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The dengue virus is currently one of the most widespread arboviruses infecting human populations, with serious negative effects on South America and South-East Asia economies and societies. Inadequate funding, wrong policies, a lack of political will, expanding mosquito vectors, and increasing urbanization and globalization are some of the factors contributing to the failure of initiatives to address this serious public health issue. Recent data estimates that there were 96 million apparent dengue illnesses globally in 2010. This figure, which is far higher than the WHO projection, shows that the disease is spreading quickly, creating a growing threat to the economy and a significant challenge for doctors and healthcare services around the world, especially in the impacted areas. In the past 17 years, dengue/dengue hemorrhagic fever has emerged as one of the most significant resurgent tropical diseases due to the geographical expansion of both the viruses and the mosquitoes that transmit them, an increase in the frequency of epidemics, the emergence of hyperendemicity (the cocirculation of multiple virus serotypes), and the spread of dengue hemorrhagic fever to new regions.

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Challenge of the 21st century the dengue virus Infections

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Abstract:

The dengue virus is currently one of the most widespread arboviruses infecting human populations, with serious negative effects on South America and South-East Asia economies and societies. Inadequate funding, wrong policies, a lack of political will, expanding mosquito vectors, and increasing urbanization and globalization are some of the factors contributing to the failure of initiatives to address this serious public health issue. Recent data estimates that there were 96 million apparent dengue illnesses globally in 2010. This figure, which is far higher than the WHO projection, shows that the disease is spreading quickly, creating a growing threat to the economy and a significant challenge for doctors and healthcare services around the world, especially in the impacted areas. In the past 17 years, dengue/dengue hemorrhagic fever has emerged as one of the most significant resurgent tropical diseases due to the geographical expansion of both the viruses and the mosquitoes that transmit them, an increase in the frequency of epidemics, the emergence of hyperendemicity (the cocirculation of multiple virus serotypes), and the spread of dengue hemorrhagic fever to new regions. The four serotypes of the dengue virus have just recently begun to establish endemic transmission in humans, with the four serotypes having originated around 1000 years ago. The level of genetic and phenotypic diversity observed in the sylvatic (primate) transmission cycle, however, as well as its genesis, remains unknown. It appears likely that stochastic processes also play a significant role in shaping viral genetic diversity, with lineage extinction being a frequent occurrence. There is some evidence that viral strains differ in important phenotypic features such as virulence and positive selection at immunologically important sites. A more complete understanding of the evolution and epidemiology of the

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dengue virus, particularly concerning the etiology of severe disease, will require large-scale prospective studies and the comparative analysis of complete genome sequences. One of the most significant new arthropod-borne pathogens is the dengue virus (DENV 1-4). The world's (sub) tropical regions are home to all four DENV serotypes, which yearly infect 50–100 million people. While most DENV infections are asymptomatic or only cause self-limited dengue fever, an increasing percentage of patients exhibit more severe symptoms, including dengue hemorrhagic fever and dengue shock syndrome. The potential for the future there isn't a vaccination available yet for DF/DHF. All four viral serotypes have live, attenuated vaccine candidates; however, it will probably be at least ten years before they are made accessible for widespread use. Mosquito control is the only hope for reversing the pandemic DF/DHF trend, but this doesn't look likely to happen anytime soon. If effective prevention programs are not put in place as soon as possible, new dengue virus strains and serotypes will probably continue to spread between regions where *Ae. aegypti* occurs in infected air travelers, leading to continued hyperendemicity, increased frequency of epidemic activity, and increased incidence of DHF. To achieve this, government representatives, public health practitioners, and the general public must adopt an epidemic prevention mindset as opposed to an emergency response mindset.

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1. Introduction

Dengue is among the most widespread arthropod-borne viral infection worldwide **[1, 2].** More than 100 nations in Africa, the Americas, the Eastern Mediterranean, South-East Asia, and the Western Pacific are affected by the disease. The dengue virus (DENV) has four different serotypes, and each of these serotypes can result in a variety of clinical symptoms, including dengue fever (DF), a self-limited febrile illness, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) **[2–4].** Protective immunity against one serotype is given by infection with that serotype but not against other serotypes. According to several retrospective and prospective investigations, subsequent infection with a heterologous serotype increases the likelihood of developing DHF/DSS **[4].** Additionally, infants delivered to moms who are immune to dengue are more likely to experience an initial dengue infection that is more severe **[5, 6].** This shows that antibodies are crucial in regulating how an infection develops. It is thought that antibodies drive virus particles directly to Fc-receptor-bearing cells, such as monocytes, macrophages, and dendritic cells, which serve as the virus's natural targets and are receptive to DENV infection. As a result, these cells become more infected, resulting in large viral loads and widespread T-cell activation early in the infection process. High levels of cytokines and chemical mediators are consequently released, which could cause endothelial cell injury and ensuing plasma leakage. Other elements connected to illness pathogenesis include viral virulence, a person's age, and ethnicity, as well as certain epidemiological situations **[7].** Around the world, dengue fever is a leading cause of illness and death. The disease is brought on by the dengue virus, which is spread to people through the bites of *Aedes (Ae.) aegypti* and *Ae. albopictus* mosquitoes **[8].** Since the illness is endemic in almost 100 nations, the majority of

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which are in tropical and subtropical regions, it poses a threat to world health. The incidence rate and geographic distribution of dengue have dramatically expanded (almost 30-fold) over the past few decades. According to World Health Organization (WHO) data, dengue fever cases could reach 100 million per year **[9].** However, a newly released study by Bhatt *et al.* (2013) indicates that the impact of dengue is far more than the WHO estimate and that 390 million dengue virus infections may have occurred annually **[10].** The WHO has proposed a new dengue categorization system based on disease severity in response to changes in the epidemiology of the disease and an increase in incidence rates (with and without co-morbidities) **[11].** DENV is a member of the family *Flaviviridae* and the genus *Flavivirus.* There are four different but related serotypes of DENV: DENV-1, DENV-2, DENV-3, and DENV-4 **[12].** A wide range of clinical symptoms, from mild illness to serious and sometimes fatal disease, are brought on by DENV infection. The clinical manifestations of DENV infection in this situation can range from asymptomatic forms to mild febrile forms (dengue fever—DF) and severe forms, accompanied by hemorrhagic episodes and increased vascular permeability, which can cause shock and death (dengue hemorrhagic fever/Dengue Shock Syndrome—DHF/DSS) **[13].** In 2009, the World Health Organization (WHO) recommended a new classification for dengue patients based on a multicenter study called Dengue Control (DENCO). Dengue without warning signs (DwoWS), dengue with warning signs (DwWS), and severe dengue (SD) were the three classifications used by the WHO in 2009. Significant bleeding, plasma leakage, and/or organ dysfunction are characteristics of SD **[14].** In-depth studies have focused on aspects of the virus and host to comprehend the various clinical problems that infected patients present with. Various theories have been considered, including initial antigenic sin, cytokine

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storm, and antibody-dependent enhancement (ADE). Severe disease has also been linked to virulent strains and coagulation factor abnormalities. Despite this extensive research, an agreement regarding the precise immunopathogenic pathways implicated has not yet been reached, how,ever these are probably complex **[15].** Pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) are conserved microbial components that are recognized by pattern-recognition receptors (PRRs). The primary PRRs that identify viral PAMPs, such as nucleic acids obtained from viral genomes or produced during viral replication, are known as toll-like receptors (TLRs). TLRs serve key roles in both innate and adaptive immune responses to viral infection because their detection of an antigen causes the generation of cytokines and type I interferon (IFN) **[16].** Numerous immune cells and inflammatory mediators carry out the innate antiviral response to dengue virus (DENV). Viral clearance is effective if these factors are in balance, and as a result, infections will either be asymptomatic or only show minor clinical signs. However, an imbalance between these factors may exacerbate the immunological response and make the condition more severe.

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2. Dengue Virus (DENV)

DENV, a pathogenic arthropod-borne *flavivirus* (arbovirus), is a single-strand and positive-sense RNA molecule belonging to the family *Flaviviridae.* The family *Flaviviridae* contains viruses that infect people severely and are spread by arthropods. A single genus, *Flavivirus*, with numerous types, makes up the family **[17].** A third split of the family into three genera has recently been suggested. Arboviruses, including the dengue and yellow fever viruses, are members of the family *Flavivirus.* Animal pathology viruses are members of the genus Pestivirus, while the genus Hepacivirus is the proposed name for many hepatitis C virus variations **[17].** There are 47 different DENV strains known as of right now. The four closely related DENV serotypes (DENV -1 to -4) that have been found so far are all closely related, but they are only marginally antigenically distinct **[18],** and these can be further split into multiple genotypes based on their gene sequences **[19].** Because DENV chose different receptors based on cell types and viral strains, these serotypes generally evolved from a common ancestor and are all regarded as the causative agent of a roughly similar disease spectrum in humans **[20].** Completed viral particles are spherical, 11 kb long, and 40–50 nm wide. They comprise a single, positively stranded RNA molecule with a 5-methyl cap and a single open reading frame **[21].** Dengue virus and its four prevalent serotypes have been identified in **(Figure 1&2).**

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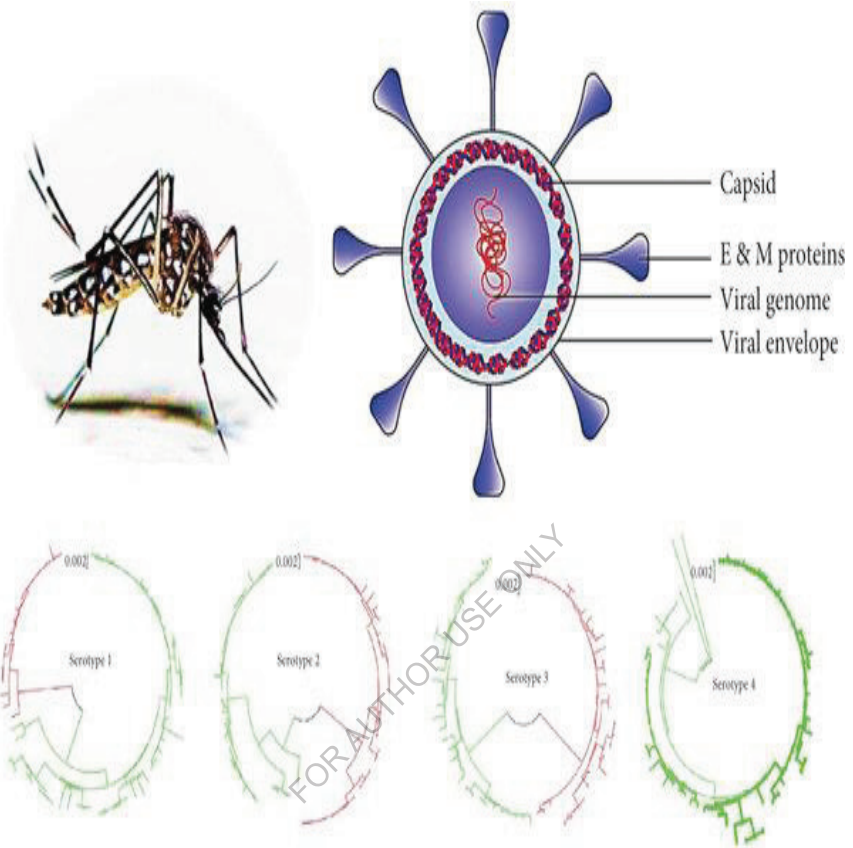

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Figure 1. Diagram with dengue virus and its four serotypes.

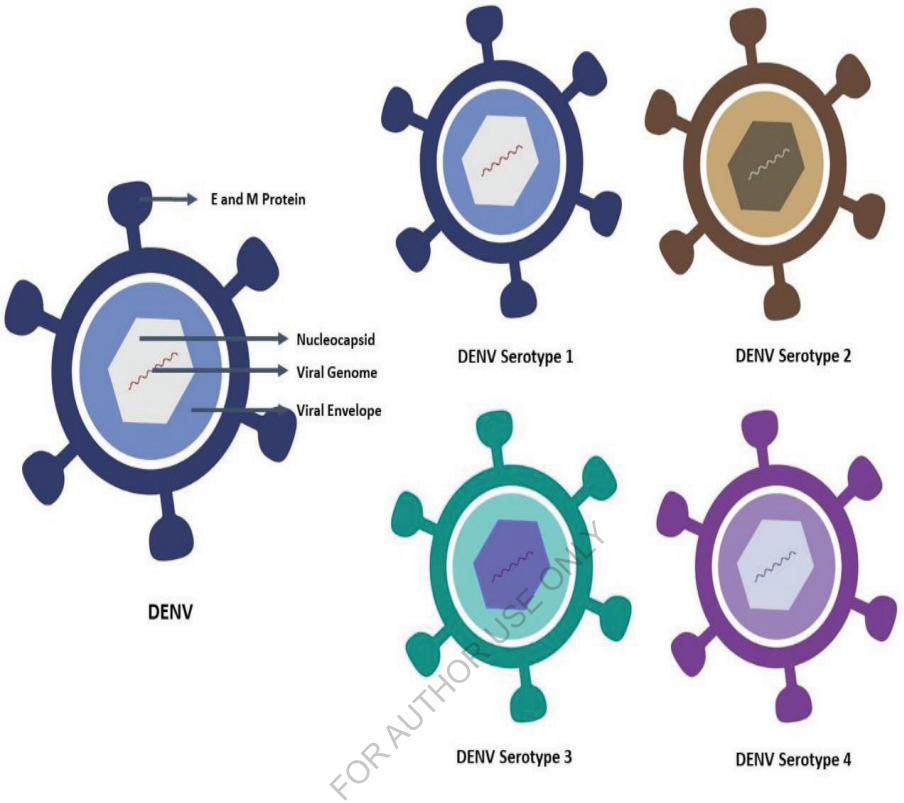

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Figure 2. The dengue virus, also known as DENV and DENV serotype, is generally spherical. DENV-1, DENV-2, DENV-3, and DENV-4 are four closely related viruses that cause dengue illnesses. After recovering from an infection, a person gains immunity to a particular dengue virus.

3. Epidemiology and Global Expansion

Although the first dengue epidemics were reported in Asia, Africa, and the Americas at roughly the same time, Benjamin Rush delivered the first case report and gave the disease's arthralgia and myalgia symptoms the name "Break Bone fever" in 1789 **[22]**. Individual serotypes were introduced serially, and serotype-specific epidemiological reports of dengue were only accessible in the 1960s. But across continents, the epidemiology of these illnesses, either separately or together, has been highly diverse **[23].** We want to highlight significant occurrences of these reports worldwide in the sections that follow: **South America:** The first confirmed case of concurrent dengue infection in the history of the world was reported from Puerto Rico in North America in 1982, despite reports over the years of numerous serotypes spreading in the area **[24].** Since then, there has been a persistent push to look at the serotype diversity in people exhibiting dengue symptoms. In one patient, serotypes DENV-1 and 4 were responsible for the illness **[24].** No concomitant illnesses have been documented, despite reports of dengue infection in several regions of the remainder of America **[25].** Data from Mexico from 1980 reveals that only mono infections through DENV-1 and 2 were reported to be widely circulating among the people in the south and southeast of the nation. The first instance of concurrent dengue illness from the Central American region was only ever documented in 1983 **[24]** in Brazil. Over 100,000 persons became ill with dengue during this significant dengue outbreak in 1987, which also marked South America's first concurrent dengue infection record. A trend can be observed when examining the serotypes of concurrent infections during the early years, showing that the introduction of a new serotype resulted in a stereotypic shift that led to a concrete infection in the years that followed **[24].** For instance, all DENV strains except for DENV-4 were present in Puerto Rico before the 1981–

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1982 outbreak, but DENV-4 and DENV-1, which had been in circulation since 1977 **[24],** were discovered in the first concurrently infected patient. Interestingly DENV-3 and DENV-2 serotypes were said to have circulated separately in the area during the outbreaks in 1963 and 1969, respectively**.** Following that, it has been established that all four serotypes are circulating in this area [39]. In Mexico, only DENV-1 or 2 were known to be circulating as mono infections until 1982; however, during an outbreak in 1983, DENV-4 was initially recorded as a concurrent infection together with DENV-1. This case followed a similar pattern **[24],** Following this, DENV-1 and DENV-4 infections occurred singly as well as concurrently, and they were the most frequently isolated serotypes in Mexico (47 and 37%, respectively). Once more, a serotypic change occurred in the area in 1995, with DENV-2 being the most common serotype, followed by DENV-4 and 1. The DENV-3 serotype also started to spread throughout the area at the same time. More than 35,000 cases of concurrency involving DENV-1, 3, and 4 were documented after 1996 due in major part to the emergence of DENV-3 **[26].** South America reported dengue mono-infections involving DENV-1 and 4 in 1981, similar to Central America. However, the introduction of DENV-2 in 1987 caused a significant outbreak in Brazil that affected over 100,000 people. **In Brazil,** numerous patients have had multiple concurrent illnesses found since 1990 **[27].** DENV-1 and DENV-3 were seen in northeastern Brazil from 1998 until 2003 **[27].** Following the reemergence of DENV-4 in the nation in 2010 and 2011, concomitant infections with DENV-1, 3, and 4 have been documented **[28].** All four DENV serotypes were found to be co-circulating in Guatemala and Mexico in 2021, while DENV-1, DENV-2, and DENV-4 serotypes were co-circulating in Colombia, French Guiana, Martinique, and Paraguay **[29]. Asia*:*** The four DENV serotypes have remained endemic

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in Asia, particularly South-East Asia, since the 1960s, and their geographic distribution patterns have rarely changed **[30].** The first DENV infection case to be virologically verified in India was reported in 1963–1964 **[31].** Dengue has remained an endemic disease in India ever since. Delhi became one of the most severely affected hyperendemic places in the country in 2003 when all DENV strains were discovered to be circulating there**.** Since then, all four serotypes have produced multiple outbreaks in India, and a significant number of concrete infections have been reported nationwide. The highest-ever rates of dengue infections in the area were recorded in Delhi in 2006, where 19% of patients had concurrent illnesses. DENV-1/3 strains accounted for the majority of cases. However, similar cases of DENV-1/4, DENV-2/3, and DENV-3/4 were also reported here by different serotype combinations. Before an outbreak in South India in 2017 where 56 (82%) samples out of 68 were found to be positive for DENV-4 and 18 (26%) were concurrently infected with at least one of the other stereotypes, DENV-4 was seen to be on the decline. The last report of the serotype was documented in 2009 **[31].** Because all DENV serotypes were circulating in Manipur during 2007**[32],** there have been reports of the first DENV outbreak in northeast India. The investigation also noted concurrent infections in the area, including DENV-1/ 3, DENV-2/ 3, and DENV-1/ 4. Interestingly, only cases of DENV-1 infection were noted, with DENV-2 and 4 not present separately **[29].** Arunachal Pradesh's Pasighat region experienced a significant dengue outbreak in 2015, with DENV-1 accounting for nearly 90% of the region's 66 confirmed cases of the disease. This outbreak was another significant one from northeast India. Only one incidence of simultaneous DENV-1 and 2 infection was infection shale five cases of DENV-2 infection were reported **[33].** All four dengue serotypes were discovered to be circulating during the 2017–2018 dengue outbreak in the

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Theni district of Tamil Nadu, and cases of multiple serotype illnesses were also discovered **[34].** Sri Lanka is another nation in South Asia with a high dengue prevalence. All four of the serotypes that are present on this little island nation in the Indian Ocean have an impact **[35].** Intriguingly, unlike in its neighbors, DENV-3 has remained the predominant serotype in Sri Lanka, where outbreaks of dengue virus infections have been common ever since **[36].** According to a study carried out in 2011–12 in three separate provinces, DENV-1 and 2 infections in concrete have been reported in as much as 10.3 percent of cases **[37].** Since 1994, Pakistan has mostly only had dengue epidemics in some regions of the Sindh and Punjab provinces **[38].** However, Pakistan experienced a significant outbreak in 2011 that resulted in 290 fatalities in the city of Lahore alone **[39].** The report provided dengue statistics broken down by district from two provinces. Punjab province reported the detection of all four serotypes, with DENV-2 (41.64%) and DENV-3 (41.05%) being the most prevalent. Additionally, the data shows that DENV-2 and DENV-3 mixed infections occurred in Punjab (3.81%) and Khyber Pakhtunkhwa (8.33%) in the same year. Another dengue outbreak with all four serotypes circulating among the populace occurred in Pakistan in 2013 **[40].** About dengue serotype co-circulation, Southeast Asia exhibits a trend resembling that of South Asia. Two patients in Thailand were the subjects of the first report of concurrent infection in 1990 **[37].** Several cases of concrete infections have since been made in Thailand, Malaysia, Taiwan, Vietnam, China, and Indonesia **[41].** It should be emphasized that DENV-2 is the most prevalent serotype overall, with DENV-1 and 3 occasionally coexisting and producing concurrent infection **[42].** Malaysia recorded the most concurrent infections with DENV-1 or 3 during an epidemic among the Southeast Asian nations in 2014. Since the disease was first identified in 1968,

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dengue has become a significant public health concern in Indonesia and has spread to all 34 of the nation's provinces **[43].** The examination of preserved DENV-infected samples from the 1970s, tested from various regions of the nation, revealed concurrent serotype-based infection. Despite reports of concurrent illnesses in Indonesia in 2011–12, their serotypes were not identified. More recently, Jakarta has been the site of concurrent DENV-2 and 3 infections **[44].** In adult *Ae. aegypti* mosquitoes collected from the homes of suspected dengue patients as well as confirmed dengue patients, the study found concurrent circulation of three serotypes DENV-1, 2, and 4 during the peak dengue season in 2015 in Tarlac City, Philippines.**[45].** Other regions of the world: In addition to Asia and the Americas, concurrent infections have also been documented in other regions of the world. A DENV-1 and DENV-3 pandemic was documented in 1989 on the South Pacific island of New Caledonia **[46].** Similarly, Yemen recorded its first dengue case in a tourist in 1984, and the sickness has since become a well-known ailment in the nation. The most recent outbreak in 2012–2013 found that more than 14% of the population under study tested positive for concurrent infection due to all four serotypes, either as dual infections or, in rare circumstances, triple infections **[47].** During the late 19th and early 20th century, dengue was documented in Africa. Twenty dengue outbreaks with scientific confirmation were recorded in 15 African nations between 1960 and 2010 **[48].** Concurrent infections from Somalia during an outbreak in 1993 were documented in a single investigation **[47].** Dengue infections reached their peak in 2019 across the globe, and for the first time, dengue cases were also identified in Afghanistan. Due to the COVID-19 pandemic, the effects of these diseases in the following year, 2020, have not been thoroughly recorded. Travelers are more likely to contract dengue illnesses and contract DENV from endemic nations.

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When they return to their home countries, these individuals transfer new serotypes into a non-endemic country if they are in the viremic phase **[49].** These cases are significant because they mark the beginning of the introduction of novel serotypes to the nation and, depending on other environmental and vector-related conditions, may lead to their long-term establishment. There have been reports of imported concurrent infections in Japan, Belgium, China, and the Netherlands **[49].** All of the travelers in each of these cases contracted the disease while visiting dengue hyperendemic nations.

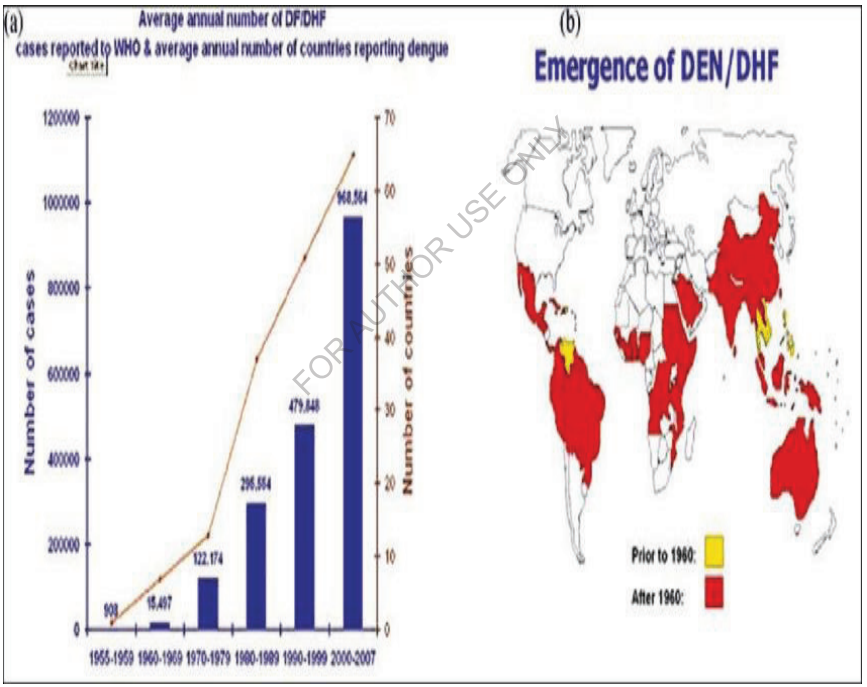

Figure 3: According to data from the WHO, dengue fever (DF) and dengue hemorrhagic fever (DHF) are quite common worldwide. (a) Since 1955, the average number of cases and the total number of afflicted countries. (b) A map of the world depicting dengue virus frequency from 1960 to 2006.

4. Transmission of Dengue Virus/Fever

After the initial midgut infection, DENV spreads systemically via the body cavity of Aedes vectors (often referred to as the hoemocel) and then disseminates in secondary tissues. The amount of time (7 to 14 days at 25–30°C) between the initial midgut infection and subsequent transmission of DENV by its vector, such as *Ae. aegypti*, is known as the extrinsic incubation period. Due to the viral genome's stability in the midgut of the vectors, DENV persists there **[50].** An essential mechanism known as the ubiquitin-proteasome plays a crucial role in the control of infectious DENV production in vectors **[50].** Finally, during the DENV transmission to the host, the salivary glands become infected and virions are released into the host's saliva **[51].** The four DENV serotypes can spread into the host through plasma and blood cells. Huerta et al. **[52]** have discovered a connection between DENV-II envelope glycoprotein domain III and human plasma proteins. After the dengue virus attaches to the target cell through contact between multiple cell surface receptors and the viral envelope (E) protein, the person becomes infected with DENV **[53].** All classified serotypes engage in interactions with mannose, heparan sulfate, nLc4Cer, and DC-SIGN/L-SIGN receptors in mammalian cells. Furthermore, it has been discovered that the DENV-2 serotype strongly binds to GRP78, CD14-associated protein, HSP70/HSP90, and two other unidentified receptor proteins. Serotypes DENV 1-3, on the other hand, bind to the laminin receptor, while serotypes DENV 2-4 attach to an unidentified protein receptor **[54].** DENV after receptor-mediated endocytosis, virion fuses with acidic lysosomes, and its genomic RNA is released into the cytoplasm and translated into a polyprotein of ~3400 amino acids (genome is about 11000 bases of positive-sense, a single-stranded RNA (ssRNA)) that are further cleaved by viral and host proteases into three

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structural (capsid: C, membrane: M, and envelope: E) and seven nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) proteins **[55].** The cytoplasm and nuclei are where the C protein, a key DENV structural component, is found **[56].** This protein's nuclear localization is assumed to be essential for its orderly replication **[56].** Between the nucleocapsid core and E/M outer shell, lipid (around 17% of the weight) forms the lipid bilayer of the virus **[57].** A membrane-bound replication complex formation aids in the incorporation of host components, viral proteins, and genomic RNA during the replication of DENV **[58].** The positive-strand (+) DENV genomic RNA in this instance serves as a template for the synthesis of complementary negative-strand (-) RNA, which is successively used for the manufacture of numerous (+) RNA genomes that are useful for translation and the regulation of replication cycles or packaging into virions **[59].** However, Raquin and Lambrechts found in a study **[60]** revealed DENV genomic RNA in the *Ae. aegypti* salivary glands, indicating active DENV replication in the vector before transmission **[60].** Although DENV itself encodes RNA-dependent RNA polymerases, other cellular components accelerate this virus' infection cycle **[61].** Alix apoptosis-linked gene-2-interacting protein X is one of the crucial proteins that DENV infections can change. As a result, limiting this phase may be one of the creative and effective methods to lessen DENV replication in the host **[62].** Instead, several genes have been discovered that when silenced by at least 60% in DENV's most significant vector, *Ae. aegypti*, limit infection. One of these, SeqID AAEL000379 (CRVP379) silencing, has been found to drastically lower DENV infection in the cells of the midgut tissues of *Ae. aegypti* **[63].** Only *Aedes* mosquitoes possess the gene Loqs2, which is necessary for RNA interference to function properly in this species of mosquito. Without Loqs2, however, the viruses can proliferate and subsequently

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infect their salivary glands **[64].** The localization and concentration of the viral replication complex in the perinuclear location by the DENV nonstructural protein 4A (NS4A) and host cellular vimentin have been shown to facilitate the efficient replication of viral RNA **[65].**

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5. Dengue Viruses and Vectors

Single-stranded RNA viruses of the genus *Flavivirus*, family *Flaviviridae*, make up DENVs. Seven non-structural protein genes (NS) and three structural protein genes, referred to as core, membrane, and envelope, respectively, make up the RNA genome **[66]** [DENV-1 through DENV-4]. Each of these serotypes has several genotypes with variable levels of pathogenicity. In endemic tropical and subtropical nations, as well as in both urban and semi-urban settings, all four DENV serotypes can circulate concurrently **[67].** Except some rural areas in Southeast Asia and West Africa, where non-human primates may also be impacted, humans are the only reservoir hosts. When a mosquito bites a person during the viremic phase, the mosquito is infected, and the virus multiplies in humans. The time between the time the mosquito consumes an infected blood meal and the time the mosquito becomes infectious is known as the extrinsic incubation period. Only when this temperature-dependent extrinsic incubation period has passed do mosquitoes have the virus in their salivary glands andcano infect and spread the virus to people when they are bitten? Once infected, mosquitoes carry the virus for the duration of their typical lifespan (30–45 days) and can pass it on to their offspring via the transovarial route**.** Dengue infection is known to be spread by Aedes mosquitoes, specifically *Aedes aegypti, Aedes albopictus, Aedes scutellaris, and Aedes polynesiensis*. The most significant vector, *Aedes aegypti,* originated in Africa and then expanded to tropical and subtropical regions as a result of global trade. In the South Pacific*, Aedes polynesiensis and Aedes scutellaris* are often encountered insects. Originally from Southeast Asian woods, *Aedes albopictus* has evolved to thrive in urban and semi-urban settings **[68].** Through the recent trafficking of used tires, *Aedes albopictus* has also recently migrated from Asia to other continents; mosquito eggs laid down stay

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viable for many months even in the absence of water collecting. The species *(Aedes Albopictus)* may survive far from habitations since it can adapt to low temperatures and feed on other animals and birds. As a result, *Aedes albopictus* is proving to be an emerging health concern for the spread of DENV in temperate regions, including certain Eastern European countries of the Mediterranean basin **[68].**

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6. The Dengue Virus Life Cycle

The *flaviviruses* are icosahedral, spherical, and encapsulated tiny (50 nm in diameter) viruses. The viral genome is made up of an 11 kb positive-sense RNA that codes for seven non-structural viral proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) that are necessary for viral replication and maturation in addition to the three structural proteins (capsid protein C, membrane protein M, and envelope protein E) that make up the virion **[69].** The mosquito vector injects DENV into the skin's epidermis, where it comes into contact with skin cells that are receptive to the virus, including keratinocytes, fibroblasts, mast cells, and immature dendritic cells (Langerhans cells) **[70].** These cells migrate to lymphoid organs, where they are more likely to spread to tissues and peripheral blood mononuclear cells (PBMCs). Hepatocytes, kupffer cells, cardiac fibers, tissue endothelial cells, monocytes, lymphocytes, and platelets were shown to contain DENV antigens in human postmortem tissue studies, indicating that these cells are susceptible to DENV infection **[71].** Dendritic cells (DCs), monocytes, and other mononuclear phagocyte lineages are thought to be the main targets of DENV infection **[72].** These cells were referred to as the primary target cells of DENV infection in vivo and serve as excellent in vitro models of DENV infection **[73].** The virus attaches to its primary target cells (monocytes, macrophages, and DCs) via cell receptors expressed in the plasma membrane to begin the replication cycle. Heparan sulfate**,** the mannose receptor (MR)**,** CD14 **[74],** heat shock protein 70 (Hsp70), Hsp90 **[75],** and dendritic cell-specific intercellular adhesion molecule-3 grabbing non-integrin (DC-SIGN/CD209) are only a few of the various mammalian cell receptors that have been proposed. One of the top potential receptors, essential for DENV infection, is DC-SIGN **[76].** Similar to other flaviviruses, the virus penetrates tolerant host

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cells by receptor-mediated endocytosis. The virus is subsequently endocytosed in a clathrin-mediated mechanism following binding to cell receptors. Endocytic vesicle acidification causes glycoprotein E to be reorganized, the viral envelope to fuse with the endosomal membrane, and the release of the viral ssRNA into the cytoplasm once it has entered the cell. After that, the rough endoplasmic reticulum (RER) membrane translates the viral ssRNA, While the viral ssRNA is reproduced by a viral replication complex, the host protein is first produced as a polyprotein that is later cleaved into distinct proteins (structural and non-structural). Then, in a process known as budding, the proteins E, PrM, and C as well as the freshly generated RNA participate in the assembly of the immature virus. The host enzyme furin finally mediates viral precursor membrane (prM) proteolysis in the trans-Golgi network, initiating E protein homodimerization rearrangement and producing fresh, mature virus particles that are released from the host cells **[77].**

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6.1 Virion structure

The *Flaviviridae* family includes the enveloped positive-strand RNA virus known as DENV **[78].** The capsid protein C, membrane protein M, and envelope protein E are the three structural proteins found in mature virions. To create the viral nucleocapsid, multiple copies of the C protein (11 kDa) encase the RNA genome **[79].** 180 copies of M and E are anchored in a lipid bilayer that is produced by the host cell and surrounds the nucleocapsid. The M protein is a tiny (about 8 kDa) proteolytic fragment of the prM protein, which is roughly 21 kDa in size. The 53 kDa E protein has three different structural domains **[80].** Between domain II, the homodimerization domain, and domain III which resembles an immunoglobulin, is where domain I am located structurally. The virus has a spherical nucleocapsid core and an icosahedral envelope architecture, according to structural studies of mature DENV virions **[81].** In fully

developed virions, E is arranged into 90 head-to-tail homodimers that are arranged in sets of three and roughly parallel to the viral surface, resembling a smooth "herringbone" pattern. As a result, DENV virions don't have genuine T = 3 symmetry, which means that each icosahedral asymmetric unit's three E monomers exist in three chemically unique contexts and could thus play different roles at various stages of infection **[81].**

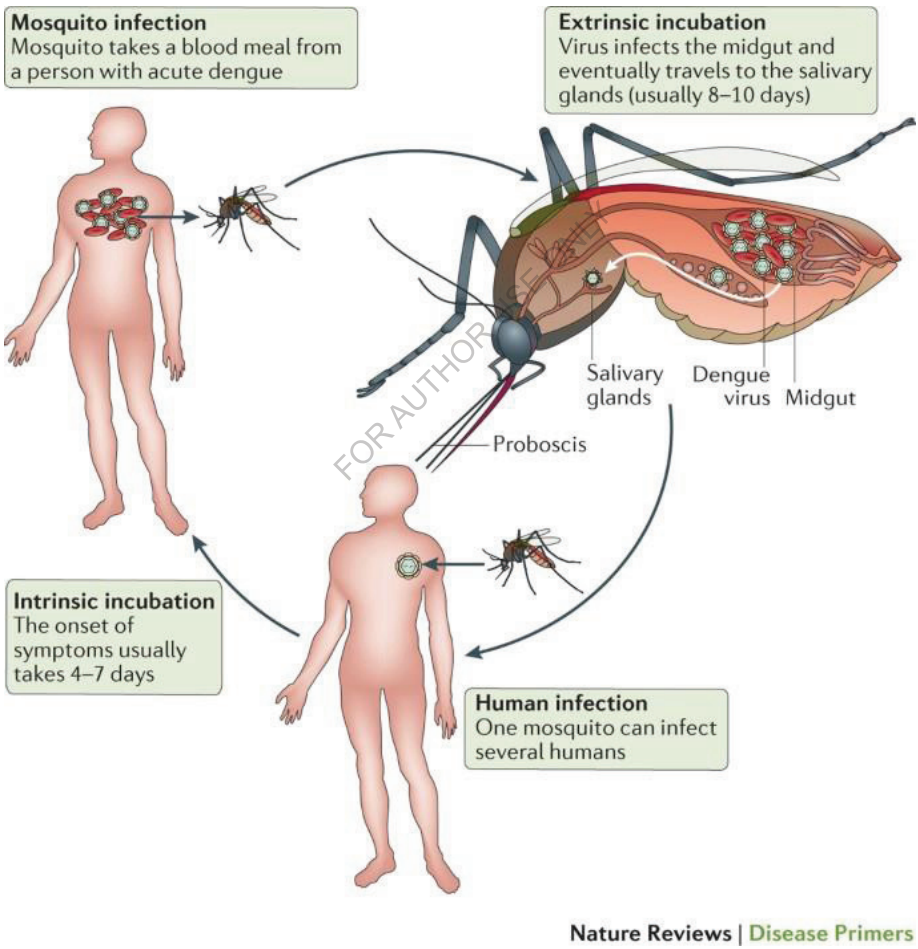

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Figure 4: The human and mosquito dengue virus life cycle. By feeding on a person who is experiencing the viraemic stage of an infection, an Aedes aegypti mosquito can get infected. Dengue viruses first infect midgut cells and other tissues in mosquitoes during the extrinsic phase of the cycle before spreading to the salivary glands. As it feeds or tries to feed on humans, an infected mosquito can then spread the dengue virus to a number of them. It typically takes 4 to 7 days after infection for symptoms to appear and for an individual to be able to pass the dengue virus to a fresh mosquito. Affected people and those who are asymptomatic can both spread the dengue virus to mosquitoes.

6.2 Replication and assembly of dengue virus particles

The translation of the viral proteins is shown schematically, as is the DENV genomic RNA. The RNA molecule is translated into a single polyprotein after nucleocapsid uncoating and virus-cell entrance **[82],** and the endoplasmic reticulum (ER) membrane is traversed back and forth by the polyprotein under the control of its signal- and stop-transfer sequences. Proteases produced by cells and viruses break down the polyprotein into seven nonstructural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) and three structural proteins (C, prM, and E). To ensure appropriate protein folding, the E protein is glycosylated at the amino acid residues Asn67 and Asn153 **[83].** There are other possible N-linked glycosylation sites in prM at locations 7, 31, and 52 as well as in NS1 at 130 and 207 **[84].** The NS proteins start viral genome replication after protein translation and individual protein folding **[82].** The C protein then bundles the freshly created RNA to create a nucleocapsid. The prM and E proteins combine to produce heterodimers that face the ER lumen. Then, the prM/E heterodimers group together to form trimers, and it is thought that these oligomeric contacts cause a curved surface lattice to be induced, which directs virion budding **[81].** Since no particular

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interactions between C and prM/E proteins have been discovered thus far **[85],** it is unclear how this is coordinated with the engulfment of the nucleocapsid. Interestingly, since the synthesis and release of capsid subviral particles have frequently been seen, the nucleocapsid's encapsulation during virus assembly is not essential **[86].** A single immature virion has 180 prM/E heterodimers that shoot out vertically from the viral surface as 60 trimeric spikes, according to structural studies of newly assembled immature virions **[85].** The secretory route allows the immature particles created in the ER to develop. The trans-Golgi network (TGN)'s slightly acidic pH (5.8–6.0) causes the prM/E heterodimers to dissociate, resulting in the creation of 90 dimers that lay flat on the particle's surface with prM capping the fusion peptide of the E protein. The cellular endoprotease furin can cleave prM as a result of the

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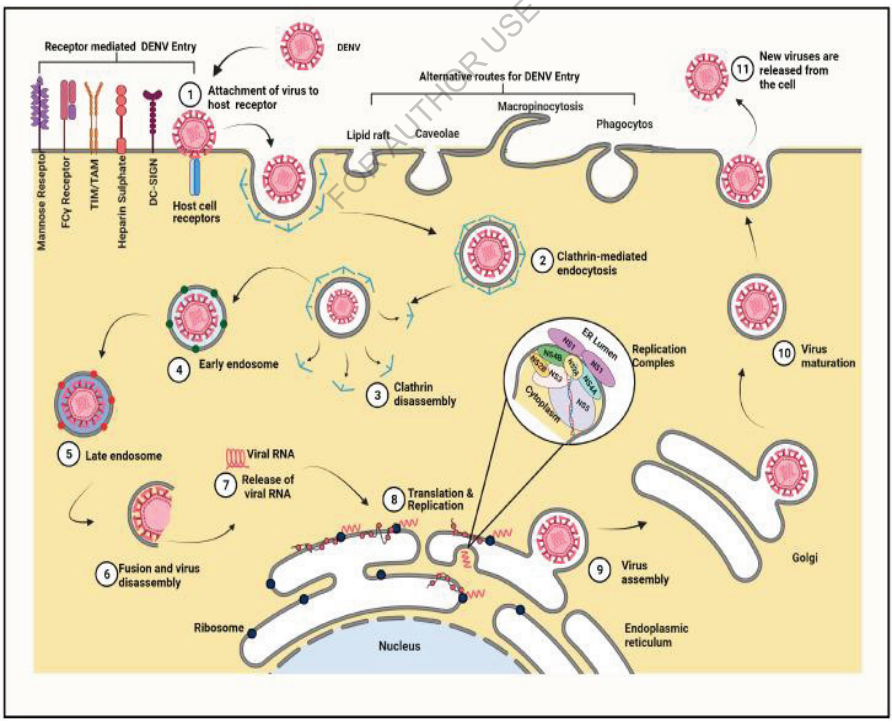

Figure 5. Dengue virus reproduction cycle: The virus binds to receptors on the host cell to start the replication cycle, which starts with entry into the host cell. (1) While additional methods of the entrance are also identified, the DENV bind can host a variety of receptors for entry via receptor-mediated endocytosis. The most extensively researched and documented kind of endocytosis is described here. (2) The virus is endocytosed into a perforated bubble coated in clathrin, which acts as the site of internalization. (3) In addition, the release of clathrin starts the somal processing. (4) The early endosome, which progresses toward endosome maturation, is identified by the presence of the Rab5 protein (green solid spheres). (5) Rab7 (red solid spheres) is a marker for the mature endosome. At a lower pH, or around 5.5, this acidic pH causes an unseveral formational changes. (6) The union of the viral envelope and host membrane is brought on by conformational changes. (7) The virus's breakdown causes the release of the encapsidated viral genome (RNA attached to the capsid) into the cytoplasm. (Since it is unclear how the viral genome is uncoated, it is not shown in the picture.) (8) In the ER, translation and replication are aided by the viral RNA, which acts as a template. The Replication complex is the unique structure for replication created by the convolution of the ER membrane in the presence of NS proteins. (9) An immature virus particle is formed in the ER from the assembled viral proteins and replicated DNA. (10) In the Trans-Golgi Network (TGN), this immature virus proceeds through furin-mediated maturation. The infection cycle is subsequently finished by the mature virus being exocytosed from the infected cell (11).

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6.3 Receptor Interaction and viral entry

The principal targets of DENV infection during a natural infection are cells of the mononuclear phagocyte lineage [monocytes (MO),

macrophages (M), and dendritic cells (DCs), including the skin-resident Langerhans cells] **[88].** DENV was discovered to initially infect the midgut of insects, from which it travels and replicates in numerous bodily parts and organs **[89].** Additionally, it has been demonstrated that DENV may infect a wide range of cell lines, including human (K562, U937, THP-1, HepG2, HUVEC, ECV304, Raji, HSB-2, Jurkat, LoVo, KU812), mosquito (C6/36), monkey (Vero, BS-C-1, CV-1, LLC-MK2), hamster (BHK), and murine M (Raw, P388D) **[90].** A large variety of DENV-permissive cells suggest that the virus must connect to a common cell-surface molecule or utilize several receptors to mediate infection. The discovery of numerous potential receptors and/or attachment factors during the past ten years implies that DENV can utilize a variety of molecules to enter cells.

7. Pathogenesis

As previously established, dengue can be caused by any of the four serotypes, DENV1 to DENV4. Dengue fever can result from an infection brought on by one of them. The infection confers lifetime immunity against infections caused by that subtype but not by other serotypes **[91].** When subsequently infected with viruses of various serotypes, a history of an infection with a different serotype increases the risk of getting dengue hemorrhagic fever. The "antibody-dependent enhancement" (ADE) is a perplexing and counterintuitive phenomenon **[92].** In the aftermath of the SARS-CoV-2 pandemic, a distinct kind of issue may be anticipated to arise about ADE **[93].** The characteristic of severe dengue, which manifests as shock and encephalopathy, is endothelial dysfunction leading to vascular leakage and increased permeability **[94].** Although there could be catastrophic repercussions, frequent rapid and complete reversals show that this is more likely caused by inflammatory mediators than by endothelial infection. Numerous elements could be

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in action **[94].** The most likely options are cytokines like tumor necrosis factor-a (TNF-a), which are frequently raised during the crucial stage of dengue. The endothelium glycocalyx can also be damaged by dengue NS1, a soluble non-structural viral protein, which can lead to vascular leakage. This theory might be refuted by some discrepancy between the timing of NS1 antigenemia and the appearance of vascular leakage symptoms. Additionally, during the acute stage of the illness, levels of several inflammatory lipid mediators, including platelet-activating factor (PAF) and leukotrienes, are elevated.

8. Neuro-pathogenesis

It is yet unclear how DENV infection affects the nervous system. Likely, both virus and host variables contribute significantly to the development of dengue-related neurological disorders. Direct CNS invasion by the virus, immunological responses, and metabolic changes may all be at play. Even though DENV has historically been thought to be non-neurotropic, neurological involvement, the presence of viral particles in the CSF, and BBB degradation all seem to point to direct viral neurotropism **[95].** Recent research suggests that dengue involves neuroinflammation **[95].** The secreted glycoprotein known as the non-structural 1 antigen (NS1Ag) serves as a cofactor for viral RNA replication and stimulates the release of cytokines. As shown by their early activation, natural killer cells actively contribute to the development of neurological symptoms. These cells then activate T helper (Th) cells. Interferon-gamma, interleukin (IL) 12, IL-4, and transforming growth factor-beta are a few examples of pro-inflammatory cytokines that are released as a result of these Th cells' division, transformation, and differentiation into Th17 and Th9 cells. These cytokines exacerbate the blood-brain barrier (BBB) damage, which makes it easier for other immune mediators to enter the brain and cause neuroinflammation **[91].**

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Before now, the pathogenesis-based classification of the neurological consequences of DENV infection into three groups was used **[96]** those brought on by a change in metabolism, such as encephalopathy; **[97]** those brought on by a viral invasion, such as encephalitis, meningitis, myositis, and myelitis; and **[98]** those brought on by autoimmune reactions, such as ADEM, optic neuritis, myelitis, and GBS. Solbrig and Perng documented three categories of neurological involvement more recently: CNS and ocular involvement, PNS syndromes, convalescent stage involvement, and post-dengue immune-mediated syndromes **[99].**

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9. Immune Response to DENV

9.1 Innate and Adaptive Immune Responses to Dengue Virus (DENV)

The immune system is made up of specialized cellular and molecular structures with specific roles in the body's defense against foreign invaders. It is essential to the upkeep of body homeostasis. **[100].** Innate and adaptive host defenses serve as the immune system's two main lines of protection against pathogenic microorganisms **[101].** Controlling DENV infection requires both innate and adaptive immune reactions. DENV enters susceptible hosts' skin while a mosquito feeds on their blood **[102].** The first cells to contract the infection are the skin's tissue-resident macrophages or Langerhans cells. In the early phases of DENV infection, type I IFN production is triggered by the expression of PRRs that are capable of recognizing viral PAMPs. Additionally, in response to infection, additional inflammatory mediators are generated, creating an inflammatory microenvironment. Acute-phase proteins, chemokines, and cytokines work together to directly destroy pathogens and communicate with effector cells **[102].**

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9.2 Immunopathogenesis versus Protection

The two basic phenomena related with dengue severity are "original antigenic sin" and "antibody-dependent facilitation of infection." There is general agreement that the "cytokine storm," or enhanced production of cytokines, chemokines, and other inflammatory mediators by various immune system cells, is responsible for the increases in endothelial permeability seen in severe dengue. Endothelial permeability, tissue harm, and multiple organ failure are all related to cytokine storm **[103].** Prior to inducing an adaptive immune response during a dengue infection, the host's innate immune response serves as a first line of defense in limiting virus proliferation. Through PRRs, the innate

immune system's cells identify pathogens. IFNs and other pro-inflammatory cytokines are produced when TLRs are activated to identify certain viral components. These cells' effector function mostly induces an antiviral response, although unbalanced responses may result in disease pathogenesis. DENV has the ability to avoid the innate immunological response of the host, particularly type I responses. Greater viral replication occurs in target cells as a result of the host's innate immune response being subverted. As a result, innate immune cells are drawn in and produce inflammatory mediators more vigorously, leading to endothelium damage and organ failure. The increased release of inflammatory mediators causes the vessels' permeability to increase, which might result in fluid leakage and severe dengue symptoms **[104].**

9.3 Pattern Recognition Receptors

Innate immune responses are triggered and an effective warning is given when PRRs identify PAMPs and/or DAMPs. The immune system can identify different kinds of infections thanks to PRRs. They only share tiny amounts that have been identified as "patterns" with one another, making this conceivable. Lipopolysaccharide (LPS), a component of Gram-negative bacteria's cell walls, is one example of a target PAMP for mammalian host cells. TLR4 recognizes LPS, which causes the inflammatory response to be triggered. PRRs are characterized as innate immune receptors, which sets them apart from particular lymphocyte receptors (adaptive immunity). These receptors are expressed constitutively on the cell surface, in the endosomes, and/or in the cytoplasm of all innate leukocytes **[105].**

9.4 Toll-like Receptor Signaling

TLRs were first identified in Drosophila, and they were later described as being crucial for immunity and embryonic development. 13 members of the TLR superfamily of receptors, numbered TLR1 through TLR13, have

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been identified in mammals. There are now 10 distinct TLRs known to exist in humans. According to their placements on the evolutionary tree, the TLR superfamily has also been divided into five subfamilies: subfamily TLR1, subfamily TLR3, subfamily TLR4, subfamily TLR5, and subfamily TLR7. They are transmembrane receptors that can be distinguished by whether they are found in the cytoplasm (homologous to the IL-1 receptor (IL-1R) and also known as the toll IL-1R (TIR) domain) or the extracellular domain (leucine-rich domain). The TIR domain interacts with adaptor proteins, transmits TLR signals, and starts signaling cascades that result in the synthesis of immune mediators. These procedures cause microbial resistance **[106].** Five distinct adapters can communicate with the TIR domain: MYD88, short for myeloid differentiation primary-response gene **[107],** MAL, or MyD88-adaptor-like protein **[108],** Interferon- (IFN)-inducing TIR-domain-containing adaptor protein (TRIF) **[109],** The proteins sterile - and armadillo-motif-containing protein (SARM) and TRIF-related adaptor molecule (TRAM) **[110]**. Every year, new studies are offered to discuss the role of TLRs in both the direct infection of cells and the progression of dengue infection from the standpoint of DENV infection. In response to DENV infection, some cell types stand out depending on the TLR subfamily. The TLR1 subfamily has been linked to coagulopathies, pro-inflammatory abnormalities, and mostly activates monocytes and dendritic cells **[111].** Increased vascular permeability and bleeding are two vascular illnesses linked to the TLR4 subfamily; the key routes causing those events may be platelet and endothelial cell activation **[111].** Through NK, MC, DC, and pDC cells, the TLR3 and TLR7 subfamily are linked to protective antiviral responses **[112]** (**Figure 6).**

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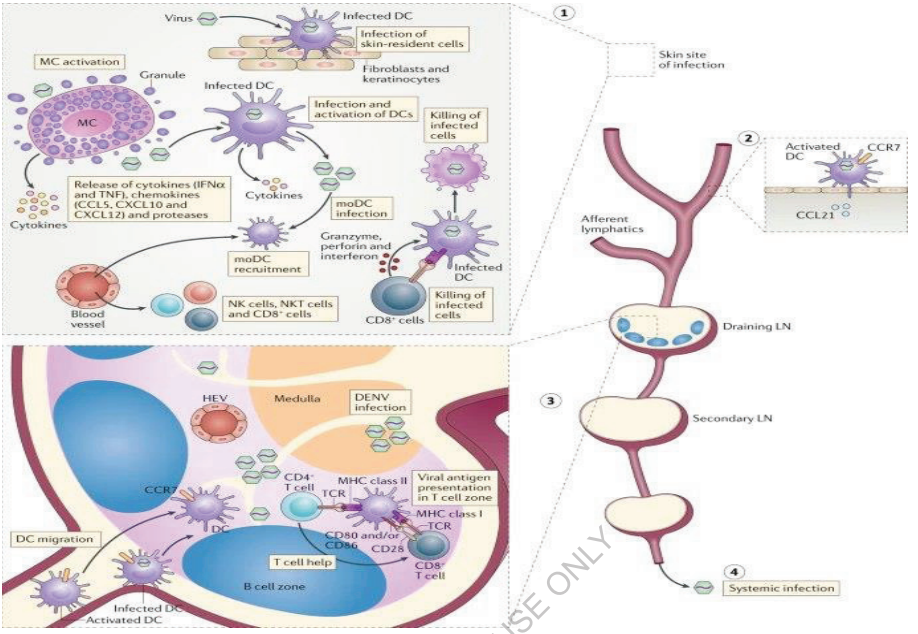

Figure 6: Beginning of skin and lymph nodes that drain the dengue virus immunity. (1) Immune sentinel cells, like mast cells (MCs), dendritic cells (DCs), and Langerhans cells (LCs), which are found in the epidermis, are the first immune cells to come into contact with the dengue virus (DENV) following infection by a mosquito bite. Within minutes of the DENV being detected, MCs degranulate and release proteases, chemokines, and cytokines like IFN and TNF. The chemokines aid in the recruitment of cytotoxic cells, including as CD8+ T cells (cytotoxic T lymphocytes), natural killer (NK) cells, and natural killer T (NKT) cells to the site of cutaneous infection. These cells have a TH1 cell phenotype. Infected cells include keratinocytes, macrophages and/or monocytes, DCs, and possibly other cell types. As a result, these infected cells release cytokines in addition to MCs. NK, NKT, and CD8+ T cells have the ability to destroy DENV-infected cells and aid in the clearance of the virus, whereas monocyte-derived DCs (moDCs), which are drawn to the

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site of infection, can act as targets of infection and permit the virus to multiply in the skin. (2) Skin-resident dermal DCs, macrophages, and recruited moDCs serve as both antigen-presenting cells and targets for DENV infection and replication. DCs move in a CCR7- and CCL21-dependent way to the draining lymph node (LN). (3) DENV-activated DCs deliver antigens to CD4+ and CD8+ T cells in T cell zones to trigger the adaptive immune response. They also upregulate the expression of co-stimulatory molecules such CD80 and CD86 in the LN. CD8+ T cells receive assistance from activated CD4+ T cells. Due to the infection of cells like DCs and macrophages, secondary LNs also contract an infection sequentially after the discharging LN. (4) LNs are DENV's amplification hubs, which it uses to spread to systemic infection. T cell receptors and high endothelial venules are both used.

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10. Challenge and Confrontation Issues

10.1 Pros and Cons of Evaluating Disease Parameters

Dengue is one of the most challenging vector-borne human diseases for medical professionals to correctly identify due to the dynamic nature of dengue clinical presentations. Dengue is an acute illness that is spread by the bite of mosquitoes carrying the contagious dengue virus. Patients frequently know how many days of fever they had to endure before visiting a clinic or hospital, even if they typically cannot remember when they were bitten. At that point, samples are collected, and variables are examined. As a result, the conclusions obtained from these reports frequently disagree, resulting in arguments over the clinical results **[113].**

10.2 Antibody Status in Acute Dengue Patients

It is common knowledge that antibodies are crucial in preventing infections caused by a variety of bacteria. As a result, one of the important indicators for determining the effectiveness of a vaccination is the humoral immune response. Not an exception is the dengue vaccination. Unfortunately, an agreement on the importance of a neutralizing antibody response in dengue virus infections has not been achieved due to the virus' complex viral biology and potential for interference in antibody formation between different serotypes **[114].** The discrepancy may be caused by the temporal nature of dengue; the interval between mosquito bite and development of symptoms as well as the clinical presentation, which differs across infected people, may determine the course of the illness and the analytic profiles **[115].** Therefore, it is unknown what type of immune response occurs during dengue viral re-infection. There are numerous unanswered topics, like why preexisting antibodies in acute dengue patients vanish following reinfection. Why is it so difficult to find memory B cells during re-infection? Does this occurrence have a connection to the dengue virus or a factor that occurs

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during the viral infection? However, the mystery of the low antibody levels seen in dengue patients who were re-infected has not been adequately addressed or researched. This crucial topic necessitates more research in order to increase the reliability of forecasting and assessing the efficacy of dengue vaccinations in vaccines. The answers to these queries would enhance the effectiveness and design of the dengue vaccine.

10.3 Antibody-Producing Cells

A plasma cell despite the widespread knowledge of the significance of antibodies in the management of viral infections, little is known about the plasma cells, also known as effector B cells, the cells that produce antibodies in dengue patients. Conflicting information is found in the literature regarding the etiology of dengue and how the dengue virus interacts with plasma cells or B cells in dengue patients. According to certain research **[116],** B cells or plasma cells are receptive to dengue virus infection in vitro. However, other reports do not note this characteristic **[117].** According to a few studies using samples from acute dengue patients, viral antigens are linked to B-lymphocytes and the virus may reproduce in these cells, which could account for the existence of atypical lymphocytes **[118].**

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10.4 Molecular Mimicry

A well-functioning immune response to invaders or foreign substances is the result of the immune system orchestrating a complex network of signaling events and balancing these components to protect the host. But occasionally, the immune system might malfunction and start attacking its own parts. Generally speaking, antibodies produced against these antigens can cause negative effects on the host when the components of an intrusion show molecular or structural similarities to, or are identical to, their host. Infection presents a persistent threat to the immune system.

It is commonly known that infectious diseases and autoimmune diseases have close ties. Numerous parts of the countless viruses that have been identified as infectious agents imitate their hosts **[119].** The dengue virus's components are no exception; it has been demonstrated that broadly cross-reactive antibodies to the viral antigens react with a range of host proteins **[119].**

10.5 Autoantibody-Associated Macrophage

Activation one of the most significant participants in innate defense inside the immune system's network are macrophages. They possess a dynamic collection of defenses that, when triggered, are effective against a wide range of infections. There are numerous subtypes of activated macrophages, and each one is capable of a different set of effector actions against pathogenic pathogens **[120].** A variety of activated macrophages are likely to be present in samples taken from infected people, given the intricacy of the immune system's functioning. It has not yet been thoroughly studied how these subpopulations of activated macrophages are distributed differently and proportionally during dengue virus infection. The illness brought on by the dengue virus has distinctive traits. Acute vascular permeability syndrome and problems in hemostasis are two characteristics of DHF/DSS. Immunopathogenesis is believed to be a significant influence in the development of this disease, despite the fact that the underlying mechanisms are still unclear **[121].** However, in patients who receive the proper fluid resuscitation, DHF/DSS manifests relatively quickly, typically over the course of hours, and disappears within 1 to 2 days. Usually, no obvious sequelae are discovered. As a result, a different scenario must be possible. We have noted that exposure to the dengue virus triggers a strong immunological response. Following infection with the dengue virus, the immune system exhibits abnormal behaviors such as excessive cytokine production and the creation of

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autoantibodies that target platelets and endothelial cells **[119].** Furthermore, we suggested that the dengue virus could result in severe hemophagocytic syndrome **[123].** IFN and TNF cytokines, dengue virus-immune complexes, and other stimuli may activate monocytes or macrophages in dengue patients. One of the activated macrophages' likely roles is to carry out the phagocytosis of platelets and endothelial cells coated in autoantibodies, which helps destroy these healthy cells. Anti-dengue antibodies develop a pathogenic nature and can exacerbate the severity of the disease. The targeted specificity and distinctive characteristics of thrombocytopenia and plasma leakage seen throughout the development of DHF/DSS are explained by the anti-NS1, anti-prM, and anti-E cross-reactive antibody action on platelets, endothelial cells, and coagulatory molecules. We think that this theory can account for the distinct features of clinical, pathologic, and epidemiological evidence that are particular to dengue virus-induced disease and explain the molecular phases of immunopathogenesis. The stimulation of macrophages by autoantibodies has contributed to numerous viral infections **[124].**

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10.6 Cooperative with Platelets

The most well-known traditional function of platelets is their significance as key hemostasis mediators, primarily working in conjunction with other inflammatory processes. According to the research's cumulative findings, platelets now play a wider range of unexpected activities, including a substantial role in directing and honing immune responses **[125].** Recently, it has been shown that immunologically linked chemicals on platelets functionally influence both innate and adaptive immunity **[126].** The ability of platelets to bind leukocytes, particularly monocytes, after activation is one of their essential characteristics **[127-129].** Prokaryotes and lower eukaryotes use PolyP as a structural element, an alternative energy source during stress responses, and in basic

metabolism **[130,131].** High levels of polyP can be seen in the granules of human platelets **[132,132].** In addition to its other hemostatic properties, synthetic polyP can specifically trigger apoptosis in plasma cells **[133].** The direct interaction of the dengue virus with platelets may be the cause of thrombocytopenia **[135],** which causes them to become activated and generate byproducts like polyP. The evidence suggests that the platelet-actions should be included in the revised model for the B cell germinal center response. We argue that polyP or other related molecules may be involved in dengue pathogenesis by changing the quality and/or quantity of antibodies produced from plasma cells in dengue patients, and possibly affecting the overall immunity.

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11. Current protocols for the diagnosis of dengue infections

Through virus isolation, genome and antigen detection, and serological tests, dengue can be diagnosed. Serology is being used in routine diagnosis the most. Of course, while analyzing a laboratory result, clinical, geographic, and epidemiological information related to the patient continue to be important factors.

12. Serological diagnosis

Initial antibody response to dengue infection in a non-immune host is characterized by a sluggish and low titer antibody response. The first immunoglobulin isotype to appear is IgM antibody. At the end of the first week after the commencement of the sickness, anti-dengue IgG shows up at a low titer and slowly rises. Contrarily, following a secondary infection (dengue infection in a prior dengue or flavivirus immunological host), antibody titers grow incredibly quickly and antibody responds broadly with numerous flaviviruses **[136].** Even in the acute phase, high levels of IgG are seen, and they sharply increase during the next two weeks. IgM response kinetics are more variable; it typically manifests late in the febrile phase of illness and is frequently preceded by IgG. Secondary infections have been linked to some anti-dengue IgM false negative results. By day five of the disease, 80% of individuals have detectable IgM antibodies, and by day's six to ten, 93-99% of cases have detectable IgM that may last for more than 90 days, according to Pan American Health Organization (PAHO**) [137]** standards. One of the most significant developments is the use of the enzyme-linked immunosorbent test (ELISA) to detect anti-dengue IgM, which has become a crucial tool for routine dengue diagnosis. In more detail, MAC-ELISA (IgM antibody capture ELISA) diagnosis relies on capturing dengue-specific IgM antibodies utilizing anti-human IgM antibodies that were previously bound on a solid phase **[138].** 10% false negative reactions and 1.7%

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false positive reactions have been recorded on average. Numerous forms, including dipstick, dot-ELISA, AuBioDOT IgM capture, capture ELISA, and capture ultramicro ELISA, have been developed [375,379-382]. Blood and serum on filter paper **[138],** if samples are collected within the right window of time (after five days of the commencement of the fever), saliva and, more recently, urine are suitable for IgM detection. There are a variety of commercial kits **[139]** for the detection of anti-dengue IgM and IgG, each with a different level of sensitivity and specificity **(see Table 1).**

Table 1. There are numerous commercial kits on the market that can be used to find anti-dengue antibodies.

Commercial kits	*Immunoglobulin isotype detection*	*Format*	*References*
PanBio Dengue Duo	IgM/IgG	ELISA	**140,142,144**
PanBio Dengue rapid test	IgM/IgG	Immunochromatographic test	**139,141**
MRL Diagnostic Dengue	IgM	ELISA	**141**
Blot IgM™, Diagnostic Biotechnology Ltd.	IgM	Immunoblot kit	**140**
Venture Technologies Dengue IgM and IgG Dot Blot kits	IgM/IgG	Immunoblot kit	**142**
Integrated	IgM	Dipstick	**143**

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Diagnostics			
Dengue Duo Rapid Strip Test, PanBio	IgM/IgG	Immunochromatographic test	**145**
UMELISA Dengue IgM	IgM	Ultramicro-ELISA	**178**

The presence of anti-dengue IgM antibodies in a probable dengue patient points to a recent infection. Due to the cross-reactivity of the antibody seen even during primary infection, IgM testing is not effective for determining the dengue serotype. Only 15% and 16% of DF and DHF cases, as well as 17% and 14% of main and secondary cases, respectively, showed a serotype-specific IgM response in a collection of serum samples from dengue patients from Nicaragua, Panama, and Costa Rica (Guzman MG, unpublished data). Additionally, some flaviviruses, including yellow fever, Japanese encephalitis, and St. Louis encephalitis, cross-react with dengue IgM antibodies **[145].** Using prototype viruses, human sera with known characteristics, and broadly group-reactive monoclonal antibody conjugates, some researchers have used a standardized combined MAC-ELISA to measure IgM antibodies to arboviruses of medical importance from the Togaviridae, Flaviviridae, and Bunyaviridae virus families. This technology has produced a useful method for quickly screening samples of human serum for different arboviruses **[146].** Clinically speaking, a fourfold increase (or decrease) in antibodies in paired samples by hemagglutination inhibition (HI), complement fixation (CF), plaque reduction neutralization technique (PRNT), or ELISA is considered to be diagnostic seroconversion **[137].** In most cases, a precise diagnosis cannot be made because flaviviruses share cross-reactive antigens. PRNT is utilized when a serologically

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specific diagnosis is necessary since it is the most precise serological test for identifying dengue antibodies **[148].** Several techniques have been devised to detect the existence and amount of dengue-neutralizing antibodies; Vero and BHK21 cell lines, carboxymethyl cellulose (CMC), and agarose are widely utilized, while some researchers use peroxidase-anti peroxidase (PAP) staining**.** Few laboratories now employ PRNT in their research. In order to use PRNT, Shu et al. standardized an NS1 serotype-specific indirect ELISA in 2002 in order to distinguish between primary and secondary dengue virus infections. They also achieved a high association between anti-NS1 serotype-specific IgG (found by ELISA) and PRNT results**.** Cardosa et al. (2002) showed that the IgG reaction against membrane protein was exclusive to flavivirus, in contrast. When sera from people infected with the dengue virus or the Japanese encephalitis virus were examined, no cross-reaction was seen. Membrane protein was suggested by these authors for seroepidemiological studies. However, because it takes a lot of time, ELISA has taken over as the method of choice for serological research. In order to categorize cases according to whether they are primary or secondary infections, ELISA for anti-dengue IgG detection is being utilized widely. Some procedures titer anti-dengue IgG using serum dilutions. In some, an IgM/IgG ratio more than 1.78 is regarded as a marker of initial infection, while one lower than 1.78 is regarded as a marker of secondary infection. Anti-dengue IgA detection as a sign of recent infection has recently been shown to be beneficial by certain researchers. Anti-dengue IgM and IgA antibodies were found in 178 samples from DF patients, according to Talarmin et al**.** Both the sensitivity and specificity numbers were 100%. From the sixth day after the commencement of the fever to the 25th day, IgA antibodies were found. IgM antibodies were typically seen at day 3.8 and IgA antibodies

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at day 4.6. Alternatively, Groen et al**.** A larger percentage of IgA detection was seen in acute serum samples from secondary cases (62%) compared to main instances (17%), indicating that the diagnostic utility of IgA serum detection using an immunofluorescence assay (IFA) is also suggested. Balmaseda et al.'s ELISA application to the detection of anti-dengue IgA in serum produced similar outcomes**.** However, compared to DF cases and non-dengue patients, sera from DHF/DSS patients had considerably greater levels of dengue virus-specific IgE, according to Koraka et al. It is suggested that measuring these antibodies can serve as a predictive indicator**.**

12.1 Virus detection

The short-lived dengue viremia often manifests two to three days prior to the onset of fever and lasts for four to five days after. As a result, the first four to five days of the illness are when samples must be collected for virus isolation. The preferred sample for routine diagnosis is serum, however dengue virus can also be found in leukocytes, plasma, and organs taken from an autopsy, including the liver, spleen, lymph nodes, lung, and thymus **[149].** Since the dengue virus is heat-labile, it is crucial to handle the samples carefully and get them to the lab as soon as possible. Specimens can be stored at 4 °C for short-term storage, while low temperatures (70 °C) are advised for extended storage. The most sensitive method for isolating the dengue virus involves mosquito inoculation, which can be done with both adult and larvae mosquitoes. Toxorhynchites mosquitoes are typically preferred due to their huge size and lack of hematophagy. Additionally helpful for virus isolation are adult male Aedes aegypti and Aedes albopictus mosquitoes **[150].** Inoculating mosquitoes with mosquito repellent to detect dengue is also helpful for ensuring vaccine quality. Jirakanjanakit et al. showed that there was no interfering between serotypes in infected mosquitoes by

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injecting Toxorhynchites splendens with a tetravalent live-attenuated dengue vaccination **[151].** Despite the better sensitivity of procedures involving mosquitoes, cell culture is preferred for regular diagnosis because to the technical skill and particular confinement needed for direct mosquito injection. The early accounts of its use made it abundantly evident that mosquito cell cultures were perfect for isolating dengue virus. Although other cell clones and lines have been investigated, the Aedes albopictus (C6/36) cell line has emerged as the preferred host for routine dengue virus isolation, though the Aedes pseudoscutellaris (AP61) cell line has also been effectively utilized **[152].** When Rodriguez et al. used a quick centrifugation technique to separate the dengue virus from C6/36 cell culture, they were able to get 16.6% more isolates than they could have with a traditional procedure. Even more crucially, this technique worked well for separating the virus from tissue samples collected from dengue fatality cases **[153].** These authors stated that 42.8% of virus isolations from these tissue samples were recovered. Vero cells, LLCMK2 cells, and other mammalian cell cultures have also been used, albeit with less success **[147].** When no other options are available, the oldest and least sensitive method of virus isolation involves injecting suckling mice's brains with the virus. Although many animals show signs or symptoms of encephalitis, many species show no evidence of sickness **[154].** The most significant contribution to diagnosing dengue is the use of mosquito cell lines for viral isolation. On mosquito head squash, infected cells, or mouse brain tissues, virus identification is typically achieved using immunofluorescence techniques with serotype-specific monoclonal anti-dengue antibodies. The detection of these viruses has been made easier by the availability of certain monoclonal antibodies at the American Type Culture Collection and World Health Organization Collaborating Centers. Typically, samples are evaluated by

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IFA using a polyclonal antibody first, and those that test positive are subsequently retested using the four serotype-specific monoclonal antibodies **[155].** Some researchers have suggested one or two rounds through a cell culture system to boost the viral concentration because some strains are difficult to identify due to low virus concentration **[156].** Recently, flow cytometry has been touted as a helpful technique for identifying dengue 1. Ten hours earlier than an IFA using anti-NS1 monoclonal antibodies, it enables the virus to be detected **[157].**

12.2 Antigen detection

Although dengue viral antigens have been found using IFA and radioimmunoassay (RIA), these assays' limited sensitivity has prevented their use in routine diagnostic procedures **[158].** Some delicate systems have recently been standardised in an ELISA format. A streptavidin-biotin amplified fluorogenic ELISA was used by Malergue and Chungue in 1995 to find and identify the dengue 3 antigen in serum. When compared to virus isolation, this ELISA demonstrated a sensitivity of 90% and specificity of 98% **[159].** Later, Kittigul et al. showed that the frequency of dengue antigen detection in peripheral blood mononuclear cells (PBMC) was higher than that in sera (53.8% vs. 18.9%). Additionally, these researchers used a biotin-streptavidin ELIS **[160].** Recently, NS1 antigen detection has received a lot of interest. Young and colleagues developed a capture NS1 ELISA and showed that individuals with secondary infections had high amounts of NS1 in their acute phase serum. They proposed that the detection of NS1 antigen would be useful for early diagnosis and as a sign of viremia **[161].** Alcon et al. also obtained comparable outcomes. Finally, Libraty *et al.* showed how well NS1 detection works as a DHF predictor. In DHF patients compared to DF patients, plasma NS1 levels were greater and linked with viremia levels **[162].** Recently, two ELISAs were used to generate a commercial

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kit for antigen detection (blue kit) and identification (red kit). According to the manufacturer (Globio Blue and Red Kit for Antigen Detection, Globio Corp., Beverly, MA, USA), the blue kit's sensitivity and specificity are 84% and 89%, and 91% and 93%, respectively. Immunohistochemical techniques (with horseradish peroxidase or alkaline phosphatase labeling) are helpful for detecting dengue antigen in formalin-fixed paraffin-embedded tissue samples, despite the fact that this method is not widely used for diagnosis in dengue-endemic areas **[163].**

12.3 Genome detection

Polymerase chain reaction, or PCR, has grown in importance in recent years as a technique for molecular epidemiology investigations, laboratory screening, including entomological surveillance, and dengue diagnosis **[164].** It has also shown promise as a research tool in the study of pathophysiology, antiviral medications, and vaccines. Reverse transcription, which creates cDNA from the target RNA, occurs before DNA amplification. Serum, plasma, infected cells, infected mosquito larvae, mosquito pools, fresh and paraffin-embedded tissues, and formalin-fixed tissue have all been found to contain dengue RNA **[165].** There are numerous PCR techniques available. Many of them use a pair of universal dengue or flavivirus oligonucleotide primers followed by a second amplification using serotype-specific oligonucleotides, or they apply a mixture of four serotype-specific oligonucleotide primer pairs in a single reaction tube. The chromosomal locations of the primers used in these techniques (E, NS1, E/NS1, prM/E, NS5, NS5/3′), as well as their specificity and sensitivity, also differ. Depending on the technique, less than 50 to 100 dengue virus particles can be detected **[166].** Lanciotti et al. created the protocol in the Americas**.** Has been used extensively. These researchers created consensus primers that amplify a

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511 bp product from the C/prM genes. Using type-specific primers, DNA products of various sizes are amplified in a second round of PCR, enabling the distinction of serotypes. Both new methods and adjustments to this procedure have been employed **[167].** When used properly, PCR is an extremely effective method for diagnosing dengue. Dengue can be found in samples that have been stored for a long time thanks to the use of PCR. The dengue 2 virus was found in serum and tissue autopsy samples from DHF/DSS cases that had been kept in storage for more than 15 years by Alvarez et al **[168]** and later by Sariol et al**.** The isolates could also be categorized using genomic sequencing. Alternately, PCR has been used for entomological surveillance by many writers. During a one-year observation period in Singapore, Chow et al. in 1998 and Kow et al. in 2001**[169]** found dengue viruses in *Aedes aegypti and Aedes albopictus* mosquito pools, enabling the deployment of vector control measures. In 1995 and 1996, infected *Aedes aegypti* were found as early as six weeks before a dengue outbreak was recognized. As a dengue outbreak early warning monitoring system, these authors suggest using this technique. Additionally, PCR enables the identification of concurrent infections caused by several serotypes in serum samples as well as in isolates from tissue cultures or mosquito inoculations **[170].** During the 1989 New Caledonia outbreak, Laille et al., 1991 were able to identify dual viremia by dengue 1 and dengue 3 in six DF patients **[170].** As well, Loroo-Pino et al. were able to demonstrate that 5.5% of the 292 samples they examined contained signs of concurrent dengue infection with two or more serotypes **[171].** The analysis of genetic strain diversity to pinpoint the start of epidemics and find virulence markers is one of the most significant applications of PCR. Using PCR in conjunction with nucleotide sequencing or restriction enzyme analysis, dengue serotypes may now be divided into genotypes **[171].** A technique for comparing

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dengue 2 genomes directly from patient plasma has been developed by Rico-Hesse and colleagues. They discovered several variations in the E protein gene's amino acids and nucleotides as well as in the untranslated region that might serve as the main predictors of DHF **[172].** Kuno et al. established the genetic link between viruses of the Flavivirus genus in other research. They argued that two virus branches—non-vector and vector-borne virus clusters—evolved from the presumed ancestor, and that tick- and mosquito-borne viruses arose from the latter cluster**.** Recent investigations claim that the dengue virus undergoes intra-serotype recombination **[174].** These findings' consequences are not clearly stated. Finally, new PCR techniques and methods have emerged that enable the quick detection and measurement of RNA. To quantify virus-specific DNA amplification in 1999, Laue et al. used a completely automated amplification process (based on the TaqMan approach). This procedure exhibits great specificity and sensitivity, obviates the chance of cross-contamination, and permits the measurement of viral load **[175].** More recently, Callahan et al. (2001) used a TaqMan RT/PCR test to distinguish between dengue serotypes and groups, reporting a sensitivity and specificity of 92.5% and 98.5%, respectively, when compared to viral isolation in C6/36 cells. Less than two hours were needed to get the results **[176].** Wu et al., 2001 used a set of universal primers and serotype-specific capture probes to type the four dengue serotypes using an isothermal nucleic acid sequence-based amplification (NASBA) test. Without employing a thermocycler, nucleic acid was amplified, and electro-chemiluminescence probe hybridization was used to identify the end result. When the assay was compared to viral isolation from the C6/36 cell line, it demonstrated a sensitivity of 98.5% and a specificity of 100%**.** The NASBA approach has recently been enhanced with a biosensor, enabling the fast detection of dengue virus RNA in about 15

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minutes**.** A fluorogenic RT-PCR technique was created as an alternate method to measure and identify dengue viruses using conserved and serotype-specific 3′-non-coding regions. When compared to virus isolation in cell culture, this system's sensitivity and specificity were 92.8% and 92.4%, respectively. It can detect 20–50 pfu/ml of serum**.**

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13. Dengue Vaccine Development

History of dengue vaccines for more than 60 years, efforts have been made to develop a dengue vaccine.

13.1 Era before WWII, 1917-1940

In order to examine specific characteristics of dengue illness, Cleland et al., published the initial literature on the modulation of dengue virus pathogenicity. They planned to culture the virus by a series of injections into human volunteers. They had successfully progressed through four generations of fabricated dengue cases, but the fifth passage yielded a possible positive case. This was the first proof that repeated passages in a host could result in an attenuated dengue virus strain. Blanc and Caminopetros were able to proceed because of this who sought to create a living virus that had been attenuated and might cause low-grade dengue with no symptoms and eventual immunity. By successively passing the virus to mosquitoes and/or using anti-serum and desiccation treatments, Simmons et al. attempted to create an attenuated or dead dengue vaccine**.** By extracting phenol from infected monkey liver and spleen, St. John and Holt attempted to create a lethal vaccination. By exposing infected mosquitoes to X-ray irradiation, Holt and Kintner hoped to create a lethal dengue vaccine **[179].** When World War II broke out, the dengue vaccine's development was halted.

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13.2 During and Post WWII Era, 1940-1960

In the years following World War II, the search for a dengue vaccine continued. Despite the fact that a filterable agent causes dengue**,** the first dengue virus was not discovered until 1940, during World War II, by Drs. Hotta and Sabin in Japan and the US, respectively**.** Several novel methods were created and developed during this time. This provided an excellent chance to research the dengue vaccine in much more detail. Additionally, several attempts were undertaken to reduce the aggressiveness of the

isolated dengue virus. These included investigations of the immunological response in mice utilizing dengue vaccines that were formalin-inactivated and attenuated and produced from nursing mice. Additionally, according to Sabin's findings, volunteers who received the live attenuated dengue vaccine developed immunity that could withstand a subsequent infection with the same dengue serotype for at least 18 months. The Caribbean hosted the first dengue vaccination field testing. But additional research showed that the live attenuated dengue vaccines made from mouse brain were not suitable for use in people. In order to research immune responses to the dengue virus, biological and functional assays were developed in large part thanks to the virus's isolation. The neutralization assay was one of these novel tools and methods, assays measuring the immune response in patients, the complement fixation test, serological measurements of immunity in mice and men, and others. Additionally, the results of the neutralization assays and complement testing revealed that there were numerous dengue serotypes, and it was possible that, when supplied concurrently, the immune response to one type interfered with the development of the others **[180].**

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13.3 Major Milestone Era1960-1980

This period was notable for the establishment of several dengue virus serotypes, which were given the names DENV1, DENV2, DENV3, and DENV4. When specimens started to become available in the late 1950s and early 1960s, researchers got their first insight into the pathogenicity of dengue hemorrhagic fever. Following World War II, there was a sharp spike in the prevalence of dengue fever (DF), which in turn caused an outbreak of dengue hemorrhagic fever (DHF). Another significant achievement from this time period was the creation of dengue vaccinations, which was made possible by improvements in policy and

guidelines**.** The creation of live attenuated vaccines for each of the four dengue virus serotypes was the top goal. Serial passages in PDK cells were used to create these attenuated vaccines. On the standards for attenuation markers, however, a consensus was reached. Following plaque purification of the virus from LLC-MK2 cells, tests were conducted to determine the virus' temperature sensitivity and the absence of CPE in LLC-MK2 cells, as well as to confirm the virus' lack of neurovirulence in suckling mice. If these criteria were met, intracerebral inoculation was used to assess the putative vaccine's neurovirulence in monkeys **[181].**

13.4 Golden Milestone Era, 1980-2000

This era brought about many golden milestones for dengue vaccine researchers. The numerous techniques and strategies employed to generate a variety of dengue vaccines have recently been reviewed**.** These potential vaccinations include chimeric, subunit, mutant, and DNA vaccines. Although dengue vaccines over- or under-attenuated produced with support from the U.S. Army didn't work well in humans**,** Through clinical testing for safety, reactogenicity, and immunogenicity, some of them advanced almost instantly (at the speed of light). These phase 1 clinical trials using monovalent vaccinations in non-immune participants produced conflicting safety and immunogenicity findings**.** The seroconversion rate was astounding with incredibly low virus doses, and neutralizing antibodies were still detectable one year later with just one dosage of the live attenuated vaccination overall. Nonetheless, the live attenuated vaccine did not cause any serious side events **[182].**

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13.5 Clinical Trial Era, 2000-Present

This **Era** centered on expediting the entry of these proven vaccinations into clinical trials. There were some setbacks; for example, one of the tetravalent live attenuated vaccines was shelved because the antibody

response to each serotype was imbalanced and there was consistently high reactogenicity. A new approach to vaccine development was adopted, while some people kept looking for the ideal formulation for the tetravalent vaccination. In order to find the best formulation for the tetravalent chimeric vaccine, clinical trials assessing vaccine safety and immunogenicity have been the main focus of dengue vaccine development for the past few decades. Although vaccination efficacy studies are in progress, cumulative data show that there are issues with specific safety issues, one of which is the potential for immune-mediated augmentation. The imbalance of immune responses to all serotypes is the other issue **[183].**

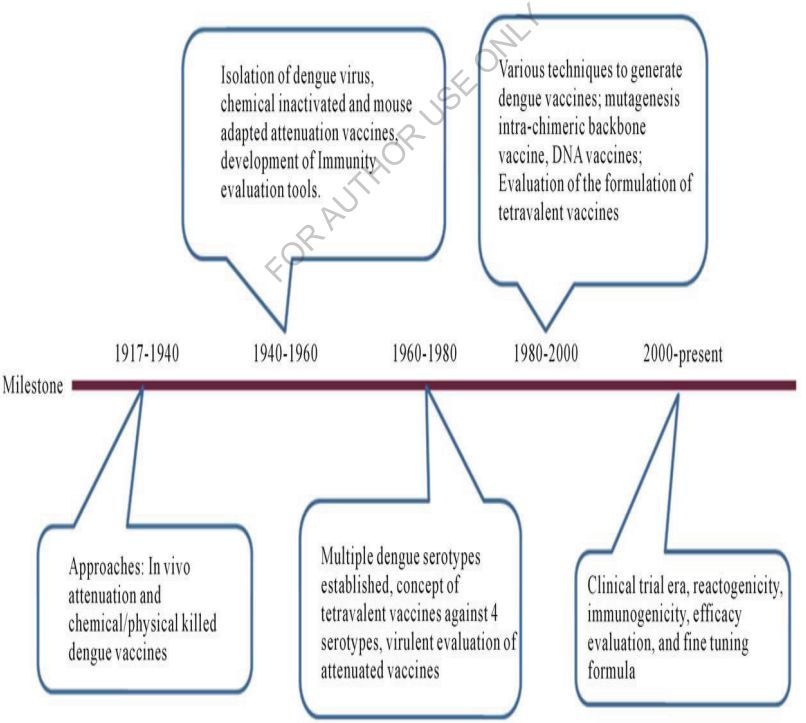

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Figure 7: An important development step for the dengue vaccine. The milestones attained in each era are used to divide up the history of dengue vaccine development. The dates for these landmarks are 1917–1940, 1940–1960, 1960–1980, 1980–2000, and 2000-present.

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14. Opportunities and challenges in computational research

Although dengue has been known since the 17th century, the enormous rise in dengue transmission is likely the result of the ecological disruption and demographic shifts that took place during and after World War II. *Aedes aegypti's* geographical range was significantly expanded as a result of ecological changes, and this, along with the movement of viraemic people, created the perfect environment for the spread of viruses between cities, nations, and continents as well as susceptible people for epidemic transmission. Dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) emerged in Southeast Asia under the optimum circumstances during the War, facilitating its spread to other geographical areas. In terms of morbidity and death, dengue fever (DF) and DHF/DSS are currently regarded as the two most significant arthropod-borne viral illnesses. More research is urgently needed to transform dengue from an emerging, uncontrolled disease into a disease that can be effectively controlled. The top research priorities as determined by the TDR and WHO are presented by A. Kroeger and M. Nathan: New fundamental understanding, including molecular explanations for the variable immune response to Den infection, identification of genetic factors causing DHF/DSS, and pathophysiologic mechanisms causing bleeding and plasma leakage, will aid in the development of safe vaccines, particular medications, early prognostic marker tests, and procedures for effective case management. Computer models will assist in the rationalization of management, while genomic studies will improve the creation of transmission-blocking in mosquitoes. The long-term struggle against dengue necessitates the research and development of new and superior weapons. A better understanding of vector dispersal and the identification of operational indicators for vector control are necessary for better assessing and designing programs related to source reduction and

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personal protection. Improved diagnostics and prognostic markers for DHF are also urgently needed. To add to the arsenal of control instruments, new ones that are efficient, secure, and affordable must be created (such as fatal ovitraps with attractants or insecticide-treated curtains, nets, plastic sheetings, and covers for water storage containers). It is necessary to establish new and improved strategies, policies, and clinical recommendations for the care of DHF in addition to innovative, evidence-based methods for effecting behavioral change. The newly developed instruments must undergo efficacy trials before being evaluated for their suitability for use in routine services. Additionally, it is necessary to examine and evaluate enhanced surveillance methods. Integrative evolutionary techniques in the context of dengue benefit from the zoonotic origin, distinct endemic and sylvatic transmission cycles, immunological serotype interactions, and the enormous diversity of dengue genotypes. However, the predominate focus on discrete biological dimensions (such as host-host transmission) has thus far constrained our ability to integrate key ecological and population genetic findings with the epidemiological behavior of the pathogen. Evolutionary frameworks do not appear frequently in dengue modeling literature, with the field of phylogenetics now dominating evolutionary techniques. This is in stark contrast to other significant viral infections, such as the influenza A virus or the human immunodeficiency virus (HIV), where evolutionary frameworks are dominant. In general, the failure to stop the introduction and establishment of sustained transmission across the globe, as well as the geographical and epidemic growth of dengue genetic variants and mosquito vectors, necessitate a renewed emphasis on and improvement in computational techniques. Frameworks based on ordinary differential equations (ODE), which are tractable and computationally cheap, have dominated the research in the area of dynamic modeling. However, these

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methods frequently make crucial assumptions without challenging them, such as homogenous mixing, competition between antigenic types, continuity, and determinism, which can hinder our comprehension of the essential processes influencing the evolution, epidemiology, and management of dengue. We here propose, discuss, and illustrate a few key computational avenues that we hope will provide beneficial research opportunities in the upcoming years. These include real-time computational analysis of genetic data, phylodynamic modeling frameworks, within-host model frameworks, and GPU-accelerated computing. By linking evolutionary, ecological, and epidemiological aspects of this biological system as well as expanding on traditional modeling assumptions. Programs for DF/DHF prevention that are effective and sustainable must include a number of elements. First, it's crucial to have a functioning, lab-based surveillance system that can give early notice of epidemic activity. Additionally, there needs to be efficient information sharing and global cooperation. In the event that the surveillance system forecasts an increase in dengue transmission, the second component is a rapid-response contingency plan to stop an epidemic before it starts. Its effectiveness depends on political backing to put this swift response into effect on schedule. The medical community's education is the third element of a successful preventative program. Experience has demonstrated that if doctors and nurses are aware of the pathophysiological changes that take place in DHF, case fatality rates can be kept to an acceptable level. As a result, early diagnosis and efficient therapy are essential to preventing fatalities in this condition. Control of *Ae. aegypti* at the community level is the fourth element. The sustainability of the preventative program will depend on reducing the reliance on government mosquito control organizations and increasing the burden of *Ae. aegypti* control on urban residents, where the majority of

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dengue transmission takes place. This will necessitate community involvement and program ownership. The demand for research and enhanced public health infrastructure is also quite high. Research on the epidemiology and illness pathophysiology of DF/DHF, as well as new mosquito control technologies and dengue vaccines, are urgently needed to develop more effective prevention tactics. The emerging epidemic of DF/DHF cannot be reversed without an upgraded public health infrastructure to support community-based preventative efforts.

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15. Conclusion

That many preventive measures—like eliminating standing water in and around the home—are free of charge. First off, living conditions in some endemic nations include extreme temperatures and humidity, erratic electricity, and the requirement for big plastic or metal tanks to store water. Thus, coexisting with mosquitoes on a daily basis becomes the norm and may give one a false sense of immunity to dengue or other diseases spread by mosquitoes. Second, the perceived benefits of devoting time and effort to consistently implementing advised vector control techniques at home are directly related to how community members see disease risk. People's perceptions of the illness can affect health-seeking behaviors since the clinical spectrum of dengue infection ranges from nonsevere (e.g., asymptomatic, cold-like symptoms) to severe (e.g., bleeding). Furthermore, a lack of a thorough grasp of the severity of dengue and other disease threats at the local level would result in erroneous risk awareness and subpar preventive measures, which will impede progress on the country's top priorities for dengue prevention and control. Particularly in impacted countries, dengue fever is a significant financial burden. In order to combat disease transmission, lower death rates, and lower healthcare costs, significant efforts must be made. More scientific investigation is required, which, in our opinion, is the best way to shed light on the pathophysiology of dengue infection and unravel the underlying molecular processes that underlie the development of the disease's more severe forms (DHF/DSS). The creation of a suitable preventive vaccine and efficient therapy will advance as a result. One of the most significant human diseases spread by mosquitoes in the world today is dengue. Numerous strategies have been used in an effort to halt the spread of the disease and lessen its impact, but to no avail. Considering the success in developing vaccines against other flaviviruses,

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such as yellow fever, dengue appears to be a disease that can be avoided. Therefore, developing a dengue vaccine seems to be a feasible and effective way to stop the spread of the dengue virus. With this idea in mind, a wide range of dengue vaccines have been developed. While some of these dengue vaccines are still in pre-clinical development, others are now through clinical trials. Importantly, if everything goes according to plan, some of these dengue vaccinations could be approved within the next few years**.** These vaccines' true safety and population-based effectiveness, however, are still uncertain. It may be necessary to revise the clinical endpoints used to gauge the safety and effectiveness of dengue vaccines. The makers of dengue vaccinations, public health organizations, as well as legislators and regulators, pay insufficient attention to other factors relating to the safety of dengue vaccines. These elements are important and have a role in the complicated illness symptoms that dengue patients experience. It has been years since a safe and effective dengue vaccine has been developed. By including these characteristics into the current vaccine design platform, a candidate may now be within reach. Despite the establishment of voluntary groups with social agendas, dengue prevention, and control face two distinct problems. First, MISPAS has depleted local and national infrastructure for public health preparation in order to fund the integrated rollout of health measures during dengue epidemics. Without the proper preparation, a quick public health response to epidemics may compromise the capacity to withstand subsequent developing disease epidemics. Second, HWs may lack motivation and believe their stakeholder role in decision-making is negligible due to the lack of follow-up, training opportunities, and professional incentives in local leadership. In response, this low morale may lead HWs to look for work in national or worldwide organizations that can offer financial security and career progress.

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