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Review

Dengue and human health: A global scenario of its occurrence, diagnosis and therapeutics



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ABSTRACT

Dengue is one of the highest and rapidly spreading vector-borne viral diseases with high mortality rates. The infection causes acute febrile illness, a major public health concern in the tropics and subtropics globally. The disease is caused by an RNA virus that belongs to the Flaviviridae family. The virus is transferred to humans by the mosquito vector called *Aedes aegypti*, which is the cause of new prevalent sicknesses worldwide. These vector-borne viral diseases spread very fast and pose public health and economic challenges that deemed various prevention and control techniques. The *Flavivirus* genus consists of five different types of viruses starting from DENV-1 to DENV-5. Thus, the present review focuses on the origin of the virus, how the Dengue virus can be detected, infection, the morphology of the virus, its classifications as proposed by ICTV, the replication and genome of the dengue virus, translation, receptor binding, and some vaccine trial volunteers. In addition, it highlights the current challenges and limitations of effective dengue treatment.

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1. Introduction

Viral infections have been of great concern with increase in the rise of epidemics and pandemics leading death of millions of people (Mujtaba et al., 2021; Irfan et al., 2017a, 2017b). Dengue is known as a mosquito-borne viral infection that has very high mortality and morbidity rates. Mosquitos are the primary transmitters of this viral infection. Globally, around 2.5 billion individuals have been affected by the virus (Khursheed et al., 2013; Caraballo and King, 2014). The Pan American Health Organization (PAHO) reported around 20,368 cases of dengue virus infection in the Americas region six weeks into 2021. PAHO reported that Nicaragua has about 4,297 cases, which is the highest. The next highest is Colombia, with 4,118 cases. Paraguay has 3790 cases, while 1,951 and 1,670 cases were reported in Mexico and Ecuador, respectively. In early 2021, DENV 1–4 variants were reported in the Americas; thus, severe cases keep going up. The Americas has seen up to 55,800 Dengue virus cases from 1st Jan 2021 to now. Out of the 55,800 cases, 210 were severe, while 13 cases resulted in deaths (PAHO, March 2021).

There has been a rise in Dengue virus infection cases in recent years, resulting in around 100–400 million cases every year (WHO). *Aedes aegypti* transmits the dengue virus through various species of female *Aedes* mosquito. The infection starts with mild fever; however, after three to fifteen days of infection, the individual can develop a very high fever and other consequences, as shown in Fig. 1 (Anderson and Rico-Hesse, 2006; Tuiskunen-B and Lundkvist, 2012; Bhatt et al., 2013; Basurko et al., 2018).

Different preventive strategies are developed to control the spread of dengue virus infection. Nevertheless, different variants of DENV can be found globally, and its transmission is made easier because of human travel globally. Deaths caused by the dengue virus increased from 960 in the year 2000–4032 in 2015. However, at present, the deaths due to DENV infections have drastically reduced.

2. Origin of dengue virus

There is no evidence yet confirming when Dengue Virus was first reported in humans. This is primarily due to the asymptomatic nature of the disease. As a result, it is not easily diagnosed. Dengue fever (DF), although it is not a new illness, was first discovered and documented in Batavia in 1779; after a year, it was deemed a pandemic in Philadelphia, USA (McCallum, 2008). The World Health Organization reported that about 1.2 million people got the DF in 1998 (Dengue: global alert response GAR). Then 50 years later, the number of infections increased by 30 times. Another case was reported in China in 1992 by a medical encyclopedia. Between the 18th and 19th centuries, world shipping expanded, bringing urbanization to port cities, which became ideal for the primary mosquito vector, *Aedes aegypti*, to transmit the disease.

Similarly, when Southeast Asia expanded very quickly, it resulted in increased transmission of the virus. The first dengue pandemic took the world by storm, happening simultaneously in Africa, Asia, and North America (WHO, 2021). At the moment,

the various reports obtainable suggest that there are around 50 million DF cases in hundred nations of the world (Guzman and Istúriz, 2010).

DENV is transmitted between individuals when an infected *Aedes* mosquito species (*Ae. aegypti* or *Ae. albopictus*) bites a human host (Carrington and Simmons, 2014). These mosquitoes can also spread the West Nile virus. Southeast Asia witnessed a dengue hemorrhagic fever (DHF) epidemic due to a change in ecology (Gubler, 2006). Its symptoms include plasma leakage, hypovolemia, an increase in vascular permeability, and shock (WHO, 1997; Gubler, 2002). In 2019, WHO reported about 4.2 million cases of the dengue virus.

3. ICTV dengue virus taxonomy

Less than fifty species of arthropod-borne viruses are contained in the Flavivirus (Gubler, 2006; Mukhopadhyay et al., 2005). These small-enveloped viruses have RNA genomes that range from 9000 to 13,000 bases (Simmonds et al., 2017).

Usually, birds and mammals are the primary hosts for transmitting this virus by ticks or arthropod vectors and mosquitoes (Simmonds et al., 2017). However, viruses transmitted by mosquitoes are mostly limited to dengue virus, yellow fever virus, West Nile virus, and Japanese encephalitis virus (Simmonds et al., 2017).

4. Virus morphology

The flavivirus has a spherical shape with a diameter of 50 nm. Mature virions consist of virus-encoded, membrane-associated proteins M and E (Fig. 2). Intracellular immature virions have the precursor prM divided into M as they mature (Stadler et al., 1997). X-ray crystallography has been used to obtain the virion structures of the dengue virus (Kuhn et al., 2002; Mukhopadhyay et al., 2003). The envelope protein, E, is a rod-shaped dimeric molecule (Yu et al., 2008). Reconstructing the image of the virion envelope with cryo-electron micrographs has shown that it has icosahedral symmetry, with the E protein dimers arranged in a herringbone-like pattern.

5. Dengue virus genome and replication

There are five different classes of dengue viruses, DENV-1, DENV-2, DENV-3, DENV-4, and DENV-5 (Mukhopadhyay et al., 2005). They have 65–70% similarity with amino acid sequence (Azhar et al., 2015). The structure of the genome has a positive-sense RNA genome of 10.6 to 11.0 kb (Shrivastava et al., 2018), is flanked by 5' UTR and 3' UTR (Wadood et al., 2017), and encodes for a single open reading frame (~3400 codons). The genome encodes for three structural proteins, C protein, prM protein, E protein, and non-structural proteins, such as NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5 as shown in Fig. 3.

The Dengue Virus lashes to the host's cell surface, entering the cell via a process called endocytosis (Acosta et al., 2014). The virus mixes with the endosomal membrane and enters the cytoplasm.

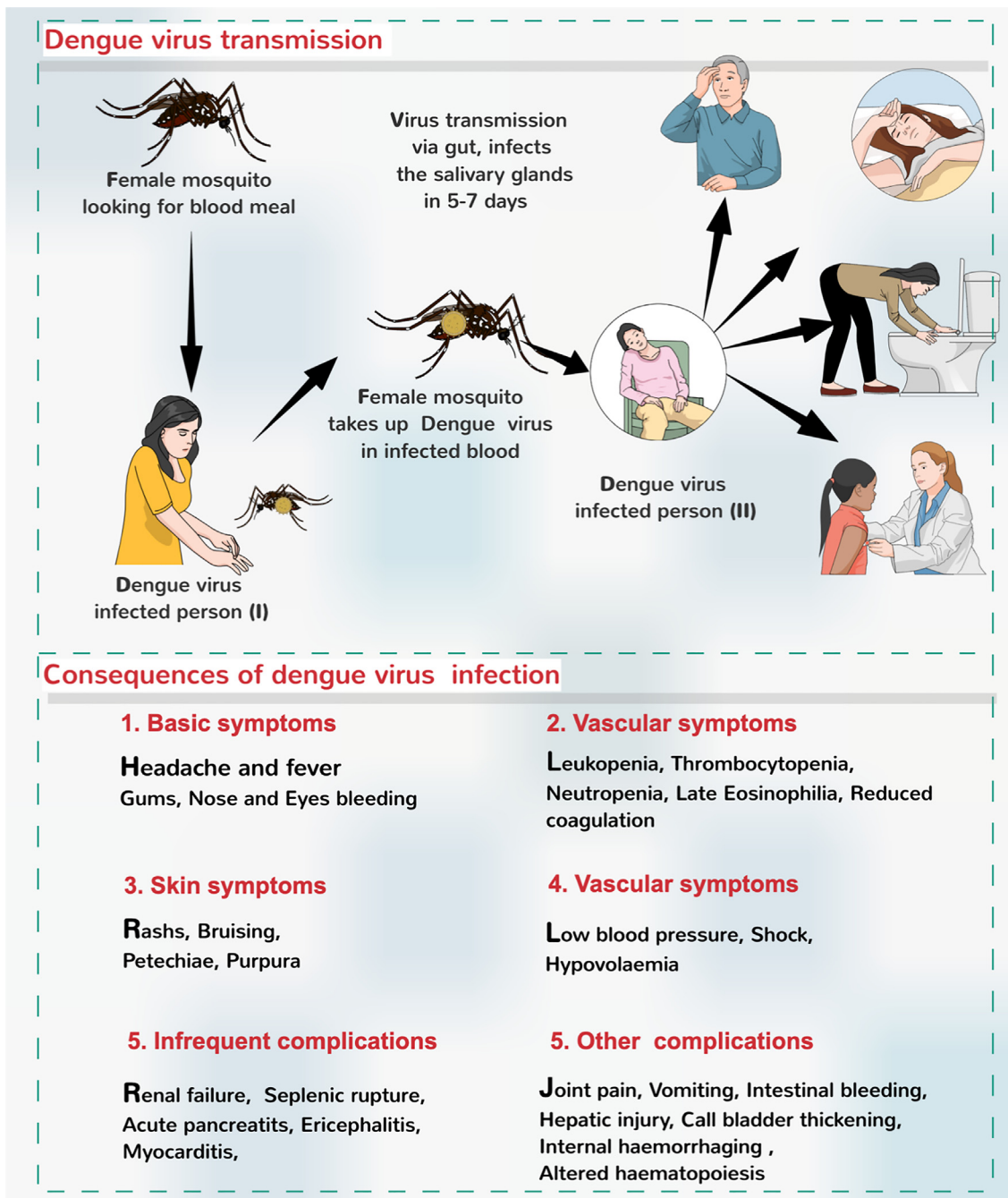


Fig. 1. Dengue virus infection and associated consequences. (Modified from Rather et al., 2017).

The virus particle releases the viral genome (Pagni and Fernandez-Sesma, 2012). The viral RNA is transformed into Polypeptide, which is divided into 10 Proteins. Afterward, the viral genome is divided into vesicle packets (VPs), which are probably the site of viral RNA replication (Welsch et al., 2009; Junjhon et al., 2014). The surface of the endoplasmic reticulum is where virus assembly occurs. The viral particles that are yet to mature are conveyed via the trans-Golgi network. In the process, they become mature and also infectious. Then the viruses are let loose, ready to damage other cells. To facilitate viral replication, the nonstructural protein 1 (NS1) of the dengue virus has to react with another viral protein known as NS4A-2K-4B (Salazar et al., 2007).

6. Detection of dengue virus

An accurate and efficient diagnosis of dengue is crucial to carry out clinical care (Dengue, 2009). To perform the diagnosis of the virus in the laboratory, either the dengue virus, viral nucleic acid, antibodies or antigens are detected, or a combination of these techniques is used. The virus can be seen in plasma, serum, blood cells in circulation, and other tissues. At the onset of the disease, the detection of either antigen, nucleic acid, or virus isolation can serve as the diagnosis of the infection. When the acute stage of the infection has ended, serology can then be used for diagnosis. The response of antibodies to infection differs depending on the

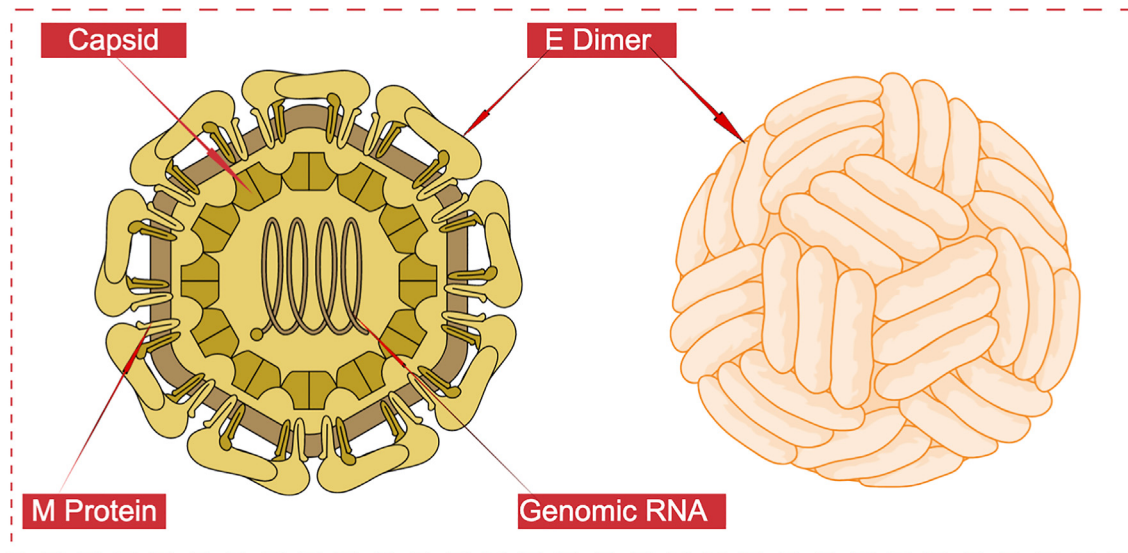


Fig. 2. Structural components of Dengue virus.

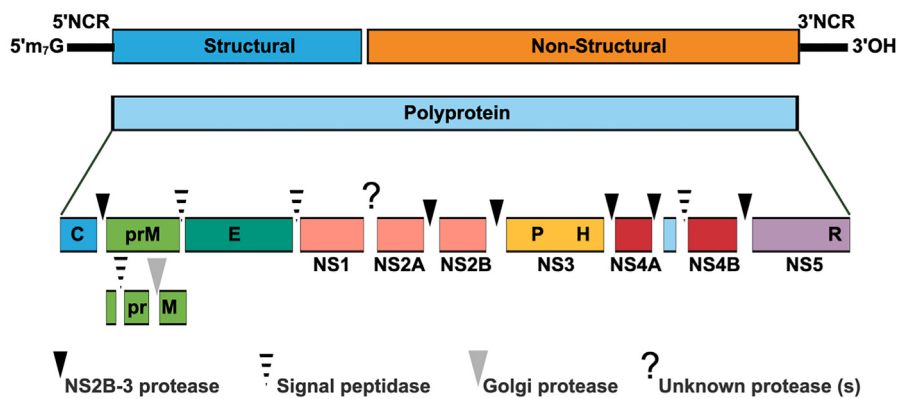


Fig. 3. Genome organization and polyprotein processing of members of the genus Flavivirus. Boxes below the genome indicate viral proteins generated by proteolytic processing. NCR, non-coding region.

immunity status of the hosts (Vorndam et al., 1997). Various laboratory diagnostic techniques have been put in place to help control the disease and manage patients.

6.1. Virologic diagnosis

For virus isolation, blood is usually taken between five and six days once symptoms are noticed in the acute phase. The collected blood sample can equally be used to detect viral RNA and NS1 antigen by RT-PCR. In addition, the isolated viruses can be distinguished through indirect immunofluorescence utilizing monoclonal antibodies to fight all five serotypes (Henchal et al., 1982).

6.2. Molecular diagnostics by RT-PCR

Specific primers are used in this method for DENV found in gene prM and gene C. A sequence common to all DENV five variants, which allow for the amplification of genomes, flank this segment. A unique primer is then used to identify each variant in a semi-nested PCR during the second amplification process. To view the cDNA, use 1% agarose gel electrophoresis, after which it is then dig-

italized (Lanciotti et al., 1992). Real-time RT-PCR can process many samples at a time and can be used both quantitatively and qualitatively.

Using the regular RT-PCR to diagnose early for Dengue virus, cases have shown that are very valuable. One of RT-PCR benefits is that its sensitivity in both primary and secondary cases is insignificant (Cordeiro et al., 2007).

6.3. Detection of NS1 antigen

Highly present in all five variants of DENV is the hexameric type of NS1 protein (Young et al., 2000). In the early diagnosis of the disease, NS1 antigen is utilized as a marker. ELISA can detect NS1 very fast and even gives the same precision as RT-PCR. However, ELISA cannot distinguish the different virus variants. The infection can affect the sensitivity of the test. NS1 test produces more accuracy when the disease has become very acute (Hang et al., 2009).

6.4. Serological diagnosis

Excluding urine, saliva, blood on filter paper, and serum can be utilized to detect IgM if within five or more days samples were

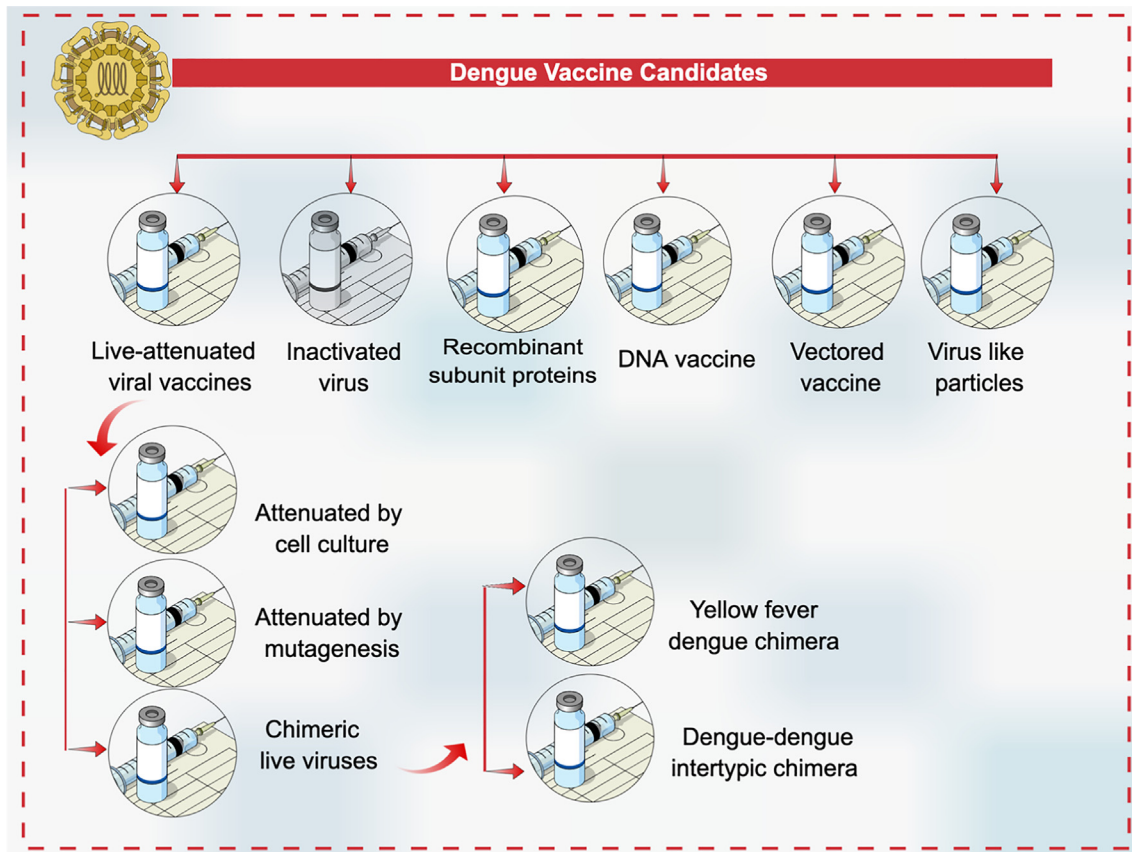


Fig. 4. Classification of dengue vaccine candidates.

taken once the fever has begun. Serum specimens may be tested at either single dilution or several dilutions. The majority of the antigens used for this test are obtained from the dengue virus envelope protein (Hunsperger et al., 2009).

7. Infection

During the viraemic and acute stage of the infection, a female mosquito can be infected when it feeds on an individual. The virus infects the mosquitoes' midgut cells and other tissues before it spreads to the salivary glands. Several people can become infected through an infected mosquito, and symptoms begin to manifest between four and seven days, after which an infected individual can also infect a new mosquito. Mosquitoes can become infected with the virus through both asymptomatic and symptomatic people (Gubler, 2014). Throughout the entire lifetime of the mosquito, it will continue to spread the virus to people as it feeds on them (Siler et al., 1926; Gubler, 2014).

8. Vaccines

Progress has been continuously made toward developing a functional and safe dengue vaccine that could help reduce the number of dengue cases globally. The first Dengue vaccine was licensed many years later. The vaccine was named Dengvaxia®; it is a dengue/live-attenuated chimeric yellow fever vaccine (Zellweger et al., 2013; Hadinegoro et al., 2015). The vaccine was produced by Sanofi Pasteur and is used in several nations like Thailand, Mexico, Brazil, Costa Rica, and El Salvador (Aguar et al., 2016). TV003/TV005 and DENVax are two dengue vaccines currently being used in efficacy trials in Latin America and Asia.

Besides, other vaccines are now being utilized in early preclinical and clinical studies. Fig. 4 below shows the classification of dengue vaccine development.

8.1. Live-attenuated virus

The only licensed dengue vaccine is Dengvaxia®, and its use has been authorized in a few countries (Guy et al., 2015). Efficacy varied in Phase 3 trials in both the Americas and Asia depending on age, serotype, and dengue serostatus at baseline (ClinicalTrials.gov. in National Library of Medicine US). The method that is now used involves attenuation by serially passing it through cell lines. This method was started by both Mahidol University (Thailand, Bangkok) and the Walter Reed Army Institute of Research (WRAIR), where they target mutagenesis and the construction of chimeric vaccine viruses (Bhamarapavati, et al., 1978; Bhamarapavati and Sutee, 2000). The candidates for the Mahidol vaccine have not achieved a balanced immune response to the four components. However, systemic symptoms were observed in the recipients of the tetravalent vaccine (Kanesa-Thanan et al., 2001; Kitchener et al., 2006).

8.2. Chimeric dengue vaccine

The vaccine was designed using two approaches: the chimeric vaccine is produced by combining an attenuated flavivirus with a strain of an attenuated DENV. Sanofi Pasteur is the manufacturer of this vaccine, which is licensed as "Dengvaxia" (Osorio et al., 2014). The phase I clinical trial of high and low doses of DENVax in healthy recipients shows that the vaccine recipients were immunogenic and safe (Martinez et al., 2015).

8.3. Purified inactivated virus

This inactivated virus is safer. At the moment, only a few quantities of purified formalin-inactivated virus (PIV) are undergoing clinical trials. So far, the results have shown that the vaccine, which WRAIR manufactures, was safe and produced an immune response in Rhesus macaques and mice after measuring the antibody responses by ELISA and carrying out neutralization tests in a few volunteers (Kanesa-Thanan et al., 2001; Kitchener et al., 2006; Guy et al., 2015).

8.4. Recombinant subunit vaccine

Insect and yeast expression systems are utilized to express Recombinant E proteins of dengue and are assessed for vaccine efficacy in monkeys and mice. Twenty percent of the E protein is deleted at the C-terminal. This deletion paves the way for purification and extracellular secretion, allowing it to retain its antigenicity. Also known as r80E, the recombinant 80% E proteins of the four DENV variants are now being manufactured by Hawaii Biotech Inc., HI, USA, and Merck and Co., NJ, USA (Kitchener et al., 2006).

8.5. Dengue DNA vaccine

A plasmid vector in the vaccine contains the genes for encoding an antigen. The plasmid codes the antigen associated with the MHC class I molecules once it enters the cell. It is also displayed on the surface of the cell, resulting in a protective immune response. The NMRC produced the DENV-1 DNA vaccine cloning the E and prM genes of DENV-1 into a plasmid vector (Putnak et al., 1996). The number of responders and the antibody titers were not adequate even though the vaccine was received well (Putnak et al., 1996; Putnak et al., 2005).

8.6. Replication-defective virus vectored vaccines

This vector transmits antigenic genes that can induce neutralizing antibody reaction as the virus itself. This virus can be transmitted by adenovirus vectors (Osorio et al., 2014). One example of the virus-vectored dengue vaccine is the cAdVax. This vaccine contains prM and E proteins obtained from two dengue variants, DENV-1 and DENV-3, in one construct in another construct (Raviprakash et al., 2008). When the two constructs are mixed, it has proven to offer more protection against all variants of DENV (Raviprakash et al., 2008).

8.7. Virus-like particle (VLP) vaccines

In heterologous hosts, the DENVs E and prM proteins could co-express, and they can also co-assemble into VLPs (Clements et al., 2010). The yeast system could be more appropriate for using VLPs for vaccine production because it could produce higher yields and glycosylated antigens (Danko et al., 2011).

9. Conclusion

According to several findings, DENV has killed many people worldwide, and most of the increasing cases of Dengue fever have been linked to urbanization. There are no known origins of the virus, which can be transmitted between people. Several methods have also been proposed for detecting the virus. In addition, different vaccines are being licensed and produced to tackle the virus. However, the most pragmatic advice is to maintain and strengthen existing dengue control practices, particularly during the current pandemic of Covid-19 and associated lockdowns. Of note, public

health organizations and community-based associations in tropical and subtropical countries should address COVID-19 and dengue.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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