and 19th centuries (Tuiskunen Bäck & Lundkvist, 2013). Also, dengue virus spread rapidly to and through Southeast Asia during world war 2. Human settlements by destructing environment and mostly troop movement have contributed to the spread of mosquito vector and therefore, dengue virus Infection throughout Southeast Asia (Tuiskunen Bäck & Lundkvist, 2013).

1.2.1 Host Range and Transmission

There are only three known natural hosts for dengue viruses: *Aedes aegypti*, Humans and in some cases lower primates (B.W.J. Mahy, 2008).. More specifically, the species of the subgenus Stegomyia remains the most important vectors in terms of human transmission, and include *Ae. aegypti*, the principal urban vector worldwide, *Ae. Albopictus* in Asia, the Pacific, Americas, Africa, and Europe, *Ae. scutellaris* spp. (Pacific), and *Ae. africanus*, and *Ae. luteocephalus* in Africa (B.W.J. Mahy, 2008).

Dengue virus cannot spread directly from one person to another as mosquitoes are necessary for transmission of the dengue virus thereby called as vector for dengue transmission. Usually, a person develop viremia four days after being bitten by an infected *Aedes* spp mosquito (Tuiskunen Bäck & Lundkvist, 2013). When any of these mosquitoes specially *Aedes aegypti*, bites a person who has dengue virus in his or her blood, the mosquito becomes infected with the dengue virus. After this, the mosquito being a dengue vector transmit that virus to healthy people by biting them. When the infected person has high number of the dengue virus in the blood, the mosquito take its blood meal and subsequently, the virus spreads through the mosquito's body over a period of eight to twelve days when it enters the mosquito's system through the blood meal of dengue infected person (Tuiskunen Bäck & Lundkvist, 2013). Now, the infected mosquito is perfectly able to transmit the dengue virus to another person while feeding off blood meal. Infected mosquitoes can continue transmitting the dengue virus to healthy people for the rest of their life spans, generally a three-to four-week period (Geo F. Brooks, 2007)

1.2.2 Dengue Virus Structure & Genome Organization

The DENV is characterized by a surface that is approximately 50 nm in diameter and carries a single strand of RNA as its genome, whereas the immature virion is characterized by a spiky surface that is of approximately 60 nm in diameter(Harapan et al., 2020). The DENV genome carries about 11000 bases encoding a single and large polyprotein. This polyprotein is further cleaved into several structural proteins and non-structural proteins (Sim & Hibberd, 2016) .The virus comprises three structural proteins that form the coat of the virus. The outermost structural protein known as the envelope protein forms the envelope around the virus with a lipid membrane where 180 identical copies of envelope protein are attached to the surface of the membrane (Gebhard et al., 2011).The main function of envelope protein is to attach to the host cell and initiate the processs of infection. The seven non-structural proteins which contributes to the production of new viruses once the virions get inside of the cell (B.W.J. Mahy, 2008).

The genome of DENV consists of a single copy and positive stranded RNA of approximately 11 kb in size (Tuiskunen Bäck & Lundkvist, 2013). It contains a single open reading frame encoding for the viral polyprotein, 5′ untranslated region (UTR; ~100 nucleotides), a 3′ UTR approximately 400 nucleotides in length. the DENV-genome is not 3′ polyadenylated unlike cellular mRNA, and attached to the 5′ end of the viral genome is a type 1 7-methyl guanosine cap structure(Harapan et al., 2020).

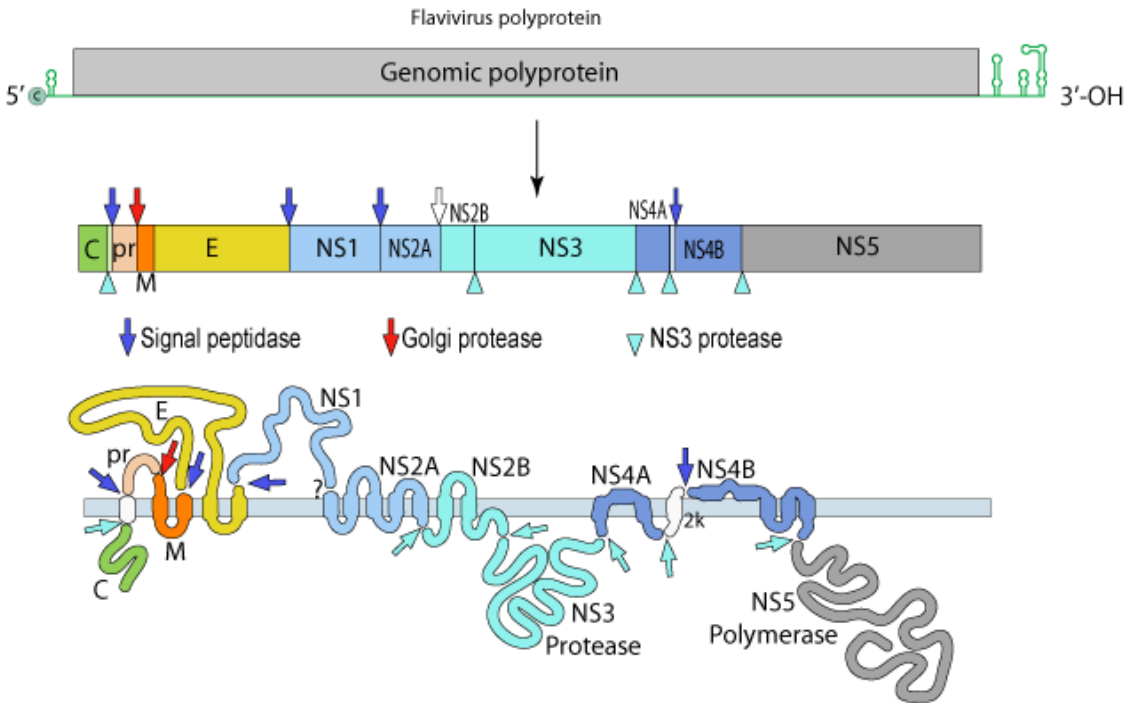

Figure1.1: Dengue Virus RNA Genome (Harapan et al., 2020)

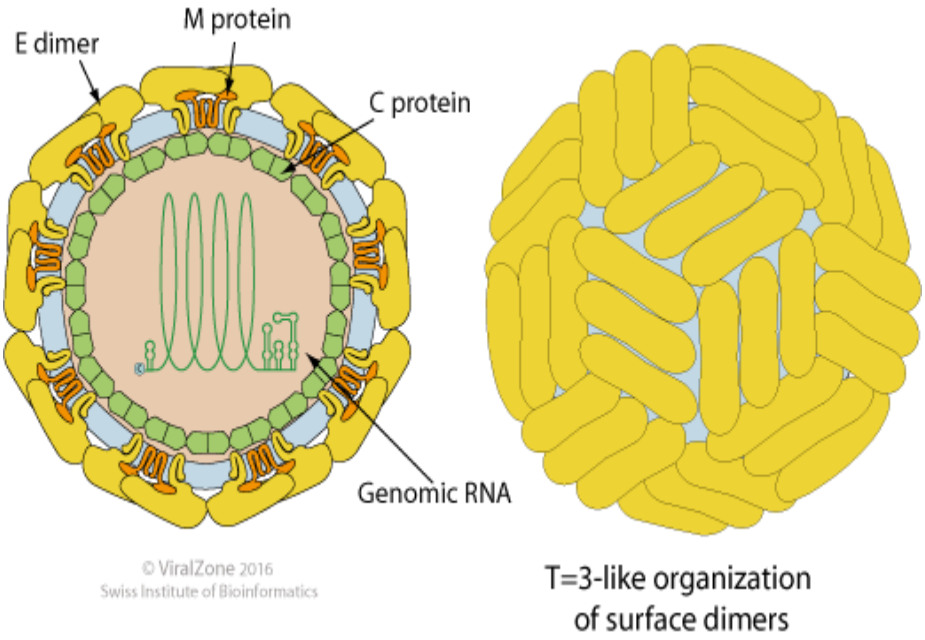

Figure 1.2: Dengue Virion Structure (Leidy, 2011)

1.2.3 Dengue Virus Life Cycle

Lifecycle of dengue virus starts with the attachment of DENV with specific receptors which is mediated by dengue envelope protein. In only few hours after infection, tens of thousand of copies of viral molecules are produced from a single viral molecule, leading to cell damage and in severe cases, to death (Tuiskunen Bäck & Lundkvist, 2013). Two transmembrane viral proteins are inserted in the lipid bilayer which forms a glycoprotein shell. The shell is composed of 180 copies of the envelope (E) and membrane proteins (prM /M). The nucleocapsid of the virus exist in the core which is formed by one copy of single stranded capped RNA genome finally forming complex with multiple copies of the capsid protein. The DENV particle enters the host cell through the process of receptor mediated endocytosis with the help of envelope protein (E). After the internalization and acidification of the endosome, conformational changes in the E protein occur which allows NC release into the cytoplasm with the help of the fusion of viral and vesicular membrane (Gamarnik, 2016). Capsid is dissociated from the virus particle and genome uncoating occur immediately that allow viral RNA to be directly involved in translation process. A large polyprotein is produced with a complex topology on ER membranes(Uno & Ross, 2018). The whole translation process takes place in rough endoplasmic reticulum. Hereafter, the viral polyprotein is cleaved into ten mature proteins : three structural proteins (capsid, prM, E) and seven non structural proteins (NS1, NS2a, NS2B, NS2B, NS3, NS4A, NS4B, NS5) (Pierson, 2015). Most of the non structural proteins provide enzymatic activities and help creating a proper environment for RNA replication and also suppressing antiviral response of host. NS5, a viral RNA polymerase catalyzes the viral RNA synthesis which associates with capsid to finally form nucleocapsid (NC). This ribonucleoprotein then buds into the ER lumen to acquire the lipid bilayer, envelope proteins and PrM(Tuiskunen Bäck & Lundkvist, 2013). All these immature virus particles travel along the secretory pathway where furin mediated proteolysis of PrM triggers rearrangement of E protein and allows the formation of mature virus particles.

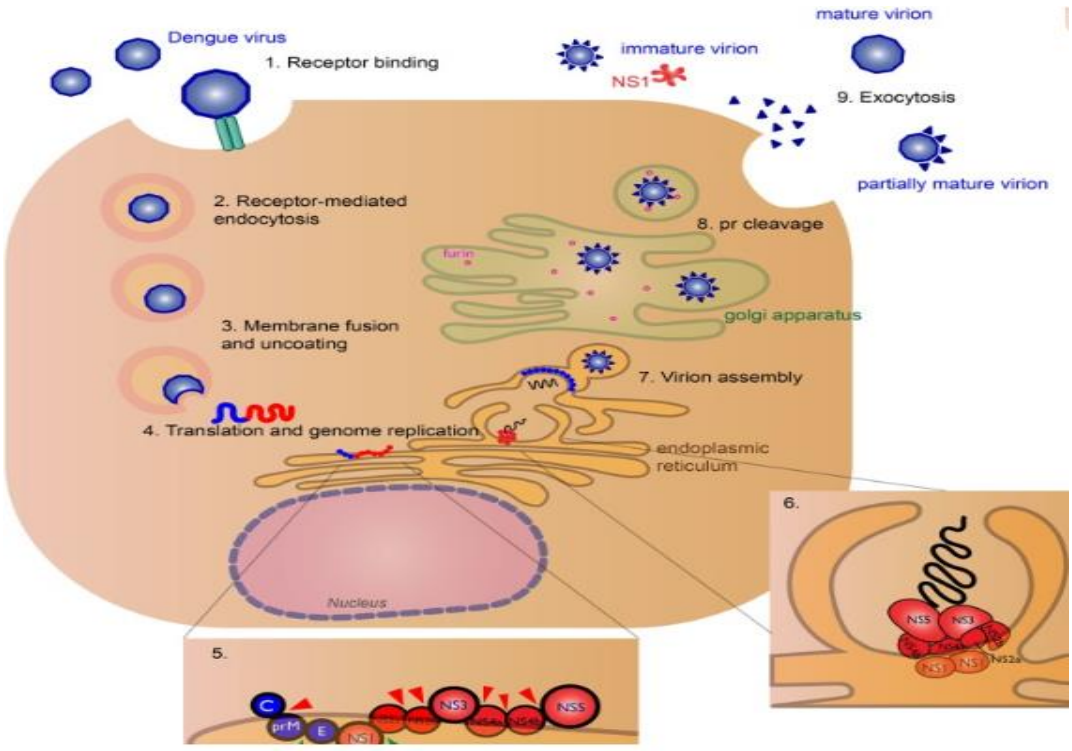

Figure1.3: Dengue Virus (DENV) life cycle(Uno & Ross, 2018)

1.2.4 Genetics

The genetic variants of dengue viruses can be documented by laboratory and epidemiological studies. The oldest DENV-1 (the Mochizuki strain) was isolated in 1943 from Japan, with subsequent reporting in the Americas in 1977 and in Africa in 1984. DENV-2 is thought to be originated from the sylvatic ancestor approximately 400–600 years ago and first reported in 1944 in Asia (Papua New Guinea and Indonesia), in Africa it was reported in 1964 and in 1953 in the Americas. DENV-3 was first reported in 1953 in Asia (the Philippines and Thailand), in 1963 in the Puerto Rico and during 1984–1985 in Mozambique and DENV -4 was first identified in Asia, subsequently in 1953 in the Philippines and Thailand and finally after long 25 years in Brazil, Cuba, Dominica, Puerto Rico, and the US Virgin Islands. (Harapan et al., 2020). However, genetic variation among dengue viruses can easily be studied by using oligonucleotide fingerprinting, primer

sequencing, and nucleotide sequence comparison(Shu & Huang, 2004). Usually, viruses circulating in the different geographic regions differ from viruses of the same serotype from other regions whereas, viruses sharing same geographic regions and timeframe show genetic homogeneity. As there is lack of good animal model for dengue it is under investigation how genetic variation can influence the clinical presentation or epidemic potential(Bennett et al., 2010). There is also a striking difference in replication characteristics between the South Pacific/American and the Southeast Asian subtypes of DENV-3 and DENV-2 (B.W.J. Mahy, 2008). Most of the viruses are now studied by partial nucleotide sequencing method. Based on 600 bp region of the envelope protein which represents amongst the total of dengue envelope protein, there are two distinct genotypes of DENV-1, four of DENV-2, four of DENV-3, and three of DENV-4. Analysis by restriction enzymes and primer extension sequencing can prove the origin of each serotype and also, there have been increasingly frequent reports of intra-serotypic recombination events among the dengue viruses (B.W.J. Mahy, 2008). However, interserotypic recombination might also occur as due to the increased occurrence of the cocirculation of multiple serotypes in an area (hyperendemicity) there have been increased reports of concurrent infections with two serotypes.

1.2.3 Epidemiology

Infection with one individual DENV serotype will exhibit long-lasting immune protection against that serotype because of the antigenic differences within all the four serotypes. According to WHO, dengue transmission is now present in all the six WHO regions and almost more than 125 countries around the world. Unfortunately, recent studies also suggested that, the number could even be 400 million per year(Powell et al., 2018). In fact, it is thought that almost 50% of the worlds population live in dengue endemic areas where they are at risk of severe DENV disease(Powell et al., 2018). Though, dengue is a a worldwide concern at present, The number of dengue cases is approximately 18 times higher in the South East Asian (SEA) region compared to other countries(Lai et al., 2018).

Almost 75% of the polulation of south east asia (SEA) experience dengue as a leading cause of hospitalization and death in both adult and children(Lai et al., 2018). In fact, eight SEA countries are now classified as hyperendemic with all four dengue serotypes circulating(Guo et al., 2017).

In Bangladesh, since 2000s the population has been experiencing episodes of dengue fever in almost every year (Mutsuddy et al., 2019). With DENV-3 predominanc, all four serotypes have been detected and more dengue cases with deaths were also reported after 2017, compared to the last 15 years(Mutsuddy et al., 2019). The Institute of Epidemiology, Disease Control & Research (IEDCR) under the Ministry of Health and Family Welfare, organization for outbreak investigation and surveillance in the country, found in 2013-2013reports that DENV-1 and DENV-2 is in circulation and also, predicted DENV-3 and DENV-4 that are circulating in neighbouring countries, have the ability to create epidemics of secondary dengue in the near future. Afterwards, re-emergence of DENV-3 was identified in 2017 and subsequently the country faced a sharp increase in dengue cases from the beginning of the monsoon season in 2018(Mutsuddy et al., 2019).

At last, the largest outbreak of dengue in Bangladesh began in April, 2019 which surpassed all previous records. A total of 70188 dengue cases had been officially recorded by the end of the August, 2019, with 67 dengue-associated deaths. In fact, the numbers increased to 81832 cases with 67 deaths by the end of Sept 16, 2019(Hsan et al., 2019). This largest outbreak which had high frequency of dengue cases was due to serotype 3, though all other serotypes are still in co-circulation(Hsan et al., 2019).

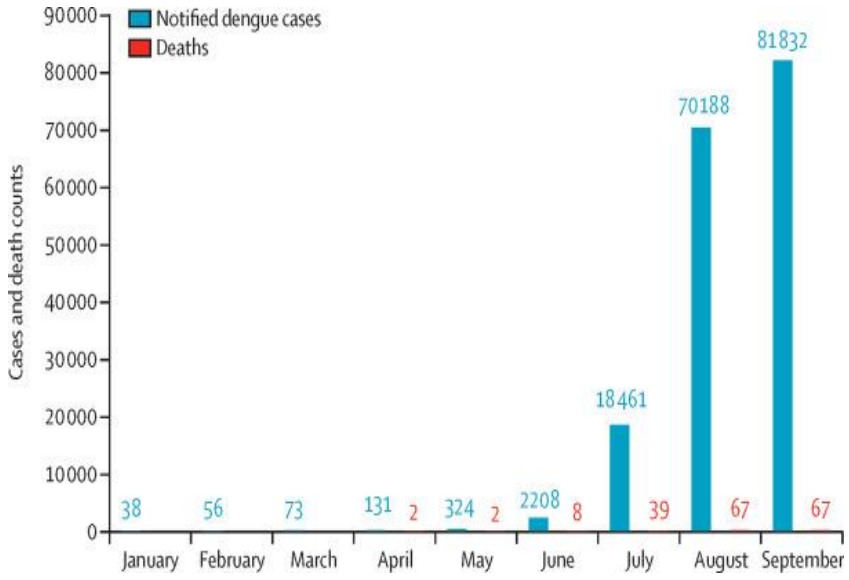

Figure 1.4: DENV cases and deaths in Bangladesh in 2019 (Hsan et al., 2019)

The poor disease surveillance, the true incidence reporting, less number of reporting cases, poor analytical skills in surveying the true incidence of DENV is much more higher than it is currently reported. Therefore, the true scenario of morbidity, mortality and associated economic burden is quite unknown. That's why, dengue surveillance, reporting and research prioritization is of paramount importance for effective dengue control and prevention of this viral diseases.

1.2.4 Seroprevalence of DENV in Bangladesh

In Bangladesh, its population have been experiencing dengue fever in almost every year. All the four DENV serotypes were detected, until 2002 where DENV-3 was the predominant serotype (Hsan et al., 2019). Later on, during 2013 to 2016, DENV-1 and DENV-2 were the circulating serotypes in major cities of this country, including Dhaka (Akram, 2019) and thus these two serotypes are now in circulation for more than a decade. This is why, it is also estimated that a large portion of the population might be

immune to DENV-1, DENV-2 serotype or both. though they are definitely at risk of developing severe dengue infection by DENV-3 or DENV-4 serotype (Akram, 2019). Thus, the high frequency of severe dengue cases in the recent years of 2018 and 2019 were due to the seroprevalence of DENV-3. (Akram, 2019). After the phylogenetic analysis of Dengue virus causing outbreak in Dhaka, Bangladesh during 2018, DENV type 2-Cosmopolitan genotype accounted for 54% and type 3-genotype was observed in 46% cases(Mone et al., 2019). In the capital of Bangladesh, around 400 and 6000 annual cases had been reported from 2008 to 2017 with an unprecedented large dengue outbreak occurring since August 2018 which involved more than 10,000 dengue cases in that year(Mone et al., 2019).

Table 1.1: Dengue serotypes Status in Different Years (Mone et al., 2019)

Year	DENV Serotype
2013-2016	DENV-2 (predominant) followed by DENV-1
2017	DENV-2 (predominant) followed by DEN1 and co detection of DENV-3 with DENV-2 (few cases)
2018	DEN 2 (predominant) followed by DEN2 and DEN1 and codetection DEN2 & DEN3 and DEN1 & DEN3(few cases)
2019	DEN 3 (predominant) followed by co-detection of DEN2 & DEN3 and DEN1 & DEN3 (few cases)

1.2.5 Clinical Manifestations

Dengue fever caused by DENV, defined by a combination of ≥2 clinical findings in a febrile person who has recently travelled or has been into dengue endemic area (B.W.J. Mahy, 2008). Clinical manifestations include nausea, vomiting, rash, aches and pains, a positive tourniquet test, leukopenia, and several warning signs like abdominal pain or tenderness, persistent vomiting, clinical fluid accumulation, mucosal bleeding, lethargy, restlessness, and liver enlargement (Tuiskunen Bäck & Lundkvist, 2013). In the case of primary infections, the ratio of inapparent to apparent infections is usually 15:1 whereas in secondary infections the ration is very lower (Geo F. Brooks, 2007). Also, the severity of dengue in a patient can be defined by the presence of some warning signs like, severe plasma leakage leading to shock or fluid accumulation with respiratory distress; severe bleeding; or severe organ impairment such as elevated transaminases ≥1,000 IU/L, impaired consciousness, or heart impairment(Harapan et al., 2020).

According to the WHO guidelines, in 2009, symptomatic dengue virus infections were classified as dengue fever (classical dengue), dengue haemorrhagic fever (DHF), and dengue shock syndrome which is regarded as the most severe form of DHF (Tuiskunen Bäck & Lundkvist, 2013). Dengue haemorrhagic fever according to World Health Organization Classification) is defined by four types of fever depending on the severity (B.W.J. Mahy, 2008). They noted that Grade I Fever is accompanied by nonspecific dengue fever symptoms, with a positive tourniquet test with haemorrhagic manifestation whereas, Grade II is almost the same as grade I, but this will include spontaneous haemorrahagic bleeding. mentioned too that, Grade III is characterized by circulatory failure which is manifested by rapid, weak pulse, narrowing of pulse pressure (20mmHg or less). Finally, Grade IV is manifested by significant shock with undetectable pulse and blood pressure.

Primary infections usually occur during the initial DENV infection of an individual. DENV infections of humans causes a spectrum of clinical conditions from inapparent disease, self limited dengue fever (DF) to severe DHF or DSS. DF is characterized by an abrupt onset of debilitating ferbrile illness, headache , retroorbital pain, myalgia, artharlgia and a maculopapular rash that appears after a 2 to 7 days incubation period after the

inoculation of mosquito where some individuals experience severe bone and joint pain where others develop haemorrhage which is associated with thrombocytopenia (Srikiatkhachorn et al., 2011). The primary or previous DENV infection represents the most important risk factor for severe dengue infection which causes a secondary infection. This results when a person previously infected with one serotype is exposed to a different serotype. DHF/DSS is quite rare Even during secondary infection, which is usually characterized by rapid onset of capillary leakage accompanied by thrombocytopenia and mild to moderate liver damage (examining aspartate aminotransferase or alanine aminotransferase) (David M. Knipe, 2001). DHF/DSS occurs as the second phase of the illness progresses after a short period of defervescence from initial fever. Gastrointestinal bleeding remains a great threat and can commonly occur if haemorrahgic manifestation are observed in a subset of DHF/DSS cases. Most importantly, fluid loss into tissue spaces with haemoconcentration and hypotension can result in shock and it carries the highest risk of mortality (B.W.J. Mahy, 2008). Laboratory findings commonly include leukopenia, thrombocytopenia, hyponatremia, elevated aspartate aminotransferase and alanine aminotransferase, and a normal erythrocyte sedimentation rate(Harapan et al., 2020). Also, Maternal anti-DENV neutralization antibody titers and infant age (peak at 7 months) is clinically correlated with the disease (Dat et al., 2018). Though there are limited data on health outcomes of dengue in pregnancy and effects of maternal infection on the developing fetus.

1.2.6 Immune Response to Dengue Virus

Both innate and adaptive immune responses are triggered in the host against DENV infection. If there is any presence of DENV infection, the innate immune system can easily recognize and takes action against the infecting virus. Though the innate immune system fails provide a long-term immune response, it activates the complement system which further makes the antibodies and leukocytes to remove dengue virus from the host. Anyways, the immune responses against DENV contribute to the protection of the host

against DENV infection and play pivotal roles in protection from reinfection yet, these responses may also play role in increasing disease severity and causing severe dengue in dengue infected patients which can be life threatening too. After infection, when there is a high level of the virus in the bloodstream, an infected person experiences the acute symptoms as the immune response fights the dengue infection. The person's B cells begin producing IgM and IgG antibodies that are released in the blood and lymph fluid and finally recognize and neutralize the dengue virus and its associated molecules. The immune response eliminates the virus, leading to recovery(Uno & Ross, 2018).

Through a mosquito bite, the DENV is transmitted to people and afterwards, the virus initially replicates in keratocytes and langerhans cells and skin cells which usually trigger a variety of host innate immune responses (Shresta, Annie Elong Ngono and Sujan, 2018). In return, these pattern recognition receptors (PRR) that recognize pathogen associated molecular patterns(Uno & Ross, 2018). This PRR recognition is followed by the production of cytokines and chemokines (Uno & Ross, 2018). Also, the activation of these receptors by DENV recognition induces type 1 interferon (IFN) responses which finally induce an antiviral state. This interferon system is triggered within hours of viral infection (Shresta, Annie Elong Ngono and Sujan, 2018). The pattern recognition receptors associated with DENV recognition are cyto-plasmic retinoic acid-inducible gene I (RIG-I) and mela-noma differentiation-associated protein 5 (MDA5), along with endosomal Toll-like receptor 3 (TLR3) andTLR7(Uno & Ross, 2018). The primary mechanism by which the innate immune system defends against viruses include the most important interferons involved in interferon system, type I interferons (IFN-α, β), type II interferon (IFN-γ), and type III interferons (IFN-λ1–4) (Shresta, Annie Elong Ngono and Sujan, 2018). Usually, When a host is infected by dengue viruses for the first time that means during a primary infection, antibodies are generated against that particular serotype(Tuiskunen Bäck & Lundkvist, 2013). These antibodies have the ability to neutralize the viruses. In fact, after a brief period of time, these antibodies remain cross-reactive for other serotypes and facilitate entry into the cell (Sarah Pagni, 2012).These Cross-reactive antibodies are able to bind to the virus and also bind to FccI and II

receptors which makes the Fc receptor-mediated endocytosis faster and more efficient than normal entry into the host cell. This in turn allows for more rapid replication and higher production of infectious particles (Sarah Pagni, 2012).

1.2.7 Dengue Virus Diagnosis

The DENV is found in the serum, plasma or other tissues that are involved in immune system after the onset of illness. They exist there approximately for 2 to 7 days depending on the course of the fever (Tuiskunen Bäck & Lundkvist, 2013). As DENV elicit a wide range of symptoms during febrile illness (Dengue Fever), dengue diagnosis only based on clinical symptoms is inappropriate. During early phase of infection (<5 days), DENV can possibly be diagnosed by virus isolation, nucleic acid amplification tests(NAAT) or detection of antigens such as NS1(Raafat et al., 2019). The NS1 antigen may be detected in some patients for several days after defervescence. After 5 days of infection, viremia usually subsides, antigens is undetectable and antibody responses are increased. At this point, specific antibody like (IgM or IgG) detection using serological methods is appropriate at this stage(Lima et al., 2012).

1.2.7.1Cell culture

The culture method can detect the virus at day 1 if 10^4 viruses are inoculated in the cell culture and this type of culture can be done in laboratories with cultured mosquito cells eg. AP-61, tRA-284, C6/36, AP64, CLA-1 cell lines and mammalian cells like LLCMK2, Vero, BHK21(Shu & Huang, 2004). Recently, a method of choice for routine based DENV isolation virus has been identified- using C6/36 line with serum or plasma from acute phase (Harapan et al., 2020).

1.2.7.2 Molecular Detection

The field of molecular diagnosis of dengue is more accurate for both the detection and characterization of dengue virus pathogen. The two step nested RT-PCR and the one step multiplex RT-PCR for the detection as well as typing of dengue are used for molecular detection of DENV (Tuiskunen Bäck & Lundkvist, 2013). These had the advantage of detecting and serotyping of all the four serotypes via analysing the unique sizes of the amplicons in the agarose gels(Tuiskunen Bäck & Lundkvist, 2013). More recently, Real Time RT-PCR is developed which has many advantages over conventional PCR technique because of the ability to provide quantitatiative measurements, lower contamination rate, a higher sensitivity, rapidity of the test result and standardization (Shu & Huang, 2004). SYBR Green real time PCR assay is also used where thereer is an advantage of designing primers simply compare to the TaqMan assay RT-PCR, but is less specific than the TaqMan assay(Raafat et al., 2019).

1.2.7.3 Serological Diagnosis

Serological detection of dengue virus specific antibodies is made possible by several techniques like hemagglutination inhibition (HI) test, the neutralization test, the indirect immunofluorescent antibody test, complement fixation, ELISA, rapid immunochromatography test using standardized kits(Harapan et al., 2020). In a study by (Lima et al., 2012), the antibodies that are mostly studied in DENV infections are IgG and IgM. Typically, IgM appears more rapidly and lasts longer than 2-3 months whereas If the HI test titer is greater than or equal to 1:2560, they are classified as having primary dengue virus infection whereas when the HI test titer is less than 1:2560, the pateints are thought to have secondary DENV(Raafat et al., 2019).

1.2.7.4 Serotyping

Recently developed E/M and NS1 serotype-specific capture IgM ELISAs can be utilized to differentiate between dengue viruses at the serotype level by using culture supernatants

of all four serotypes as the antigen sourse for final detection(Shu & Huang, 2004). Also, the NS1 serotype specific IgG ELISA could also be used for dengue virus serotype analysis using convalescent phase serum and acute phase sera from primary and secondary dengue infected patients respectively. Furthermore, immunoblot strip method utilizing recombinant antigens could also be used for serotype analysis, though it is effective for patients with secondary infections(Tuiskunen Bäck & Lundkvist, 2013). It should be noted that multiple probes and primers targeting different regions of dengue viruses should be utilized and implemented successfully to increase the sensitivity and specificity and at the same time avoiding false negative results. The diagnosis of DENV infection and the typing of DENV serotypes can be confirmed using viral isolation techniques, serology, or molecular methods. The Molecular techniques based on the detection of genomic sequences by reverse transcription-PCR (RT-PCR), nested PCR, and real-time PCR identify the four different serotypes by using genus- or serotype-specific primers or a combination of both. However, the genotypes of DENVs described within the different serotypes are based on sequence variations in gene E and NS1(Sim & Hibberd, 2016). The number of genotypes varies, ranging from three (for DENV-4) to five (for DENV-1, -2, and -3), depending on the region sequenced. In a study conducted by (Das et al., 2008), the envelope (E) and capsid (C) genes of DENV genome were chosen as targets, because they were found to have sufficient variation to be useful for serotype differentiation. However, a large number of primers had to be used to cover all anticipated sequence variations as there are inherent heterogeneity in the E region, Primers were designed for two regions in order to provide a certain amount of redundancy that would circumvent any dropouts in PCR amplification due to sequence variation(Das et al., 2008).

1.2.8 Antiviral and Preventive Approach Against DENV

1.2.8.1 Mosquito Control

Eradication of Aedes mosquitoes, which is the most effiecient mosquito vector of dengue virus, is ineffective, especially the regions where the DENV is endemic. Therefore, the

control and prevention of dengue viral infection will only be possible where there will the availability of effective dengue vaccines. Vector control can be very effective, but to let happen, the program concerning vector control must be comprehensive and sustainable also, implementation must be done thoroughly(Tuiskunen Bäck & Lundkvist, 2013). Also, insecticide-treated mosquito nets afford good protection for those who sleep during the day (e.g. infants, the bedridden and night-shift workers. The distribution of mosquito nets or bednets impregnated with insecticides such as permethrin or deltamethrin has been shown to be an extremely effective method of malaria prevention(Tuiskunen Bäck & Lundkvist, 2013).

1.2.8.2 Antiviral Drugs

While designing anti-DENV therapy, evaluation of host and viral factors are studied significantly. As, viral infections can modify many physiological and metabolical pathways, for example, lipid metabolism, change in glycolytic pathways, modification in membvrane lipid and other composition(Low et al., 2017), Targeting host factors that are mostly included in dengue pathogenesis are: dendritic cell receptors followed by specific intracellular adhesion molecule3-grabbing non integrin and glycosaminoglycans which are the first line attachment GRP-78, an immunoglobulin binding protein, the laminin receptor, T cell immunoglobiulin and mucin domain being second line attachment factors(Martina et al., 2009) are excellent antiviral potential. The most recent clinical trials for dengue have been performed using repurposed or off-patent drugs, namely chloroquine, prednisolone, balapiravir, celgosivir, and lovastatin(Sim & Hibberd, 2016). These trials have all used the conventional double-blinded, randomized, placebo-controlled design with clearly defined primary end points. The drugs were found to be safe in patients with acute dengue, but all of these compounds failed to meet a priori–defined trial end points (Shu & Huang, 2004). Two other trials involving ivermectin and ketotifen are currently have been recruited in Thailand and Singapore(Ahammad et al.,

2019). Interestingly, the preliminary findings from the phase 2 ivermectin study suggests a reduction in serum NS1 levels and body temperature with high-dose ivermectin, despite lack of significant difference in viremia levels (as measured by real-time quantitative polymerase chain reaction [qPCR])(Ahammad et al., 2019). Moreover, RNA interference is an important and effective gene silencing process which can also be used an effective therapeutics against Dengue seroptypes (Idrees & Ashfaq, 2013). It degrades targeted viral RNA by a sequence specific process(Idrees & Ashfaq, 2013). It is expected to provide a better and even next generation dengue therapeutic approach which may eradicate dengue infection.

1.2.8.3 Vaccines

Currently, a chimeric yellow fever 17D, tetravalent dengue vaccine has been approved in some countries for vaccination purpose. Five types of dengue viral vaccine are under research investigation for potentiality. which include: live attenuated vaccine, DNA vaccine, recombinant vaccine, viral vectored vaccine, inactivated vaccine (MA McArthur & MB Sztein, 2013). All of these increase the immune response against dengue viral envelope protein (E) and non structural protein NS1. Careful investigation of the immune response to DENV infection can help to contribute to the understanding of viral vaccines. There have been development of several live attenuated vaccines with recombinant DNA technology (eg. Chimeric yellow fever 17D virus tetravalent vaccine (CYD-TDV), the recombinant DENV 4 mutant bearing vaccine (rDENV4Δ30), tetra live attenuated virus vaccine (DENVax)(Raafat et al., 2019). Apart from these, live attenuated tetravalent vaccines are difficult to design as there is need to maintain attenuation of all monovalent components. Moreover, TV003 vaccine that is a mixture of four attenuated recombinant vaccine including rDEN1d30, rDEN2/4D30, rDEN3d30/31 and rDEN4d30 which is in the clinical phase of vaccine trial. Compared with the live attenuated vaccines, the recombinant vaccines can stimulate more effective immune response against all the four DENV serotypes, but there is a problem of improper protein folding and contamination of

endotoxins (David M. Knipe, 2001). Finally, after a single dose immunization in mice , it has been estimated that, adenoviral vectored vaccines can trigger a effective immunity, whereas DNA vaccines are stable, cost effective and much easy for mass production but unfortunately contains the problems of low immunogenicity (Harapan et al., 2020). However, despite several impediments, currently there are many promising vaccine candidates in clinical and pre-clinical development (Tuiskunen Bäck & Lundkvist, 2013). Most importantly, studying and understanding the immune mechanism within host after viral infection is tremendously actionable to break through the bottle neck of vaccine development.

Objective

Dengue virus epidemic is happening intermittently in Bangladesh from 2000. However, most of the study on the molecular epidemiology of this virus is focused on Dhaka city or major metropolitan cities of the country. However, significant number of Bangladeshi population lives in rural Bangladesh and it is important to explore the circulating serotypes of both urban and rural areas. The data will help to understand the transmission dynamics between both settings. Therefore, the objective of the current study was to identify the circulating serotypes of 2019 epidemic from rural Bangladesh.

Chapter 2: MATERIALS AND METHODS

2.1 Study population and Sample Collection

During the period of 2019 DENV outbreak, a total of 66 serum specimens (n=66) were obtained from paediatrics department of Faridpur Medical College Hospital (FMCH). The serum samples were received along several clinical details from suspected dengue fever patients for the characterization of dengue viral infection. The clinical information was collected from the hospital authorities. All serum samples were investigated for the presence of DENV RNA using Polymerase Chain Reaction (PCR) using viral family specific primers. The samples from clinically suspected dengue infected patients were transported to the laboratory in 2-8℃ cool boxes. Samples collected from dengue suspected patients were stored at -80℃ until examination.

2.2 Viral RNA Extraction

Total RNA was extracted from 140-µL sample using a QIAamp viral RNA mini kit (Qiagen) according to the protocol suggested by the manufacturer.

Materials

1. QIAamp mini spin columns
2. Collection tubes (2 ml)
3. Buffer AVL
4. Buffer AW1
5. Buffer AW2
6. Buffer AVE
7. Carrier RNA
8. Microcentrifuge tube(1.5ml)
9. Microcentrifuge
10. Sterile, RNase free pipette tips

11. Ethanol (96-100%)

Protocol

1. 560 µl of prepared buffer (for 140 microliter of sample) containing carrier RNA was pipetted into a 1.5 ml micro centrifuge tube. (If the sample volume is larger than 140 µl, the AVL buffer containing carrier RNA is adjusted accordingly)
2. 140 µl of sample was added to the buffer AVL-Carrier RNA containing centrifuge tube.
3. The tube was pulse vortexed for 15 seconds.
4. Incubation of the tubes at room temperature (15 -25 degree Celsius) for 10 minutes for complete lysis.
5. Brief centrifugation of the 1.5 ml centrifuge tube to remove drops of the inside of the lid.
6. 560 µl of ethanol was added to the sample and mixed by pulse vortexing for 15 seconds. After mixing, again a brief centrifugation was carried out with the tubes.
7. 630 µl of the the total volume is placed onto 2 ml collection tube containing spin column. The cap is closed and centrifuged at 8000 rpm for 1 minute.
8. Now, the QIAmp spin column is placed onto a new collection tube and the tube containing filtrate was discarded.
9. The rest of the 630 µl was placed onto the spin column and centrifuged at 8000 rpm for 1 minute. Spin column is checked for all of the lysates being transferred and placed into a new 2 ml collection tube.
10. 50 µl of AW1 was added to the spin column and centrifuged at 8000 rpm for 1 minute. The tube containing filtrate was discarded and the spin column was placed onto a new collection tube.
11. The spin column was opened carefully and 500 µl of AW2 buffer is placed into it. The mixture was centrifuged at 14000 rpm for 3 minutes.
12. The old collection tube containing filtrate was discarded and placed into a new collection tube. It was centrifuged at a full speed for 1 minute. The collection

tube was discarded and the spin column is placed into a new 1.5 ml centrifuge tube.

13. 60 µl of elution buffer (AVE) was added to the tube. The cap was kept closed and incubated at room temperature for 1 minute.
14. Centrifugation at 8000 rpm for 1 minute.
15. Finally, the eluted viral RNA was collected in a 1.5 ml microcentrifuge tube and stored at -80 degree Celsius before they were tested for DENV infection by the RT-PCR procedure

2.3 Polymerase Chain Reaction

Materials and Reagents

1. Thermostable DNA polymerase
2. Reverse Transcriptase
3. Primers
4. Template
5. dNTP solution containing all four dNTPs
6. PCR micro-well plate

Procedure

1. All reagents were arranged needed for the PCR experiment in a freshly filled ice bucket and was thawed completely before setting up a reaction. Also, a 96 well plate was placed into the ice bucket as a holder for the 0.2 ml thin-walled PCR tubes.
2. Preparation of Master Mix

Table 2.1: Reagents for Mater Mix Preparation

Reagents	**Amount (in µl)**
PCR buffer (2X, 20µmol)	5.0
Dntp	1.0
Forward Primer (Flv_F)	1.5
Reverse Primer (Flv_R)	1.5
RNase Free Water	5.0
Reverse Transcriptase and Taq polymerase mixture (25X, Applied Biosystems)	1.0
Template	10
Total Reaction Volume	**25**

1. Required agents (master mix) was set up and aliquoted into each PCR thin walled tubes. In addition, another reaction (if reagents are available) should contain a positive control using template DNA and or primers previously known to amplify under the same conditions as the experimental PCR tubes.
2. The template was added into each PCR tube separately
3. The micropipettor was set to about half the reaction volume of the master mix when mixing, and care was taken to avoid introducing bubbles.
4. The whole mixture was briefly centrifuged to collect all the components at the bottom of the tube.
5. The amplification parameters will vary depending on the primers and the thermal cycler used. It may be necessary to optimize the system for individual primers, template, and thermal cycler.
6. The PCR plate containing thin-walled PCR tubes were placed in a thermal cycler (biometraT3000). The thermal profile comprised of reverse transcription for 15 minutes at 37℃, following by initial denaturation at 95 °C for 2 min followed by

35 cycles at 95 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 2 min and final extension at 72 °C for10 min. Once the lid to the thermal cycler was firmly closed, the program was started.

7. When the program was finished, 0.2 ml thin-walled PCR tubes were removed and stored at 4 °C pending analysis by electrophoresis system.

Table 2.2: Thermal Cycling program for Dengue virus Conventional PCR

Name of the step	**Temperature**	**Time**
Reverse Transcription	48℃	45 minutes
Denaturation	94℃	1 minute
Annealing	55℃	1 minute
Extension	72℃	2 minutes
Final Extension	72℃	10 minutes

2.4 Agarose Gel Electrophoresis

The size and quantity of the PCR amplicons was confirmed by 1% agarose gel electrophoresis to detect specific bands. PCR products from each sample was mixed with loading dye. This mixture was loaded into the wells of agarose gel into the gel tray. A DNA ladder was also loaded in one well of the gel as a marker to determine the size of the amplicons. Electrophoresis was carried out on a horizontal gel electrophoresis apparatus. Finally, the bands of amplicons visualized using BioDoc UV transluminator (Biometra, Germany). Photographs were taken using a BioDocAnalyze gel documentation system (Biometra, Germany).

Equipment:

1. Casting tray
2. Voltage source
3. Gel box
4. Well combs
5. UV light source
6. Microwave

Table 2.3: Reagents needed in agarose gel electrophoresis

Reagents	Amount
Tris-Borate-EDTA (TBE buffer)	40 ml
Gel red	4µl
Agarose	0.6 g
Gel Loading Dye (6X)	2µl

Method:

1. 0.6g of agarose (for 17 wells in four slots) in a weight balance was measured (the concentration of agarose in a gel will depend on the sizes of DNA fragments to be separated) and taken into a microwavable erlenmeyer flask.
2. 40 mL of TBE buffer was poured into the flask.
3. The mixture was shaken well until the agarose powder was mixed
4. Heating in the microwave at 95℃ for 1m 30s until the agarose powder was completely dissolved. Over boiling of solution could evaporate some of the buffer.
5. After 5 minutes, the microwaved flask was set aside to cool it down to about 50℃ until it was comfortable enough to keep hands on flask.
6. In the meantime, a mold (a rectangular piece of plastic) was made. The gel tray was put into it and the combs are placed which will create wells.

7. 4 µl of gel red was added into the flask and is shaken gently. The colour of gel red is bright orange which will bind in between the base pairs and fluorescence. DNA doesn't have their own color, EtBr or Gel Red interchalates within the DNA bases and gives fluoresecent color under UV light.
8. Finally, agarose was put into gel tray slowly (to avoid bubbles, which will disrupt the gel). The bubbles produced on the gel were removed with a pipette tip by pushing the bubbles toward the edges.
9. The poured gel was allowed to sit at room temperature for 30 minutes until it was completely solidified.
10. Once solidified, the agarose gel was placed into a gel box containing buffer (electrophoresis unit)
11. The gel box was filled with TBE until the gel is covered
12. Loading dye of 2 µl was added to each of the DNA sample prior to addition into wells. A molecular weight ladder (100bp) is carefully loaded into the first lane of the gel.
13. All the samples (4 µl each) were added to the additional wells of the gel
14. A positive control was added to the last lane of the gel.
15. The gel was run for 1.5 hours at 80 V until the dye line was approximately 75-80% of the way down the gel.
16. The power was turned off, the electrodes were disconnected from power source and the gel was carefully removed from the gel box. After the electrophoresis, the amplicons were visualized in a BioDoc UV transluminator (Biometra, Germany) and using the Gel Documentation system (BioDoc Analyze Digital) were photographed.

Chapter 3: RESULTS

3.1 Clinical Manifestation Investigation for study patients

Dengue virus infection is considered as a major public health concern in the present world. Seroprevalence monitoring of circulating DENV infections is crucial for prevention of fatal outcomes in secondary dengue infections and also for patient management in a possible future outbreak. In this study, as far as clinical spectrum was concerned, all patients had come with fever along with varied clinical manifestation.

Clinical categorisation of subjects was done as shown in Table 3.5. Out of all 66 dengue suspected patients, 10 of the patients turned out dengue positive. 56 patients had fever ranging from 38.5℃ to 40.5℃ and other complications. 50% of the dengue positive patients presented with headache, 40% of the patients presented with restlessness, 70% of the patients presented with vomiting complication and 40% of patients had lethargy (Table 3.4). Hepatomegaly and skin rash were presented by 20% of the patients (Table 3.4) None of the dengue positive patients had shock and bleeding manifestations. Retroorbital pain which is considered as one of the most well-known features of dengue, was absent among the 90% of dengue positive patients in this study. Fever range of 101-103℉ was most prevalently shown in the dengue positive patients (Table 3.3). terms of seroprevalence analysis, DENV- 3 was the most prevalent serotype found in the present study followed by DENV-2 serotype. Almost 80% patients were infected with serotype 3 whereas for DENV-2, the presenting patients were 10% (Figure 3.3). Moreover, male patients were mostly affected by DENV infection compared to female patients (Table 3.4).

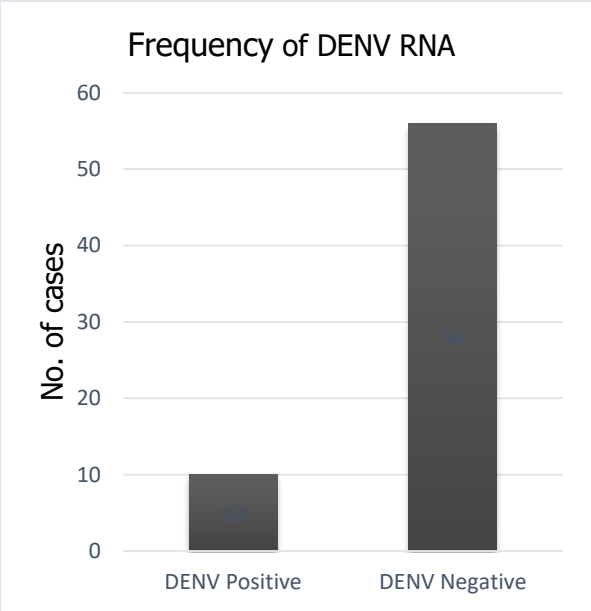

Figure3.1(a)Frequency of DENV RNA detected from the study participants

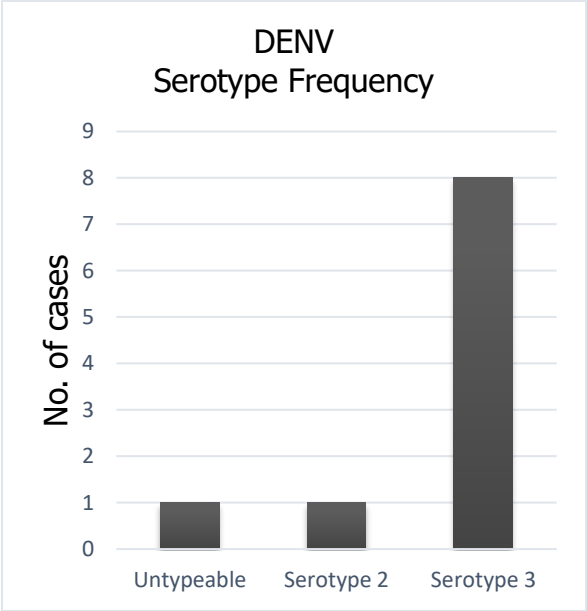

Figure 3.1(b)Circulating serotypes of DENV

Table 3.1: Age distribution of the dengue suspected patients in the present study

Age Groups	**DENV Positive Cases**	**DENV Negative Cases**
0-3 years	2	19
3-5 years	1	6
5-7 years	0	8
7-9 years	3	7
9-11 years	1	7
11-13 years	2	8

Table 3.2: Gender wise distribution of dengue positive and dengue negative patients

Category	**Male (%)**	**Female (%)**
DENV (+ve)	9 (90%)	1 (10%)
DENV (-ve)	30 (53.5%)	27 (48.2%)

Table 3.3: Difference in ranges of Body temperature in DENV positive and DENV negative patients.

Body Temperature	**DENV Positive Cases**	**DENV Negative Cases**
98°F	0	10
99°F	0	0
100°F	0	10
101°F	2 (DENV-3)	3
102°F	5 (4 DENV-3 &1 untypeable)	15
103°F	1 (DENV-2)	4
104°F	0	2
105°F	1	0

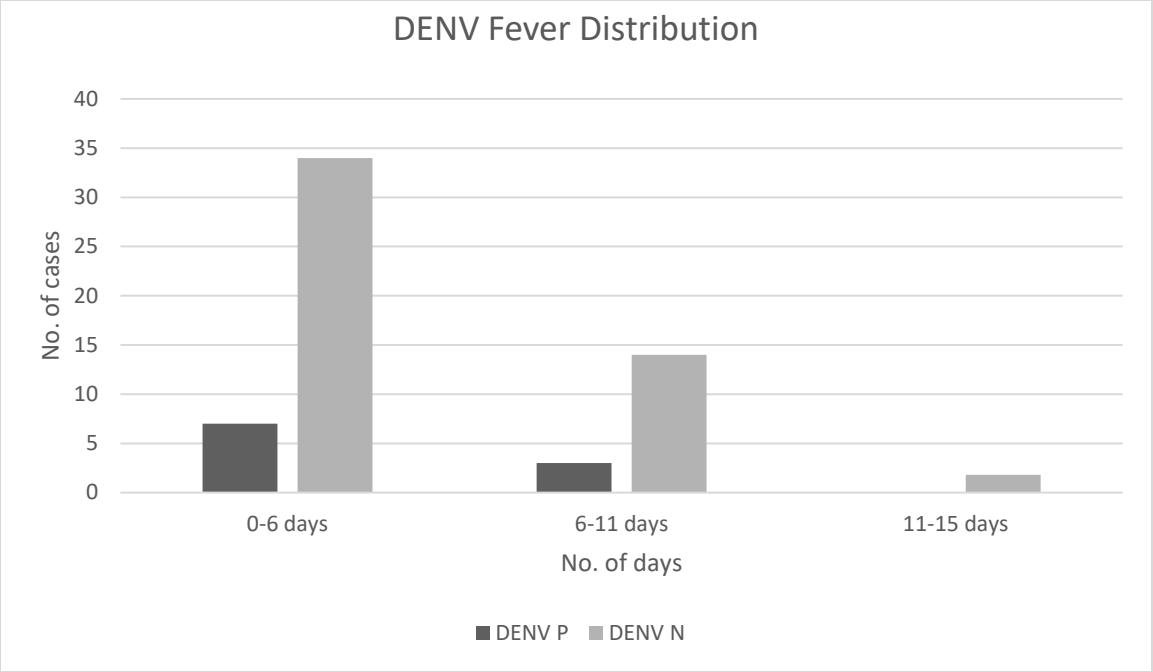

Figure3.2: Observation of DENV fever duration in dengue positive and dengue negative patients.

Table 3.4: Comparison of clinical observation among DENV positive and DENV negative patients.

Clinical Symptoms	**DENV-Negative (n=57)**	**DENV-Positive (n=10)**
Fever	46	10
Lethargy	34	4
Restlessness	21	4
Headache	25	5
Periorbital pain	6	1
Conjunctivitis	7	3
Arthritis	9	2

Arthralgia	6	0
Skin Rash	11	3
Abdominal Pain	24	1
Vomiting	30	8
Vomiting with Blood	0	0
Gum bleeding	1	0
Epistaxis	0	1
Hepatomegaly	0	2
Spleenomegaly	0	0
Shock	2	0

3.2 Agarose Gel Electrophoretic Analysis of DENV samples

Of all 66 DENV suspected samples under the present study, 10 cases (15.15%) were positive by Reverse Transcriptase [RT]-PCR assay followed by agarose gel electrophoresis.

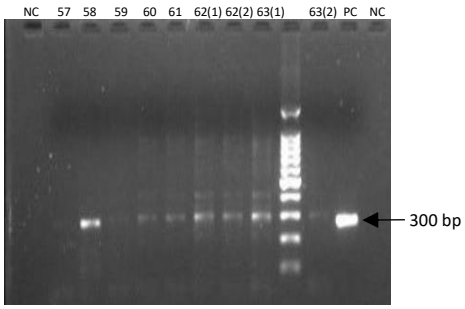

Figure3.3: Image of PCR amplicons on agarose gel captured from gel documentation

Chapter 4: DISCUSSION

Discussion

Dengue is one of the most prevalent mosquito-borne viral illnesses throughout the world which may range from asymptomatic or manifest life-threatening dengue haemorrhagic fever (DHF)/dengue shock syndrome (DSS). The present study highlights the exploration of DENV seroprevalence in rural Bangladesh along with the knowledge of varied spectrum of clinical manifestations of febrile illness. The study evaluated 66 dengue suspected patients from paediatric department of FMCH for identification of DENV RNA, serotype identification and comparison of clinical features of the dengue positive vs negative patients. In the present study, DENV-3 was the major serotype circulating around the investigated dengue positive patients during 2019 dengue outbreak in rural Bangladesh. Existence of all four DENV serotypes with predominance of DENV-3 (70.5%) was observed in 2000 dengue outbreak in Bangladesh (Hsan et al., 2019). After that, DENV-3 had been unreported from Bangladesh till 2016 (Muraduzzaman et al., 2018). From the year of 2013 to 2018, DENV-4 was the predominant serotype in Bangladesh followed by the co detection of DENV-2, DENV-3 and DENV-1 respectively (Akram, 2019). In a paediatric sero-prevalence study conducted in Indonesia by (Sasmono et al., 2018), DENV-2 was the most predominant serotype followed by DENV-1 and DENV-3 and DENV-4 being the least dominant serotype. In another study, serotyping of 151 PCR-positive samples done in 2018 showed that 41%, 31% and 9% cases were positive for DENV-2, DENV-3 and DENV-1 respectively (Shirin et al., 2019). Among the four DENV serotypes DENV-1 and DENV-2 were in the circulation in a study conducted all over Bangladesh from 2014-2018 by IEDCR (Muraduzzaman et al., 2018). In contrast, during 2013, DENV-2 was the only serotype that was in circulation in Chittagong and Khulna metropolitan cities, whereas in Dhaka, along with DENV-2, DENV-1 (31%) was also found to be circulated (Muraduzzaman et al., 2018). In the next year (2014), it was observed that DENV-1 began to spread outside Dhaka and emerge in Chittagong, but still Khulna was free from DEN1. In 2015, DENV-1 appeared in the circulation along with DENV-2. In 2016, DENV-2 became the predominant strain with existence of DEN1 in all three cities under surveillance (Muraduzzaman et al., 2018). In addition, as DENV-3 was unreported till

2016 in Bangladesh (Muraduzzaman et al., 2018), however, the presence of DENV-3 in rural Bangladesh in the present study possibly indicates that DENV-3 serotype was circulating in rural areas which was uncovered by any surveillance system. Moreover, in terms of age distribution, children within the age group between 7-9 years was mostly positive for dengue virus in the present study, whereas, the age group ranged from 10-14 years in a seroprevalence study conducted in Indonesia by (Sasmono et al., 2018).

The most common features of dengue fever in the enrolled paediatric patients were fever (100%), vomiting (80%), headache (50%), leathargy (40%), restlessness (40%), conjunctivitis (15%), skin rash (10%), arthritis (10%). (Sriram Pothapregada, 2016) in his study showed that, the most common clinical manifestations included fever (94.6%), conjunctivitis (89.6%), myalgia (81.9%), headache (75.1%), and retro-orbital pain (51.3%). In one more study on paediatric patients of Dhaka, Bangladesh by (Shultana et al., 2019), fever ever was noted among (100%) patients, followed by skin rash (48.31%), vomiting (37.07%), abdominal pain (23.59%) respectively. Similar to the study done on the investigated paediatric patients of FMCH, vomiting was the second most common clinical manifestation in a study conducted by (Hoque et al., 2019) from April 2018-March 2019.

The present study also showed that, a broad range of clinical signs and symptoms within the investigated paediatric patients were associated with the DENV-2 and DENV-3 serotypes, such as high rates of headache, lethargy, vomiting, restlessness, skin rash, conjunctivitis whereas, DENV-1 and DENV-2 was associated with multiple clinical manifestations in a study by (Sasmono et al., 2018). Another study by (Raafat et al., 2019) suggested that DENV-2 infections may cause more serious clinical issues than infections with other serotypes. Similarly, in the present study, multiple complications were associated mainly with DENV-3 and DENV-2 infected patients. None of the dengue positive patients in the present investigation experienced haemorrhagic fever, however, haemorrhagic manifestations were observed in the studies by (Shultana et al., 2019) and (Hoque et al., 2019).

Conclusion

Dengue remains to be an important public health problem affecting geographies worldwide. In several countries including Bangladesh, dengue is now considered as an endemic disease. However, data on circulating serotypes from rural Bangladesh is still very limited. The knowledge of the DENV seroprevalence among populations in rural area will help to understand the persistence and transmission of DENV among Bangladeshi population. The findings will make impact to prevent severe dengue outbreak in a country.

CHAPTER 5: REFERENCE

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